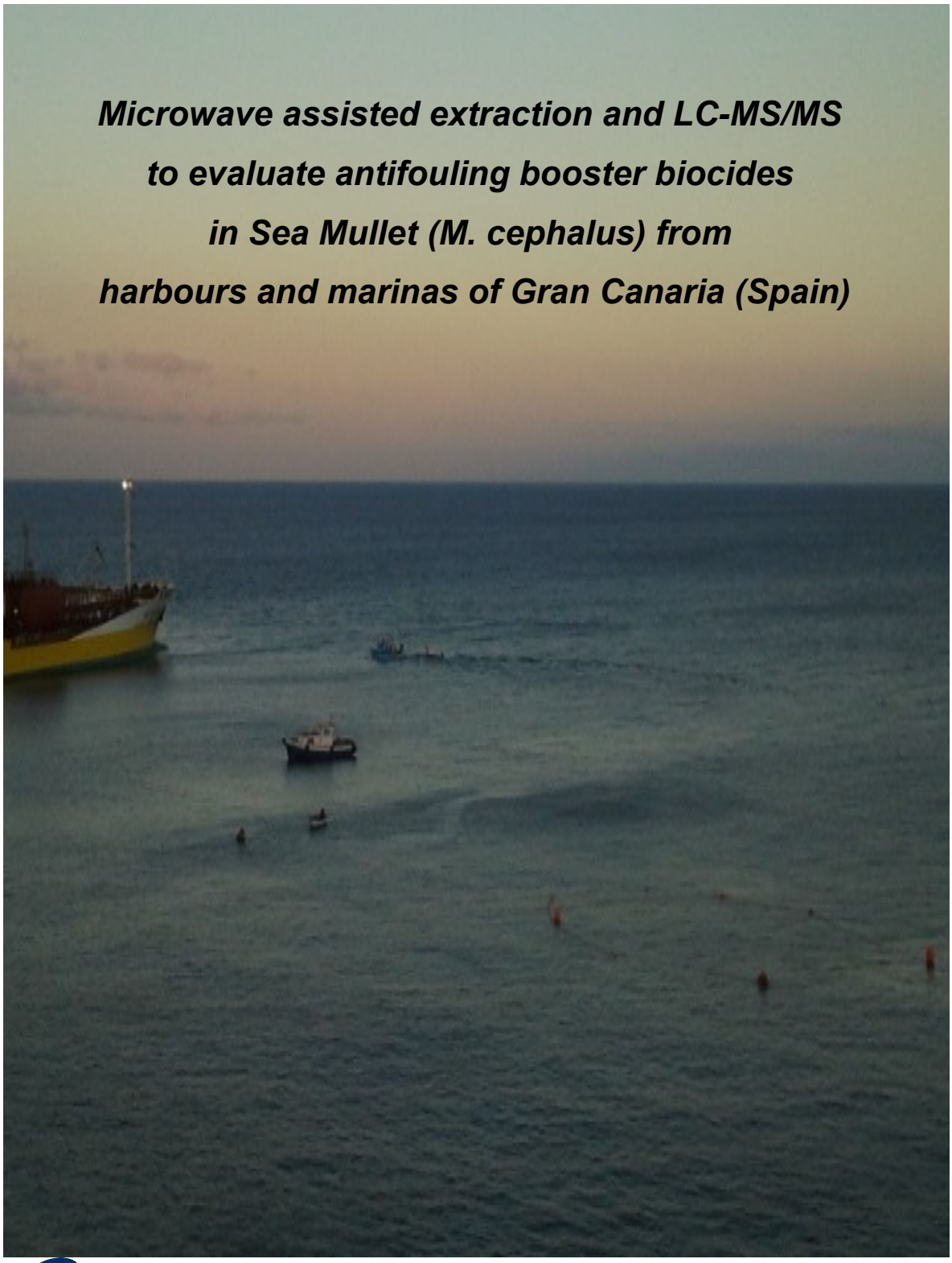
Microwave assisted extraction and LC-MS/MS to evaluate antifouling booster biocides in Sea Mullet (M. cephalus) from harbours and marinas of Gran Canaria (Spain)



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Microwave assisted extraction and LC-MS/MS to evaluate antifouling booster biocides in Sea Mullet (*M. cephalus*) from harbours and marinas of Gran Canaria (Spain)

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Abstract

Biofouling is a problem for any structure placed in the aquatic environment that it can be controlled through chemical biocides like antifouling paints. According to the Biocides Directive (98/8/EC), biocides are active substances or preparations that are intended to destroy, deter, render harmless and exert control or prevent the action of any other harmful organism through chemical or biological means. The widely use of booster biocides in antifouling paints represent an important source of pollution to the marine environment and the transfer of these toxic pollutants to the higher trophic levels is a topic of major concern.

This work present a method for the extraction, preconcentration and determination of two booster biocides commonly employed, Irgarol 1051 and Diuron, in samples of muscle tissue of *Mugil cephalus* based on microwave assisted extraction followed by solid phase extraction as preconcentration and clean-up step (MAE-SPE) coupled with liquid chromatography-tanden mass espectrometry (LC-MS/MS). Optimum conditions of MAE were established in this work and SPE clean-up and LC-MS/MS detection were optimized previously (Sánchez-Rodríguez et al. 2009). In established conditions, limits of detection (LOD) obtained were in the range between 0,1 and 0,4 $ng \cdot g^{-1}$. Recoveries, calculated at three concentration levels (0,5, 5 and 50 $ng \cdot g^{-1}$), were greater than 74%. Precision in, %RSD, was for intra-day assays less than 7,5% and for inter-day less than 12,7% respectively.

The optimized method was employed for monitoring these compounds in muscle and liver tissues of *M. cephalus* in different harbours of Gran Canaria Island. Samples were collected bimonthly and processed following the optimized method. High level of Irgarol (6,9±1,03 $ng \cdot g^{-1}$) were found in the liver whereas Diuron was undetected. However, Diuron was found in muscle (1,41±0,45 $ng \cdot g^{-1}$). The proposed sentinel organism could be used in tropical and subtropical regions for a continuous biomonitoring of booster biocides during long time of periods. This could be a useful tool to improve the ocean and coastal management.

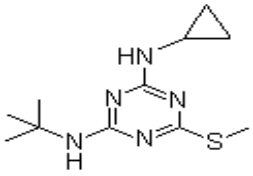
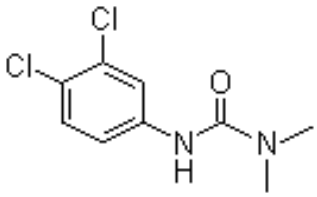
ÍNDICE

1. INTRODUCTION

The international shipping industry moves approximately 90% of world trade and represents less environment damage than its counterpart on land (ICS&ISF, 2011). This big industry is often affected by the marine fouling problem because it increases fuel consumption in proportion to the average hull roughness caused by fouling organisms. Biofouling is a problem for any structure placed in the aquatic environment but it can be controlled through chemical biocides like antifouling paints. According to the European Commission Biocides Directive (98/8/EC), biocides are active substances or preparations that are intended to destroy, deter, render harmless and exert control or prevent the action of any other harmful organism through chemical or biological means. They are used because of their potential to destroy a wide range of organisms and for their relative easy applicability to vessels (La Carbona et al. 2010). For this reason, antifouling paints are today an economic alternative for the shipping industry (Voulvoulis et al. 2002). But, at the same time antifouling paints represent a hot point of release of toxic pollutants to the marine environment. Since the restricted use of Tribultyltin (TBT) due to their ecological effects (Alzieu et al. 1986; Yohei et al. 2003), marine paint companies have been developing alternative antifouling biocides most of them are based on copper (Cu) compounds (Daffor at al. 2011).

Diuron and Irgarol 1051 are the most commonly used booster biocides nowadays leading to significant increases of these biocides inputs into the marine environment (Voulvoulis and Lester, 2006). Diuron is relatively soluble in water and has a reported log K_{ow} of 2.8, K_{ow} is the octinol/water partition coefficient, low values of which indicate hydrophilicity and high ones, lipophilicity. Diuron will be found only weakly sorbed to sediments. Irgarol is persistent in sediments whether adsorbed to sediment particles or associated with paint particles. The key

environmental properties of these booster biocides is presented in Table 1. Consequences of the massive use of these biocides are the presence of high concentrations of these compounds and their degradation products in the coastal areas, specially in marinas and harbours with heavy boating activity (Konstatinou and Albanis, 2004; Lambropoulou and Albanis, 2004; Sánchez-Rodríguez et al. 2011a, 2011b).

	Irgarol 1051 (2-N-tert-butyl-4-*N*-cyclopropyl-6-Methylsulfanyl-1,3,5-triazine-2,4-diamine)	**Diuron** (1-(3,4-dichlorophenyl)-3,3-dimethylurea)
Structure		
Log *Kow*	*2,8*	2,8
Degradation	*$t_{1/2}$ = 100 – 250 days in seawater, persistent in sediments*	*$t_{1/2}$ = 14 days in marine sediment (biotic degradation), persistent in seawater*
Restriction	No restriction	Banned for use in UK and Netherlands, and on pleasure boats in Denmark
CAS Registry Number	28159-98-0	330-54-1

From Tomas and Brooks (2010)

Table 1. Key environmental properties of Irgarol and Diuron.

The risk with booster biocides depend on their half life and degradation rate. Once they have been released to the water column, biocides can be transformed in high or less toxic compounds that persist in the environment elevating their concentrations (Thomas and Brooks, 2010). Among booster biocides, Irgarol 1051® and Diuron have been the most studied due to their persistence in sediments and water column (Eguchi et al. 2010; Thomas et al. 2003).

The problem with this kind of compounds is the lack of knowledge about their fate and effects in aquatic marine environment. Some studies found that these

compounds inhibit the growth of fresh (Lambert et al. 2006) and seawater autotrophs which include key species such as seagrasses or corals (Chesworth et al.2004). Other authors have reported toxicological assays and probabilistic risk assessment of these booster biocides (Sánchez-Rodríguez et al. 2011a) but the potential of bioconcentration and further bioaccumulation through the food web is unknown (Guardiola et al. 2012). One of the reasons of this lack of knowledge is the absence of reliable analytical methods for the extraction and determination of booster biocides in biological samples. Due to the trace concentrations of booster biocides in marine environment it is necessary to develop techniques for extraction that include pre-concentration with the purpose achieving limits of detection (LODs) on the order of $ng \cdot L^{-1}$. Liquid-liquid extraction (LLE), solid phase extraction (SPE) and microwave assisted extractiion (MAE) are the most used extraction methods in water and sediment samples (Sánchez-Rodríguez et al. 2012) and a small number of methodologies are focused on booster biocide determination in biological samples. New advances in the development of methods based on liquid chromatography with tandem mass spectrometric (LC-MS/MS) for detection of analytes in complex matrices as biological matrices have been developed. All these advances include less treatment time of samples, less solvents volumes, fast and accurate identification of the target analytes (Eeckhaut et al. 2009). Techniques reported for the extraction of Diuron and Irgarol in biological samples, like mussel include sonication with acetone and LC-MS/MS analysis (Tsang et al. 2009) or/and mechanical shaking with acetonitrile and LC-MS/MS analysis (Harino et al. 2006).

A recent survey of booster biocides in harbours, ports and marinas of Gran Canaria applying a novel method of microwave-assisted extraction combined with LC-MS/MS revealed high levels of Diuron and Irgarol 1051 in surface waters and sediments (Sánchez-Rodríguez et al. 2009, 2011b). This fact could indicate a possible bioconcentration in the marine organisms that living in the area

presumably for passive diffusion (Katagi, 2010). Also, it has been reported that Diuron poses a risk to aquatic life as other herbicides because it generates an impact in vascular plants and algae which are primary producers in the aquatic food web (Fojut et al. 2012). Fishes are in the top of trophic levels. Therefore, they could indicate food chain effects and past pollution levels in the environment (Friedrich et al. 1996). Also, they are an important human food source and despite pelagic habits of this kind of organisms, fishes can be used as good monitor for great scale assessment (Fernandes et al. 2007; Raymeny and Barry, 2000). Previous surveys have used fishes as indicators organisms of organic pollutants showing good correlations between environmental concentrations and those found in fish tissues (Harino et al. 2000; Lee et al. 2006; Muñóz et al. 2010; Restrepo et al. 2008).

The Sea Mullet, *Mugil cephalus* could be one of these indicators organisms. It presents a worldwide geographical distribution, tropical and subtropical regions, found in all oceans from 42°N to 42°S. It is a common specie in coastal water, resistant to changes in the environment, sometimes extremes and obtain their food material from the bottom sediment (De Silva, 1980; FAO, 2012). Moreover, *M. cephalus* is a benthopelagic fish, frequent and abundant throughout the area of Gran Canaria until 50 meters deep (Brito et al. 2002). It presents a long-life period of 22 years old allowing the sampling of more than one year-class (Danemann and Ezcurra, 2008). Also, it is easy to sample in harbours and provide adequate tissue for analysis.

The purpose of this study is the optimization and development of a methodology for the extraction and determination of two selected booster biocides, Diuron and Irgarol 1051, in *M. cephalus* tissue. This methodology is based on microwave assisted extraction followed by solid phase extraction as pre-concentration and clean-up step (MAE-SPE) coupled with liquid chromatography-tanden mass

spectrometry (LC-MS/MS). The optimized process will be employed for the evaluation of these compounds in the marine environment using the selected organism.

2. MATERIALS AND METHODS

2.1. *Chemicals and reagents*

Diuron (1-(3,4-dichlorophenyl)-3,3-dimethylurea) and Irgarol 1051 (2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine), were obtained from Sigma-Aldrich (Steinheim, Germany). All compounds were at 99.0 % of purity. The stock solutions (1000 $\mu g.mL^{-1}$) were prepared by dissolving appropriate amounts of the commercial products in methanol and then storing the solutions in glass stoppered bottles at 4°C in the dark prior to the use.

LC-MS grade methanol used to dissolve the standards, LC-MS water, and ammonium formate and formic acid (98%) used to prepare the mobile phase were purchased from Panreac Química (Barcelona, Spain). Ultra-high-quality water, obtained by a Milli-Q (Millipore, Bedford, MA, USA) water purification system was used in the solid phase extraction (SPE) protocol and to dilute samples. The 0,45µm syringe driven filter employed for the purification of the extract solution was provided by Scharlau Chemie S.A. (Barcelona, Spain).The SPE cartridges (Envirelut pesticide 500 mg) were supplied by Varian (Madrid, Spain).

2.2. *Instrumentation*

For the extraction, a multiwave microwave oven with a 6 EVAP rotor and 6MF 100 vessels (Anton Paar, Graz, Austria) was used. A Varian Vac Elut 20 SPE manifold (Varian INC, CA, USA) coupled to a Santorius vacuum pump was used for the clean-up step.

Chromatographic analysis was carried out using a reversed phase liquid chromatography coupled to a triple quadrupole mass spectrometer equiped with an

electrospray interface (LC-ESI-MS/MS). The equipment was composed of a Varian 320-MS TQ Mass Spectrometer (Varian Inc, CA, USA), with a Varian HPLC system consisting of a binary pump, auto-sampler and temperature controlled column compartment.

2.3. *Sample collection*

For the extraction optimization, muscle tissue of *M. cephalus* was obtained from the Central market of Gran Canaria (Canary Islands, Spain). Samples used were obtained from surrounding environment.

In function of the benthopelagic habits of the sentinel organism, two sampling stations were developed. Gran Canaria Island has a circular shape with important shipping activity from the south to the north of the island. To monitor the impact of this shipping activity two ports were chosen, Puerto de Mogán at the south-west of the island and Muelle Deportivo de Las Palmas at the north-east (Fig. 1). During a period of five months (February - June of 2012), every two months samples of fishe were taken from the internal docks of each port by a fisherman using a fishing rod. From each point, six fishes were caught and pooled to 3 or 4 individuals with the same size. Then, livers and muscle tissue were separated and mixed in the pool and carefully stored at 4°C until the preparation and analysis.

2.4 Sample preparation

Fishes bought from the market for the optimization were dissected and the muscle without skin was carefully extracted and dried in an oven at 70 °C until constant weight. Finally, it was homogenized using a mortar. These samples were previously analyzed to verify that the samples did not contain any quantity of the analytes in the study. Muscle samples were spiked with a mix of selected compounds in methanol to obtain a final concentration of 50 $ng \cdot g^{-1}$ in order to study the best conditions to extraction. Then, the samples were stirred to homogenize and air-dried for overnight in the dark at room temperature.

Samples collected from the ports were also treated the same way for the determination of diuron and irgarol in muscle and livers of *M. cephalus* for further evaluations.

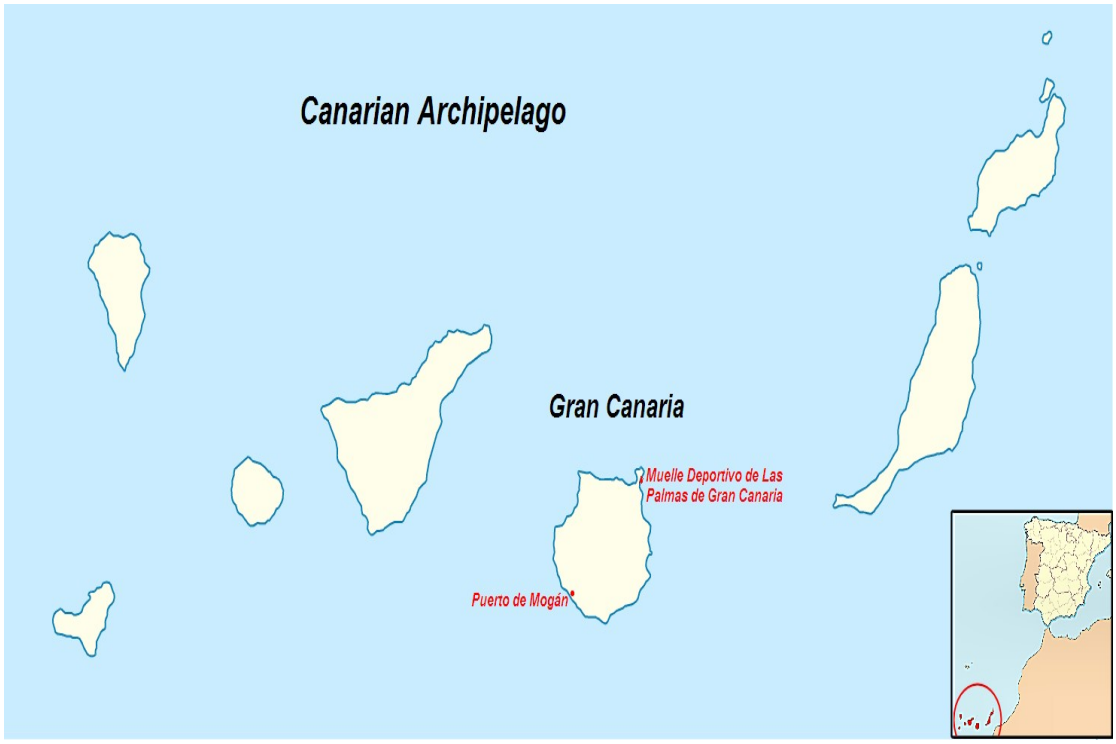


Figure 1. Sampling sites map.

2.5 MAE-SPE Procedure

For the microwave assisted extraction (MAE) process, two grams of the spiked sample were transferred to the polytetrafluorethylene (PTFE). Then, 10 mL of extract agent (methanol) was added to the samples and the vessels were closed and placed symmetrically in a rotor. Once the rotor was placed in the microwave oven, a power of 200W for 4 minutes of duration was used for the satisfactory extraction of selected analytes. At the end of the extraction the vessels were allowed to cool at room temperature for 10 minutes before open the microwave. After this, the extract solutions were filtered with a 0,45 μm syringe filters and were diluted with 100 mL of Milli-Q water previous clean-up solid phase extraction (SPE).

The SPE protocol employed in this work had been published previously by our research group (Sánchez-Rodríguez et al. 2009, 2011b). The first step was the activation of the cartridge (Envirelut pesticide, 500 mg) with 2 x 5 mL of methanol and 2 x 5 mL of Milli-Q water. MAE extracts were passed through the cartridges under a vacuum at a flow rate of 1 mL/min. The rinsing step was carried out with 2 x 5 mL of Milli-Q water and the elution step with 2 x 1 mL of methanol at the same flow rate. Final extracts were introduced into a glass vials before their analysis in the LC-MS/MS.

2.6 LC-MS/MS

The LC-MS/MS analyses were carried out in a Varian 320-MS LC/MS/MS system (triple quadrupole) equipped with an electrospray ionization (ESI) interface. The stationary phase was a Varian Pursuit UPS 2.4 C18 50 x 2.0mm (2.4 μm particle size) column set at 40°C.

The chromatographic separation was performed under isocratic conditions with 40% (v/v) of 5 mM ammonium formate, acidified with 0.2% (v/v) formic acid (Phase A) and 60% (v/v) methanol (Phase B). A flow rate of 0.2 $mL.min^{-1}$ was maintained for 8 min, and 10 μL of the sample was injected into the system.

The mass spectrometer parameters are detailed in Table 2. The housing temperature was set at 60°C, the nebulizing gas pressure at 50 psi, the drying gas pressure at 30 psi, and the shield and needle voltages at 0.6 kV and 5 kV, respectively. Nitrogen was used as the nebulising and drying gas and argon as the collision gas at a pressure of 3.78×10^{-5} psi.

Analytes determination were carried out in the multiple reaction monitoring (MRM) mode. The detector voltage was fixed at the maximum extended dynamic range (EDR) to optimise the signal, and the mass width was set at 2 and 1.5 amu for the first and third quadrupole, respectively. The dwell for all transitions was 0.1 s.

Simultaneous presence of the quantification and confirmation ions and retention times based on authentic standards were used to identify positively the presence of booster biocides in real samples.

Analite	Mass	Precursor ion	Capillary (V)*	Cuantification
				Coll. e(V)[a]
Diuron	232	233	44	71,9 (14,5)
Irgarol	253	254	48	198 (11,5)

Table 2. ESI/MS/MS parameters for studied analytes.

2.7 *Statistical analysis*

Experimental design for the optimization of MAE conditions (3^2) was performed using the Statgraphics Plus software, version 5.1 (Manugistic, Rockville, MD, USA).

2.8 Analytical parameters

Calibration curves were obtained from spiked muscle samples subjected to the optimized MAE-SPE process with six different concentrations of each compound in the range of 0,5 - 400 $ng \cdot g^{-1}$. Triplicate analysis were performed at each

concentration. Linear relationship were obtained between the peak areas and the analyte concentrations with correlation coefficients higher than 0,995 in both cases.

Inter-day and intra-day precision, as relative standard deviations (%RSDs) and recoveries were calculated at three concentration levels (0,5; 5 and 50 $ng \cdot g^{-1}$) from the six spiked samples to which the MAE-SPE extraction and LC-MS/MS were applied.

Limits of detection (LODs) and quantification (LOQs) were defined and determined to equal the concentration of analyte that yielded a signal to noise of 3 and 10, respectively.

3. RESULTS AND DISCUSSION

3.1. MAE-SPE extraction optimization

Preliminary studies of antifouling booster biocides were developed for seawater and sediments (Sánchez-Rodríguez et al. 2009, 2011b). Frequently, methods used for the determination of analytes in sediments samples are employed for the evaluation of biological samples. In this sense, microwave assisted extraction (MAE) is an efficient extraction technique for solid samples (Sánchez-Rodríguez et al. 2012).

Main parameters that influence in MAE extraction include microwave power, extraction time and extraction volume, between them (Madej, 2009; Vega-Morales et al. 2011).

Factorial designs are used in MAE optimization. The application of these strategies decrease the numbers of assays required and accounts for the interactions between variables that occurs in the extraction process (Miller and Miller, 2005). Sánchez-Rodríguez et al. (2011b) established that the time and power extraction were the most influenced variables in the MAE extraction in the screening phase. Because of the small influence of the solvent volume on the analyte extraction, this variable was fixed in 10 mL.

With the purpose to achieve better results for the extraction in muscle tissue, the weight of the sample was increased to 2 grams (Sánchez-Prado et al. 2010). In these conditions, a new factorial design was needed to establish the optimum microwave power and extraction time.

A 3^2 factorial design was performed with two variables (irradiation time and power) and three levels (2, 4, 6 minutes and 200, 400 and 600 W, respectively). This design consisted of 12 randomly distributed runs. All analyzes were performed on

2 g of sample (containing 0,5 $\mu g \cdot g^{-1}$ of each analyte) and they were carried out in triplicate using the polynomial fits of the results. The response surface for each analyte was composed. Figure 2 shows the response surface obtained for Diuron (a) and Irgarol (b). It is clear the great influence of high power during longer time in low peak areas probably due to volatilization or degradation of the analytes. Higher analytical signals were obtained at low power and less time but with small differences for irradiation time among the analites. For this reason, intermediates values of time (4 min) and power (200W) were selected like conditions for the extraction of Diuron and Irgarol using MAE.

In the light of these results, the MAE-SPE optimized procedure for these analytes were obtained with 2 grams of sample tissue in 10 mL of methanol-like solvent, during 4 minutes at 200W of power. The obtained extract was diluted in a 1/10 methanol/MilliQ (v/v) ratio. SPE procedure was described in section 2.5 section and it was applied to obtain 2 mL of methanol as the eluate.

Figure 3 shows a flow scheme of the whole analytical method.

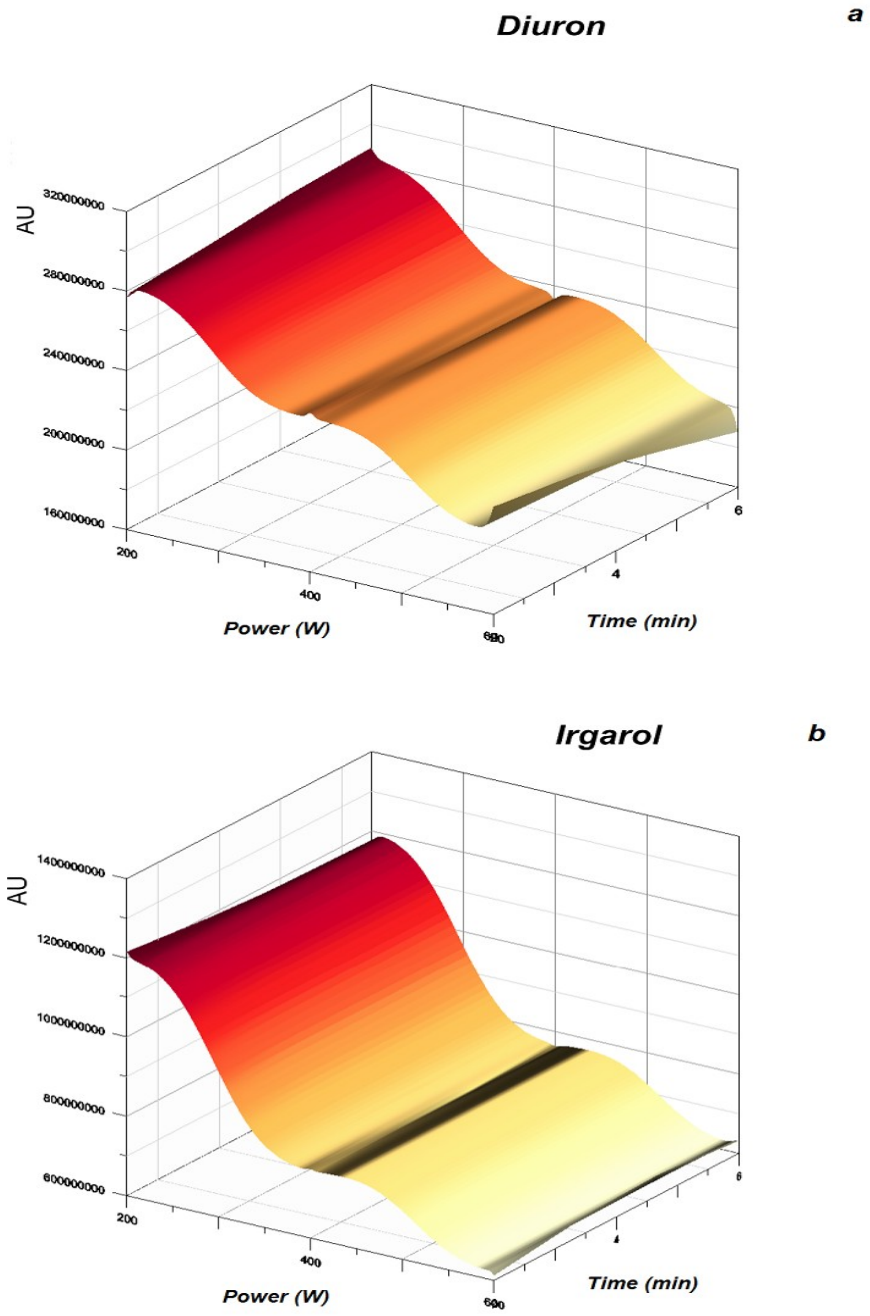


Figure 2. Response surface for the effect of time and power on the extraction of: a) Diuron and b) Irgarol.

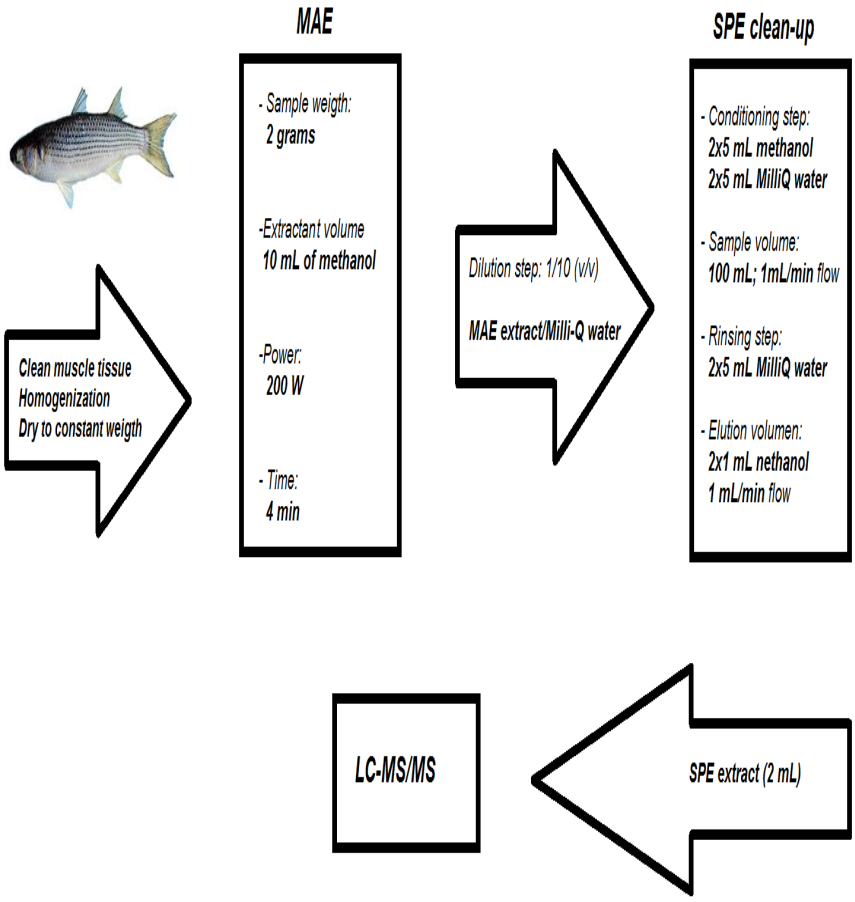


Figure 3. Flow scheme of the protocol of the proposed analytical method.

3.2. Matrix effects

Liquid chromatography coupled to tandem mass spectrometric detection (MS/MS) is currently considered as the method of choice for quantitative analyses of compounds in biological matrices. However, molecules originating from sample matrix that coelute with the compounds of interest can interfere with the ionization process, occurring ionization suppression or enhancement (Xu et al. 2007). This phenomenon is called matrix effect and it was first described by Kebarle and Tang (1993).

Matrix effects has been described by Taylor (2005) as the Achilles´heel of quantitative LC-MS/MS methods. For this reason this effect has been evaluated over the current proposed method. It is not well known the mechanics of matrix effects but it could originate from the competition between an analyte and the coeluting process. These effects appear during the access of analytes and undetected matrix components to the droplet surface for transfer to the gas phase (Matuszewski et al. 2003). The matrix effect was assessed by means of the post-extraction spiked method. This consists of the comparison of the response of the analyte in pure solution (*A*) to the response of the analyte spiked into a blank matrix sample (*B*) that has been performed through the proposed MAE-SPE LC-MS/MS method, using the mathematics expression published by Taylor (2005):

$$(B-A)/A*100$$

The results can be interpreted as suppression or enhancement according to whether the response is diminished or magnified (Eeckhaut et al. 2009). According to Taylor (2005) a value of 0% represent no matrix effect, negative values would indicate suppression and positive values, an enhancement of the signals.

The results measured from the tissue samples indicate a relative loss of signal of -28 for Diuron and -17 for Irgarol. It means a relative signal suppression, ranging from 17% to 28%. It could be due to alterations in ionization efficiency caused for

the undetected matrix components. Eeckhaut et al. (2009) review report validated LC-M/S/MS methods with matrix effects values until 40% in bio-analytical assays. According to this, we conclude that the currents values obtained could be acceptable for the proposed method.

3.3 Analytical parameters

Once the MAE-SPE procedure was optimized for muscle samples, the performance of the method was evaluated by estimating the linearity, sensitivity, precision and recovery.

Calibration curves were obtained with spiked samples in a linear range between 0,5-400 $ng \cdot g^{-1}$ using triplicate analysis of each one. The correlation coefficients were higher than 0,995% in both cases.

Limits of detection (LODs; S/N: 3) and limits of quantification (LOQs; S/N: 10) were below of the limits reported for sediments (Sánchez-Rodríguez et al. 2011b) and mussels (Harino et al. 2006, Tsang et al. 2009). They were enough to determine the presence of Diuron and Irgarol in real samples.

Recoveries (%), intra-day and inter-day precision assays were performed using three levels of concentrations (0,5; 5 and 50 $ng \cdot g\text{-}^{1}$) from the calibration curve and applying the whole method (MAE-SPE-LC-MS/MS).

Good percentages of recovery ranging between 74% and 97%, were reached. Intra-day and Inter-day precision calculated like relative standard deviations (%RSDs) were lower than 13% in all cases. All obtained results are shown in Table 3.

Chromatogram of a spiked sample tissue of M. cephalus is shown in Figure 4. Good peaks shapes and resolution were achieved for both compounds.

Level	Parameter	Compound	
		Diuron	*Irgarol*
0,5 ng·g⁻¹	*LOD*[a] (ng.g⁻¹)	0,13	0,1
	LOQ[b] (ng.g⁻¹)	0,44	0,34
	*Recovery (%)**	86,1±7,8	97,9±2,8
	Intra RDS (%)[1]	7,2	3,3
	Inter RSD (%)[2]	8,7	12,7
5 ng·g⁻¹	*Recovery (%)**	80,7±7,6	89,2±7,2
	Intra RDS (%)[1]	6,4	7,5
	Inter RSD (%)[2]	9,2	11,7
50 ng·g⁻¹	*Recovery (%)**	74,4,2±4,2	76,2±3,8
	Intra RDS (%)[1]	4,4	7,5
	Inter RSD (%)[2]	7,7	10,2

*Mean and relative standard deviation (n=6); [1]Relative standard deviation Intra Day (n=6)

Table 3. Analytical Parameters for determination of Diuron and Irgarol under study using MAE-SPE-LC-MS/MS procedure in fish muscle tissue.

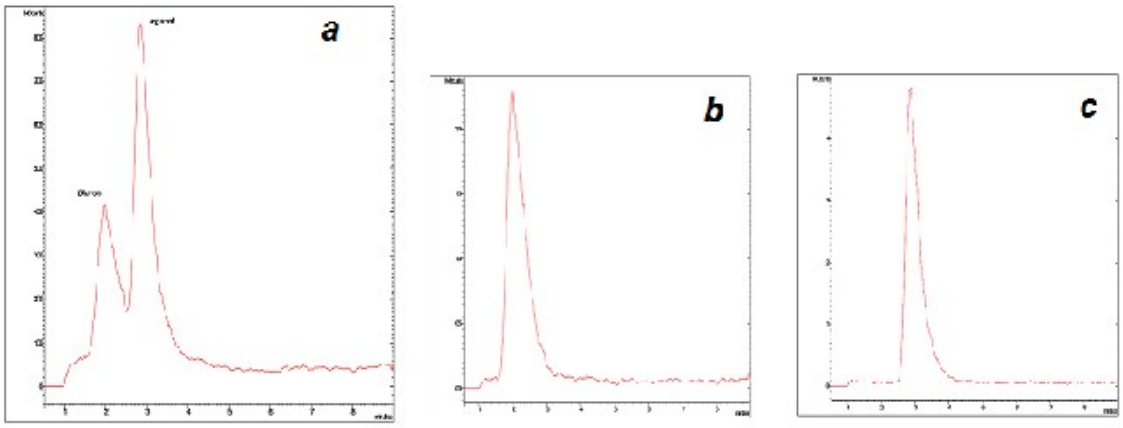


Figure 4. Chromatogram of a spiked tissue sample extract after MAE-SPE process: a) peak of both compounds, b) analytical signal of Diuron and c) Irgarol.

3.4 Determination of Diuron and Irgarol in muscle and liver of Mugil cephalus

The proposed method was applied to evaluate the levels of Diuron and Irgarol in the biota samples from harbours of Gran Canaria Island. For that, the first step was to select a good organism to serve as a monitor.

M. cephalus was chosen because it presents the characteristics of a good sentinel organism. It has a long life, it is abundant, easily identified, and is available throughout the year. Furthermore it provides enough tissue for analysis, it is resistant to stress, and is tolerant to a wide range of variations in physicochemical parameters (Phillips, 1978; Mogrado and Bebianno, 2005; Hajjaj et al., 006). Moreover, it presents a close relationship with sediments.

Concentrations of Diuron and Irgarol were assessed in muscle and liver tissues of *M. cephalus* after optimizing the MAE-SPE-LC-MS/MS method. These tissues were chosen because they showed the fate of the selected compounds once they have been released on the water column. Biocide evaluation in these tissues could represent a measure of their impact on the public health because *M. cephalus* is often a food-fish. Moreover and despite the fact that the liver is rarely consummed, this tissue is a good monitor for the pollutants in the environment because of its detoxification role. The liver is well-known to detoxify many types of organic compounds. (Kurunthachalam et al. 1995).

Obtained results are shown in Table 4. Levels of Diuron found in muscle were in the range of 0,51-1,73 $ng \cdot g^{-1}$. Highest concentrations were located in Muelle Deportivo (1,73±0,68 $ng \cdot g^{-1}$). It is noteworthy that this compound is restricted in some countries of Europe Union (Table 1). These diuron levels were not detectable during the last month of sampling.

In liver samples, diuron was not detected during the whole sampling. This fact could indicate a faster metabolism of this compound, which could be possibly to the action of microsomal enzymes (Hajjaj et al. 2006).

Month	Site	Diuron ($ng \cdot g^{-1}$)		Irgarol ($ng \cdot g^{-1}$)	
		Muscle	Liver	Muscle	Liver
February					
	Las Palmas	1,73±0,68	<LOQ[1]	0,58±0,18	<LOQ[1]
	Mogán	1,09±0,56	<LOQ[1]	0,84±0,18	1,04±0,43
April					
	Las Palmas	0,51±0,17	<LOQ[1]	0,69±0,21	0,53±0,22
	Mogán	<LOQ[1]	<LOQ[1]	0,43±0,02	0,80±0,15
June					
	Las Palmas	<LOQ[1]	<LOQ[1]	0,35±0,03	0,56±0,06
	Mogán	<LOQ[1]	<LOQ[1]	0,42±0,01	6,9±1,03

*mean of two determinations and standard deviation; [1]Below Limit of Quantification.

Table 4. Concentrations levels of selected biocides in *Mugil cephalus.*

However, unlike diuron, highest levels of Irgarol, 6,9±1,03 $ng \cdot g^{-1}$ and 1,04±0,43, were concentrated in the liver. Hajjaj (2006) report the same pattern for Phenyltin and Butyltin compounds in *M. cephalus*, the first chemicals used as antifouling and ban since 1980.

In all cases, samples collected in February presented the highest concentrations of both compounds in muscle samples. This fact could be related with the main season for tourism and with major shipping activity in Gran Canaria (Gobierno de Canarias, 2012).

In a attempt to understand better the presence of Diuron and Irgarol in *M. cephalus*, Table 5 show the mean of all measures (n=18) and the last reported concentration in sediments in the studied area. The comparison between concentrations found in *M. cephalus* and sediments could suggest an efficient transfer of these compounds from the environment to the biota.

M. cephalus is a secondary specie in the food web and any accumulation of some pollutants in their tissues could transfer to predators or higher trophic levels in the process known as biomagnification (Connell, 1988, Katagi, 2010). This process

could have important implications in the health and conservation of the marine biodiversity.

This approach of the booster biocides determination in marine biota could be the first step to adopt a tool for the study of the monitoring of changes in the concentrations of these selected compounds in different matrices (water, sediment and biota) through the time and different geographical-scales. The proposed specie as sentinel organism is a cosmopolitan specie which would permit a extend monitorization in tropical and subtropical regions where it is present.

Measures in biota could relate all concentrations of booster biocides present in water and sediments. Furthermore, accumulation of these contaminants in tissue may be useful for evaluating the potential trophic transfer of contaminants (Chapman, 1992). In this sense, biomonitoring booster biocides could help to trace the final fate of these compounds in the marine environment.

***Mugil cephalus* – Gran Canaria This reserch**						**Environment – Gran Canaria (Rodríguez-Sánchez et al., 2011b)**
Muscle			**Liver**			**Sediments**
X	**D.E.**	**Min-max**	**X**	**D.E.**	**Min-max**	**Min-max**
1,41	0,45	0,51 – 1,73	-	-	-	1,0 – 6,4
0,55	0,18	0,35 – 0,84	1,96	2,7	0,53 – 6,9	0,7 – 26,4

Mean concentration in (ng.gr^{-1}) dry weight; D.E.= Standard deviation;

Min-max= Minimum and maximum values; [1]LOD=Limit of Detection; *= One Determination

Table 5. Overview of concentration levels of diuron and irgarol in *M. cephalus* versus concentration levels reported for sediments in the same area.

4. CONCLUSIONS

In this work, a trace analytical method based on microwave-assisted extraction and SPE clean-up (MAE-SPE) combined with LC-MS/MS was developed for the determination of Diuron and Irgarol in fish samples. The best extraction conditions were obtained with 2 grams of sample tissue in 10 mL of methanol-like solvent, during 4 minutes at 200 W of power, an extract dilution of 1/10 methanol/MilliQ (v/v) ratio, and the clean-up and pre-concentration previous to elution step with 2x1 mL of methanol.

Low LODs and LOQs were reached. The proposed method offers a combination of sensitivity and simplicity when compared with conventional methods.

Applications of the developed method to the analysis of muscle and liver tissues of *M. cephalus* indicate the presence of Irgarol in both studied tissues with concentrations in the order of $ng \cdot g^{-1}$. However, Diuron was not detected in liver tissue of *M. cephalus.*

Preliminary results could suggest a transfer of these pollutants from the study environment to the organism by bioconcentration and bioaccumulation processes. This evidence would indicate that the biomagnification process could be possible. It will require further study to confirm this aspect. In this sense, results demostrate that the optimized methodology could represent a powerful tool in the evaluation of Diuron and Irgarol in the fish samples. *M. cephalus* could be used like monitor organism in marine environment to study the fate of these biocides in the food chain.

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