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Multiresidue method for the analysis of five antifouling agents in marine and coastal waters by gas chromatography–mass spectrometry with large-volume injection

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Abstract

A simple multiresidue method has been developed for the determination of five pesticides, commonly used as active ingredients in antifouling paints, in seawater samples. The pesticides studied were: chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), dichlofluanid (*N*-dimethyl-*N*-phenylsulphamide), Sea-Nine 211 (4,5-dichloro-2-*n*-octyl-4-isothazolin-3-one), Irgarol 1051 (2-methylthio-4-*tert*.-butylamino-6-cyclopropylamino-*s*-triazine) and TCMTB (2-thiocyanomethylthiobenzothiazole). The analytes were extracted from 200 ml water samples, using solid-phase extraction. A copolymer with hydrophilic–lipophilic balance was used as sorbent yielding good recoveries (82–95%) for most compounds except dichlofluanid and Sea-Nine 211 (<60%). Large volume injection (10 μl) gas chromatography and electron impact ionization MS (selected ion monitoring mode) detection enabled these compounds to be identified and quantified at the 1.2–3.0 ng/l level. Analysis of samples performed in three marinas in Almería (Spain) revealed the presence of Irgarol 1051 in all the cases, at concentration levels between 25 and 450 ng/l.

Keywords: Water analysis; Environmental analysis; Pesticides; Chlorothalonil; Dichlofluanid; Sea-Nine 211; Irgarols; Thiocyanomethylthiobenzothiazole

1. Introduction

To prevent biofouling of submerged surfaces in the sea, antifouling paints are widely used. Since the restriction imposed by European Union regulations [1,2] on the use of tri-*n*-butyltin (TBT) as an active ingredient, new formulations have been introduced in the market. Typical registered antifouling paints contain metallic compounds such as copper in amounts of 50% (w/w) and pesticides such as Irgarol 1051, Sea-Nine 211, diuron, chlorothalonil, zineb, TCMTB (2-thiocyanomethylthiobenzothiazole), etc., in amounts of around 5% (w/w).

Unfortunately, these authorised alternatives to TBT have also been revealed as a risk for the aquatic environment due to the slow dissipation of these compounds to the surrounding waters [3]. A recent toxicological study of Irgarol 1051 [4] shows that this compound is highly toxic to non-target marine algae and that it is sufficiently stable to reach toxic concentrations in certain areas in the marine environment. In this sense, a few studies have been published about the presence of Irgarol 1051 in environmental samples [5–10], but scarce information exists about the presence of other herbicides, which can contribute to combined effects. Recent papers have emphasised the lack of established analytical

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multiresidue methods to cover these target compounds in seawater samples as well as the lack of data available on their presence in marinas and coastal areas [11].

Most of the analytical procedures reported are based on classical liquid–liquid extraction (LLE) [10,12] or solid-phase extraction (SPE) [5,6,8,10] followed by GC–MS or LC–MS detection [13–15]. Solid-phase microextraction [16] and inmunochemical techniques has also been applied [17,18]. Although GC–MS is a very sensitive technique, detection of the low levels of pesticides present in water samples often requires the application of preconcentration techniques that include the processing of large volumes of water (1–2 l). This results in tedious and time-consuming procedures, especially for routine laboratories that will finally carry out the extensive monitoring seawater programmes.

A considerable gain in total analysis time and, therefore, an increase in sample throughput can be achieved by reducing sample volume (200 ml only) and compensating the generated loss of sensitivity by injecting a volume of sample that is larger than the typical 1–2 μl [8,19,20]. The use of more selective analytical techniques such as GC–MS with chemical ionisation (positive and negative) may also contribute to improve the system's performance and yield very highly sensitive (low ppt) analytical procedures.

The aims of the present investigation were: (i) to develop a rapid and reliable multiresidue method for the analysis of a group of pesticides that is usually present in antifouling paint formulations (chlorothalonil, dichlofluanid, Irgarol 1050, Sea-Nine 211 and TCMTB) by GC–MS and (ii) to determine the presence of these compounds in different marinas in Almería (Spain).

2. Experimental

2.1. Chemicals and reagents

From the pesticides studied, Irgarol 1051 was obtained from Ciba-Geigy (Barcelona, Spain); Sea-Nine 211 from Rohm & Haas (Philadelphia, PA, USA); dichlofluanid, chlorothalonil and TCMTB from ChemService (West Chester, USA). In all the cases purity was higher than 98%. Individual stock standard solutions (100 mg/l) were prepared in ethyl acetate and stored in the dark at −20°C. Working standard mixtures in ethyl acetate, containing 10 mg/l for each pesticide, were used for spiking samples. Calibration standards were prepared in ethyl acetate and in SPE extracts from blanks of seawater samples. In the last case, 200 μl aliquots of the extract were evaporated to dryness under a gentle stream of nitrogen and dissolved again, with sonication, in 200 μl of ethyl acetate containing the pesticides studied.

Pesticide grade ethyl acetate and methanol were obtained from Merck (Darmstadt, Germany). Helium (99.999% purity) used as carrier gas, methane (99.9995% purity) for CI experiments and nitrogen (99.999% purity) for drying were from Air Liquide (Madrid, Spain).

2.2. Sample preparation

Water samples were taken from three marinas located in Almería, a province in the south of Spain on the Mediterranean coast. Samples were also taken at a reference site, out of the marinas, where the presence of the pesticides studied was not detected. Samples were collected at a depth of 30–40 cm in 2.5-l glass bottles, which were previously washed and rinsed in ethanol. Pesticides were extracted on arrival at the laboratory by SPE.

Off-line SPE experiments were performed using an automated sampler processor from Gilson (Villiers-le-Bel, France). This system includes: an Automated Sample Preparation with Extraction Columns system (ASPEC XL) fitted with an external LC pump for dispensing samples through the SPE cartridges and with a switching valve for the selection of samples. The LC and the switching valve are devices from Gilson (models 306 and 817 respectively).

We used Oasis hydrophilic–lipophilic balance (HLB; divinylbenzene–*N*-vinylpyrrolidone copolymer) cartridges (200 mg, 6 ml) from Waters (Milford, MA, USA) to carry out the preconcentration of the sample. The conditioning step was performed with 5 ml of ethyl acetate follow by 5 ml of methanol and finally 4 ml of distilled water, all at 4 ml/min. The HLB cartridges can dry between the conditioning and loading step, which is not possible with sorbents like styrene–divinylbenzene. A 200 ml

seawater sample was loaded at 10 ml/min in the Oasis cartridges. After the preconcentration, the sorbent was completely dried with nitrogen with the pressure set at 2 atm (positive pressure) (1 atm= 101 325 Pa). The time used for drying was 15–20 min. The elution step was performed by adding 2×2 ml of ethyl acetate to the cartridge at 1 ml/min and waiting 5 min between the two aliquots in order to keep a good contact time between the solvent and the trapped analyte. The final evaporation of the extra solvent was carried out with a stream of nitrogen at 30°C. The extracts were preconcentrated to a final volume of 1 ml in ethyl acetate.

2.3. GC–MS analysis

GC–MS analyses were run on a HP 6890 Series gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) interfaced to a HP 5973 mass-selective detector. Data acquisition and processing, and instrument control were performed by the HP MSD ChemStation software. Analytes were separated in a Hewlett-Packard HP-5MS capillary column (5% diphenyl/95% dimethylsiloxane), 30 m×0.25 mm I.D., 0.25 μm film thickness. A 2.5 m×0.25 mm I.D. uncoated retention gap (Hewlett-Packard) was coupled to the front of the analytical column, via a press fit connector. A split/splitless injector was used in splitless mode. Two different injection-port liners were assayed: (a) a deactivated single-tapered liner prepacked with glasswool (Hewlett-Packard), and (b) an empty liner that was filled with 0.5 cm Carbofrit (Restek, Bellefonte, USA) placed at 3.6 cm from the upper part of the liner. After several tests, the injector operating conditions were optimised as follows: injection volume 10 μl; injector temperature 250°C; initial pulse pressure 30 p.s.i. (1.5 min); split flow 50.0 ml/min and split time 1.5 min (1 p.s.i.= 6894.76 Pa). The helium carrier gas flow was maintained at 1 ml/min. The oven temperature programme was 1.0 min at 105°C, 25°C/min to 180°C, 5°C/min to 230°C (1 min) and the transfer line temperature was set at 280°C.

Typical MS operating conditions were optimised by the autotuning software. Electron impact (EI) mass spectra were obtained at 70 eV electron energy and monitored from m/z 50 to 400. The ion-source and quadrupole analyser temperatures were fixed at 230°C and 106°C respectively.

Using methane as reagent gas, analyses were performed in positive (PCI) and negative (NCI) chemical ionisation mode. The autotuning software performed the reagent gas flow adjustment and the lens and electronic tuning. The quadrupole temperature was fixed at 120°C (PCI) and 106°C (NCI) and the ion-source temperature at 250°C (PCI) and 150°C (NCI).

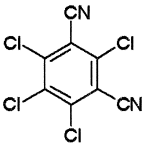
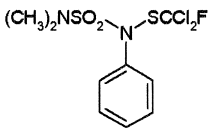
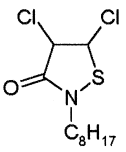
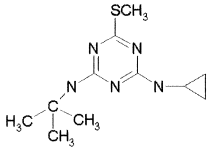
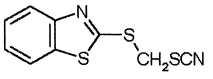
3. Results and discussion

3.1. EI and CI spectra

Table 1 shows the main mass fragments and relative abundance obtained by GC–MS with electron-impact ionisation (EI) and chemical ionisation in positive (PCI) and negative (NCI) modes. Abundant fragmentation was observed in most cases in the EI-MS spectrum where the molecular ion (M^+) was present with a very low abundance (10–25%). Only chlorothalonil and Irgarol 1051 showed a M^+ ion which coincided with the base peak. The TCMTB spectrum presented a base peak at m/z 180 which corresponds to the $[M–SCN]^+$ ion. The loss of the SCN group has also been observed in the standard solutions from the appearance of 2-(methylthio)-benzothiazole as degradation product. Dichlofluanid showed a base peak at m/z 123 that can be assigned to the $[PhNS]^+$ ion. Less abundant was the fragment at m/z 224 originated by the loss of the $(CH_3)_2NSO_2$– radical. Abundant fragmentation was also present in the Sea-Nine 211 EI mass spectrum with a base peak at m/z 169 (loss of the alquil chain) and abundant fragments at m/z 246 and 182. Irgarol 1051 presented a base peak corresponding to the M^+ ion (m/z 253) and an important fragment (98% abundance) at m/z 182 {ion $[M–NC(CH_3)_3]^+$}. Mass spectra published by others authors [5,8] present some differences in the relative abundance of the fragments, especially for the peaks at m/z 253 and 182.

PCI mass spectra exhibited the characteristic $[M+H]^+$ ions as a base peak for Irgarol 1051, Sea-Nine 211 and chlorothalonil. In these cases, little fragmentation and typical adduct formation of the molec-

Table 1
Chemical structures and mass spectral data for the five compounds studied, obtained under different ionization modes

Compound	Chemical structures	Main ions (m/z) (% abundance)		
		EI	PCI	NCI
Chlorotalonil		266(100), 264(76), 268(49), 229(10)	267(100), 231(35), 295(13), 307(8)	266(100), 264(81), 230(30)
M_w 264				
Dichlofluanid		123(100), 224(60), 167(48), 332(10)	201(100), 99(86), 224(48), 313(18)	99(100), 155(50), 199(45)
M_w 332				
Sea-Nine 211		169(100), 246(82), 182(80), 283(11)	282(100), 284(75), 310(48), 322(8)	281(100)
M_w 283				
Irgarol 1051		253(100), 182(98), 238(75), 196(32)	254(100), 282(27), 294(10), 198(25)	252(100), 253(20)
M_w 253				
TCMTB		180(100), 238(25), 136(30), 108(20)	182(100), 210(20), 222(9), 136(41)	166(100), 58(28)
M_w 238				

ular ions, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$, was also observed. The base peaks of the other compounds corresponded with fragment ions at m/z 201 for dichlofluanid and at m/z 182 for TCMTB.

The NCI mass spectra of all the compounds studied presented very little fragmentation. $[M]^+$ and $[M-H]^+$ ions were observed in some cases (chlorothalonil, Sea-Nine 211 and Irgarol 1051). TCMTB showed an intense peak at m/z 166 and dichlofluanid exhibited a higher fragmentation with the base peak at m/z 99.

3.2. GC–MS analysis

Adequate resolution of the analytes was obtained by using the chromatographic conditions previously described, with a total analysis time of 14 min. This short analysis time per sample contributed to an increase of sample throughput. The retention times obtained are included in Table 2.

The analyses of the pesticide mixture in matrix extracts were performed in EI, PCI and NCI ionisation modes and under full scan and selected ion monitoring (SIM) conditions. In this way it was possible to establish the best conditions with respect to sensitivity and selectivity. The low pesticides level in natural waters made the use of the SIM mode necessary, which provided response factors from 10 to 150 times higher than full scan mode. In a quadrupole instrument the use of the SIM mode

Table 2
Analytical data for SPE–GC–MS of target analytes in seawater samples

Analyte	t_R (min)	Quantitation masses		Dwell time	Mean recovery (%) (RSD, %)		LOD (ng/l)	
		EI	NCI	(ms)	100 ng/l	10 ng/l	EI	NCI
Chlorothalonil	8.31	266	266	100	82 (7)	78 (8)	2.5	0.5
Dichlofluanid	10.03	224	99	100	58 (10)	53 (15)	2.5	1.5
Sea-Nine 211	11.17	246	281	100	42 (9)	45 (8)	2.5	1.5
Irgarol 1051	11.50	253	–	100	90 (10)	95 (9)	1.2	20 000
TCMTB	12.54	180	166	100	95 (6)	98 (7)	3.0	1.5

provides highly selective methods, but reduces the identification power. However, for a number of target compounds around thirty, this SIM mode can fulfil the objective as multiresidue method. In order to achieve a compromise between sensitivity and identification capability, the three most abundant fragments in the spectra, whenever possible, were selected as diagnostic ions (see Table 1). Dwell times for single ions used in the SIM method are shown in Table 2.

The analyses in PCI mode did not significantly increase sensitivity, on the contrary it decreased for most compounds. Nevertheless, the use of NCI enhanced the detection sensitivity between 1.5 and 5 times for all compounds except Irgarol 1051 which showed a dramatic increase in the limit of detection of four orders of magnitude (Table 2).

Considering the different limits of detection (LODs) obtained for the pesticides studied, an optimum sensitivity it would be obtained with a combination between GC–EI-MS and GC–NCI-MS methods. Nevertheless, as time requirements are important in the development of a routine method, the GC–EI-MS method has been selected because of its capability for simultaneous identification and quantitation of the selected compounds. Analysis in NCI mode can be used as additional confirmation, specially at low concentration levels, with the exception of Irgarol 1051.

3.3. Optimisation of the injection volume.

To avoid the loss of analyte detectability produced by the use of only a 200-ml sample volume, higher injection volumes than the typical 1–2 μl were necessary. In order to observe the effect of increasing injection volume and to establish the adequate injection conditions, volumes of 2–15 μl of a blank matrix extract containing the pesticides at a spiked level of 250 ng/l were analysed.

The injection of sample volumes larger than 6 μl in a conventional split–splitless injector, in splitless mode, lead to peak tailing, poor peak shape, and low recoveries. Such a large injection volume rapidly expands into a large gas volume, causing part of the sample to be blown into the gas lines filling the injection port. This undesirable effect could be avoided by using the electronic pressure programming (EPP), that permits the programmed elevation of column head pressure just before the beginning of a run and return it to the normal value after a specified amount of time. In this way the initial gas volume is reduced and the sample is introduced into the column faster, avoiding possible losses of the analytes. Parameters such as the injection pulse pressure, time of the pulse, split vent time and initial oven temperature had to be optimised for large injection volumes. After several tests the injection pulse pressure was fixed at 30 p.s.i. until 1.5 min. At higher injection pressures (45 p.s.i.) the appearance of split peaks was observed. A splitless time of 1.5 min was necessary to introduce the whole sample into the column, otherwise losses of the analytes in the split flow were detected. An initial oven temperature of 105°C helped to obtain good peak shapes.

Fig. 1 shows the effect of increasing injection volume from 2 μl to 10 μl in the analysis of an SPE extract spiked with the biocides mixture at 250 ng/l level. The signal clearly increased with the injection volume following a linear relation. Nevertheless, the rise in signal (approx. ×4) did not correspond with the rise in the injection volume (×5), probably due to overloading in the capillary column. Together with an increase in the signal a rise in the baseline

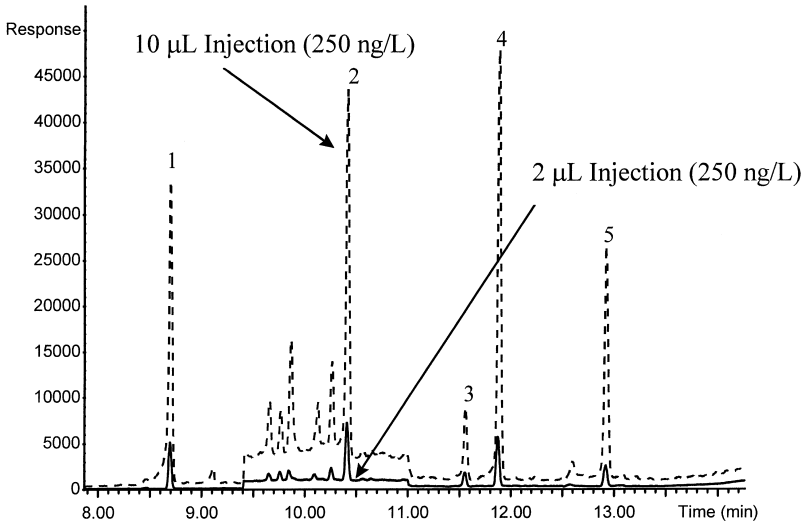


Fig. 1. GC–EI-MS chromatograms obtained by the injection of 2 μl and 10 μl aliquots of an SPE water extract spiked with (1) chlorothalonil, (2) dichlofluanid, (3) Sea-Nine 211, (4) Irgarol 1051 and (5) TCMTB at 250 ng/l level.

was also observed (Fig. 1). This fact advised against the use of injection volumes higher than 10 μl. On the other hand, the S/N ratio did not follow the same behaviour as the signal and it was strongly dependent on the quantitation mass selected. In the case of dichlofluanid, the S/N ratio varied from 57 (2 μl) to 86 (10 μl) when the base peak in the spectrum (m/z 123) was selected as quantitation mass. Nevertheless, if the peak at m/z 224 (55% abundance) was selected, the increase in the S/N was from 59 to 178.

3.4. Matrix effect and linearity by injecting a volume of 10 μl

In order to maximise the trapping of non-volatile components present in the extracts and to avoid extensive matrix effects when using high injection volumes, two different packings for the injection-port liner were investigated: glasswool and Carbofrit. Carbofrit had previously been reported as an alternative insert packing material [21] and its efficiency has been proved in the present work. The reproducibility of the injection with the two packings was studied by the analysis ($n=10$) of a blank matrix extract containing the pesticides at a spiked level of 5 μg/l. Good coefficients of variation (3.5–7.2%) were obtained in both cases for all the compounds studied (Table 3). Nevertheless, when glasswool was used as packing material, the appearance of a broad matrix peak was observed after five sample injec-

Table 3
Calibration and precision data for the GC–EI-MS detection of the biocides studied

Analyte	Range of concentration calibration graph (ng per 10 μl)	Calibration equation		Correlation coefficient R^2		Precision of the injection ($n=10$), RSD (%)	
		Pure solvent	Blank matrix	Pure solvent	Blank matrix	Carbofrit	Glasswool
Chlorothalonil	0.005–10.00	$y = 17649x - 195547$	$y = 15388x - 139831$	0.9992	0.9991	3.6	6.9
Dichlofluanid	0.005–10.00	$y = 5840x - 24378$	$y = 5217x - 10768$	0.9991	0.9998	3.5	4.8
Sea-Nine 211	0.005–10.00	$y = 3929x - 29798$	$y = 2539x - 21641$	0.9994	0.9991	5.2	7.2
Irgarol 1051	0.005–10.00	$y = 20918x - 169459$	$y = 18440x - 77396$	0.9995	0.9995	5.1	3.8
TCMTB	0.005–10.00	$y = 16244x - 321401$	$y = 16002x - 1167038$	0.9980	0.9990	6.1	6.7[a]

[a] $n=5$.

tions, preventing the detection of TCMTB. This fact reveals the lack of efficiency of glasswool in retaining the accumulated non-volatile components from samples that finally flow to the analytical column. No similar effect was observed with Carbofrit, where only a slight rise in the baseline was observed after ten injections. Nevertheless, the injection of 10 μl of sample extract requires changing the Carbofrit every fifty samples in order to avoid a gradual decrease in the GC–MS response.

The linearity in the response was studied with standard solutions prepared in both blank matrix extract and ethyl acetate solvent. In both cases a linear correlation was observed for all the compounds in the studied range (from 0.5 to 1000 μg/l), with correlation coefficients of at least 0.998 (see Table 3). The matrix effects observed were only important starting from concentrations of 500 μg/l and contrarily to what we may expect, the signal decreased lightly in the presence of the matrix with respect to the solvent. In any way, deviations between both curves at the highest concentration level were lower than 15% for most of the cases. Only Sea-Nine 211 exhibited a deviation higher than 30%.

The influence of the matrix in the pesticide spectra was also evaluated. For this purpose, variations in the relative intensities of the diagnostic ions were observed at different concentration levels. Between 10 to 1000 μg/l the relative abundance of the ions remained constant, but at lower concentrations significant variations were observed from 10% to 30%. This effect has to be considered for identification purposes.

3.5. Recoveries and limits of detection

The recoveries of the SPE procedure were studied by spiking seawater samples ($n=5$) at two fortification levels: 10 ng/l and 100 ng/l. The treatment of water amounts of 200 ml decreases sample handling times considerably. The total time per sample was estimated at 45 min approximately (less than 1 h including GC–MS analysis). Oasis HLB sorbent was selected for its efficiency in the extraction of moderately to highly polar compounds. Table 2 shows the mean recoveries obtained for the five pesticides studied. Chlorothalonil, Irgarol 1051 and TCMTB were quantitatively extracted but lower recoveries ($<60\%$) were obtained for Sea-Nine 211 and dichlofluanid. The coefficients of variation ($n=5$) obtained were lower than 15%, indicating a good performance of these procedures. No significant differences were observed in the recoveries obtained at the two spiked levels.

The calibration curves generated from the standards in blank sample extracts were used for quantitation. In this way, possible quantitation errors originated by the matrix effect observed were eliminated. Integrated peak area data of one selected mass were used to construct the curves. The quantitation masses in EI mode analyses were selected based on selectivity instead of intensity criteria. So, in some cases these did not correspond to the base peak in the spectrum but they provided the best S/N ratio in the presence of matrix. The selected quantitation masses in EI and NCI modes are indicated in Table 2. When a series of samples are going to be analysed, the sequence of injection is prepared including the analysis of a check sample each four samples. These check samples correspond to one calibration standard (5 μg/l). In this way the response of the detector is checked by matching the response of the calibration standard with the response of the same concentration level of the calibration curve. The response is satisfactory when the difference in peak areas between the check sample and the correspondent level in the calibration curve is lower than 15%. Otherwise, the last group of samples was reanalysed In this way validity of the calibration curve is assured.

The LODs were determined as the analyte concentration that gave a S/N ratio of three and empirically verified by analysing pesticide mixtures at these concentration levels in matrix extracts. Table 2 shows the lower LODs obtained (in ng/l).

3.6. Application to environmental sea water samples

As an application, the presence of the studied pesticides was investigated in three marinas in Almería. Sampling was carried out at two different locations inside each marina at the end of August, when the influx of boats is highest. The presence of Irgarol 1051 was detected in all the samples analysed in a concentration range from 25 to 450 ng/l. Fig. 2 shows a GC–EI-MS (SIM mode) chromatogram

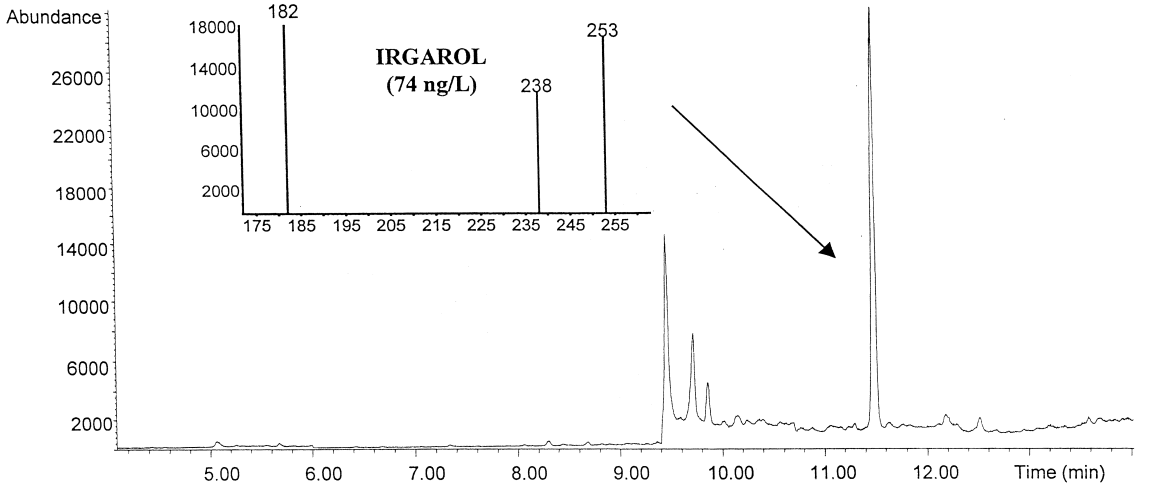


Fig. 2. Chromatogram obtained by GC–EI-MS under SIM mode of a water sample from a marina in Almería where Irgarol 1051 has been detected at a concentration of 74 ng/l. The insert shows the spectrum of the Irgarol 1051 peak.

obtained for one of the samples where Irgarol 1051 was present at a concentration of 74 ng/l. The inset in the Fig. represents the SIM mass spectrum of Irgarol 1051 in the sample. Good concordance in the relative abundance of the diagnostic peaks was observed between this spectrum and the one obtained with the calibration solutions, this way confirming the correct identification of this compound. Other pesticides were not detected at significant concentrations.

4. Conclusions

The analytical method developed in the present work allows the simultaneous identification and quantification of a group of antifouling pesticides in aquatic samples in a simple and sensitive way. SPE extraction of only a 200 ml sample volume, allows us to reduce the analysis time considerably respect classical methods which use ≥1 l sample. Losses in detectability of the analytes originated by the reduction in the sample volume are avoided by the combined use of a 10 μl injection volume and highly selective GC–MS-SIM detection in EI and NCI modes. PCI-MS did not improve the sensitivity of the method and its use was discarded.

The applied SPE method provided good recoveries for most of the compounds except dichlofluanid and Sea-Nine 211. The coefficients of variation obtained are lower than 10% ($n=5$). The detection limits reached by the method were 0.5–2.5 ng/l.

The analyses performed in three marinas revealed the presence of Irgarol 1051 in all the cases, at concentration levels between 25 and 450 ng/l. Further studies will allow the establishment of spatial and temporal trends.

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