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Determination of traces of five antifouling agents in water by gas chromatography with positive/negative chemical ionisation and tandem mass spectrometric detection

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Abstract

A highly selective and sensitive gas chromatography–mass spectrometry methodology has been developed for the determination of five antifouling compounds, currently licensed for use in marine antifouling paints. The procedure uses an ion trap mass spectrometer provided with an external ion source that allows the combined use, in the same analysis, of positive (PCI) and negative (NCI) chemical ionisation and tandem mass spectrometric fragmentation (MS–MS). Ionisation and fragmentation processes were optimised individually for each compound, thus, permitting maximum sensitivity and selectivity to be obtained. A complete validation study, including those aspects that affect both correct quantification and unequivocal confirmation, demonstrated the good performance of the proposed method. Detection limits obtained were lower than 0.005 $\mu g\ l^{-1}$, except for Irgarol 1051 (0.050 $\mu g\ l^{-1}$). The method was applied to real seawater samples from different marinas to prove their use in routine analysis.

Keywords: Water analysis; Chlorothalonil; Dichlofluanid; Sea-Nine 211; Irgarol 1051; 2-Thiocyanomethylthiobenzothiazole

1. Introduction

The search for the presence of organic compounds in the environmental field is becoming more and more frequent and intensive, provided that these compounds are potential contaminants and could be hazardous for human health and for the natural development of ecosystems. The sources by which these compounds reach environmental compartments are varied: agricultural practices, industrial effluents, dumping of urban residues, and human activities in a multitude of different fields. Together with the wide range of compounds that can be present, an additional problem arises from the low concentration levels at which these compounds have to be detected because of the dilution capacity of the environment. In this sense, the challenge for the scientists lies in the development of new analytical methods able to determine such compounds in the low picogram levels with enough guarantees about their identities.

A selective preconcentration step, generally by solid-phase extraction (SPE) and gas chromatography–mass spectrometry (GC–MS) analysis, is the preferred primary approach to multiresidue pesticide analysis of environmental samples [1,2]. The ion trap mass spectrometers provide very good sensitivity and full scan spectra an unequivocal confirmation, nevertheless, when complex extracts are analysed, matrix

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ions that can coelute with the target compounds very often damage both the sensitivity and identification capabilities. More selective methods are required and, so, other modes of operation, such as selected ion monitoring (SIM), chemical ionisation (CI), tandem MS–MS or combinations of them have been applied. In MS–MS mode a precursor ion is isolated inside the trap and later fragmented to obtain a characteristic product spectrum. The sample matrix ions are excluded from the trap during the isolation step so improving the signal-to-noise ratio of the peaks, providing cleaner spectra. On the other hand, the use of CI also provides an improvement in the selectivity and, for some compounds, a higher response. That is the case for halogen containing pesticides for which the response can be even 100 times higher in negative chemical ionisation (NCI) [3].

In light of the advantages of these modes of operation (CI and MS–MS), it is easy to think that a combination of both of them can provide analytical methods with good performance characteristics for the analysis of contaminants, such as pesticides, in environmental samples. With this interest, a GC–CI-MS–MS analytical method has been developed for the analysis of a group of five antifouling biocides, commonly used as active ingredients in antifouling paints, to prevent biofouling of submerged surfaces in the sea (see Fig. 1). The leaching of these compounds into the waters in certain areas of the marine environment, together with the resistance to degradation observed in some of them, such as Irgarol 1051 [4,5] has been revealed as a risk for the marine biota [6] and extensive monitoring programmes have been applied in different coastal zones in Europe [7–15].

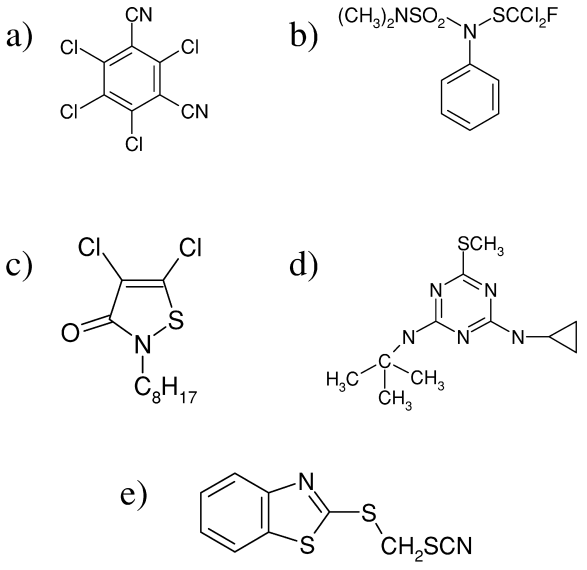

Fig. 1. Structures of the biocides studied: (a) Chlorothalonil (M_r=264), (b) Dichlofluanid (M_r=332), (c) Sea-Nine 211 (M_r=283), (d) Irgarol 1051 (M_r=253), (e) TCMTB (M_r=238).

The different analytical techniques applied over the last years for the determination of biocides in seawater go from GC–MS or liquid chromatography–mass spectrometry (LC–MS) [15–17] to solid-phase microextraction (SPME) [14] or more selective immunochemical techniques (ELISA) [13]. A GC–EI-MS –MS method has been also described for the analysis of Irgarol 1051, combined with large-volume injection and GC–PCI-MS–MS analysis for analyte confirmation [11].

In this paper, a GC–CI-MS–MS chromatographic method has been also selected, but with the special feature that the use of an external source ion trap mass spectrometer (ITMS) allows the system to change the ionisation mode from positive (PCI) to negative (NCI) during the analysis, thus, allowing one to select the more sensitive CI conditions for each compound [3]. With this procedure, very low detection limits can be reach (low ppt), even when reduced volumes of water samples (200 ml) are used during the preconcentration step by SPE, providing a rapid and reliable, multiresidue method.

The proposed method was tested for the analysis of seawater samples from three marinas located in different points of the province of Almería, a city located in the southeast of Spain on the Mediterranean coast.

2. Experimental

2.1. Chemicals and reagents

Pesticide standards were purchased as follows: Irgarol 1051 (2-methylthio-4-*tert*.-butylamino-6-cyclopropylamino-*S*-triazine) from Ciba-Geigy (Barcelona, Spain); Sea-Nine 211 (4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one) from Rohn & Haas Company

(Philadelphia, PA, USA); dichlofluanid (*N′*-dimethyl-*N*-phenylsulphamide), chlorothalonil (tetrachloroisophthalonitrile) and TCMTB (2-thiocyanomethylthio-benzothiazole) from ChemService (West Chester, USA). In all cases, purity was higher than 98%. Individual stock standard solutions (100 mg/l) were prepared in ethyl acetate for pesticide residue analysis (Scharlau Chemie, Barcelona, Spain) 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 both pure ethyl acetate solutions and blank extracts of seawater samples.

Methanol, HPLC-grade, for instrumental analysis was obtained from Panreac (Barcelona, Spain). Helium (99.999% purity) was used as the carrier gas, methane (99.95% purity) as the reagent gas in CI analyses and nitrogen (99.999% purity) for drying were from Air liquide (Madrid, Spain).

2.2. Apparatus and equipment

2.2.1. GC–ITMS

All the experiments were performed on a TRACE™ 2000 gas chromatograph (CE Instrument, Austin, TX, USA) interfaced to an ion trap mass spectrometer GCQ™ (Finnigan, Austin, TX, USA) equipped with external ionization, positive and negative chemical ionisation and MS–MS capabilities. Analytes were separated in a crosslinked 5%-diphenil–95%-dimethylsiloxane (HP-5MS, Hewlett-Packard, Palo Alto, CA, USA) capillary column (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. The temperature programme was 1.0 min at 70°C, 35°C/min to 180°C, 10°C/min to 240°C (5 min). The helium flow-rate was maintained at 1 ml/min. A split/splitless injector was used in splitless w/surge mode. The injection volume was 2 μl; injector temperature=250°C; surge pressure=207 kPa for 1.00 min; split flow=50.0 ml/min and splitless time=1.00 min. The transfer line temperature was set at 275°C.

Typical ITMS operating conditions were optimised by the software at the following values: electron multiplier at 1125 V, trap offset at 7 V, lens 1 at 35 V, lens 3 at 23 V and gate lens at −108 V. The external ion source worked in CI mode (methane as reagent gas) at a temperature of 200°C. Source pressure was optimised at 6.0×10^{-4} Torr. MS–MS conditions such as isolation (wideband application for clusters, isolation time) and excitation (resonance excitation voltage, excitation time) were optimised for the individual analytes and the results are listed in Table 1.

2.3. Sample preparation

Water samples were taken from three marinas located in Almería, a province in the south of Spain on the Mediterranean coast. Blank samples employed in the validation studies were taken from 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

Table 1
Ionisation mode, precursor ions and MS–MS fragmentation conditions used[a]

	Ionization		MS–MS fragmentation		
	Ionization mode	Main ions (*m/z*) (RA, %)	Precursor ion (isolation window)[b]	Excitation rf voltage (mV)[c]	Product ions (*m/z*) (RA, %)
Chlorothalonil	NCI	266(100), 264(50), 268(40)	266(6)	1700	229(100), 231(90), 266(20)
Dichlofluanid	NCI	199(100), 155(60), 99(10)	199(2)	800	155(100), 91(51), 199(25)
Sea-Nine 211	NCI	245(100), 209(30), 281(10)	245(4)	800	160(100), 162(56), 245(25)
Irgarol 1051	PCI	254(100), 282(25), 198(20), 294(10)	254(2)	900	198(100), 254(30)
TCMTB	NCI	166(100), 58(20)	166(2)	1100	134(100), 166(25)

[a] Main ion fragments and relative abundances (RA) obtained under CI-MS and CI-MS/MS.
[b] Isolation time=10 ms in all the cases.
[c] Excitation time=15 ms.

bottles, which were previously washed and rinsed in ethanol. Sample extractions were performed on arrival at the laboratory by off-line solid-phase extraction (SPE), by using an automated sampler processor from Gilson (Villiers-le-Bel, France). Oasis™ HLB (divinylbenzene–*N*-vinylpyrrolidone copolymer) cartridges (200 mg, 6 cc) from Waters (Milford, MA, USA) were used for the sample preconcentration. The extraction procedure used had been previously applied with good results to the group of compounds studied [18]. The procedure was as follows: The conditioning step was performed by pumping, at 4 ml/min, of ethyl acetate (5 ml), methanol (5 ml) and distilled water (4 ml) consecutively. After that, 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 (2 Atm, positive pressure). 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 evaporation of the extra solvent was carried out with a stream of nitrogen at 30°C, reaching a final volume of 1 ml in ethyl acetate.

3. Results and discussion

As the aim of this work was to obtain maximal sensitivity for each one of the analysed compounds, operational detection conditions had to be individually optimised. For that reason, it was very important to obtain a good separation for all the compounds by the adjustment of the separation chromatographic conditions. The application of the chromatographic conditions proposed in the experimental section yielded an adequate separation of the analytes studied, as can be seen in Fig. 2, which shows selected ion chromatograms corresponding to a spiked seawater sample (pesticides spiking level of 50 mg l^{-1}). Once the analytes were separated in a reasonable total analysis time, optimisation of the detector operating conditions was performed as follows:

3.1. Optimisation of the CI-MS–MS method

In order to get the very low limits of detection required for these kinds of analyses (low ppt), operating conditions of the detection system have to be carefully optimised. Two aspects in the development of a GC–MS–MS method have to be considered: (i) selection of the ionisation mode and (ii) optimisation of the MS–MS parameters, which includes the precursor ion isolation step and the further dissociation of the isolated ion to obtain the product ion spectrum.

In a previous work [18], we had shown the improvement in the analyte response when negative chemical ionisation (NCI) was used in the analysis of most of these compounds. If we consider the signal-to-noise (S/N) ratio of the peaks, the enhancement could be estimated at 10–20 times, with respect to positive chemical ionisation (PCI) or electron impact (EI). This is a well known behaviour of the chlorinated compounds. Nevertheless, Irgarol 1051 showed a dramatic decrease in the sensitivity in NCI due to the absence of highly electrophilic atoms in its molecule. This is a problem when we want to set a single multiresidue method for all the compounds because an analysis combining NCI with other ionisation modes (EI or PCI) usually requires a change in the hardware or carrying out more than one injection. The use of an ion trap analyser with external ion source can be an answer to the problem because it allows the alternative injection of the cations and anions produced in the external source into the trap, so allowing a change in the ionisation mode from PCI to NCI during a run. So, the NCI mode was selected for all the biocides except for Irgarol 1051, which was analysed in PCI (see Table 1).

The best sensitivity conditions in CI were obtained after optimising parameters such as source pressure and source temperature, which can affect the response of the compounds and even the fragmentation pattern. The influence of these parameters was studied in the range of 5.0–9.0×10^{-4} Torr, for the source pressure, and in the range of 150–230°C for the source temperature. In general, no significant differences in sensitivity were observed and the conditions selected, 6.0×10^{-4} Torr and 200°C, were

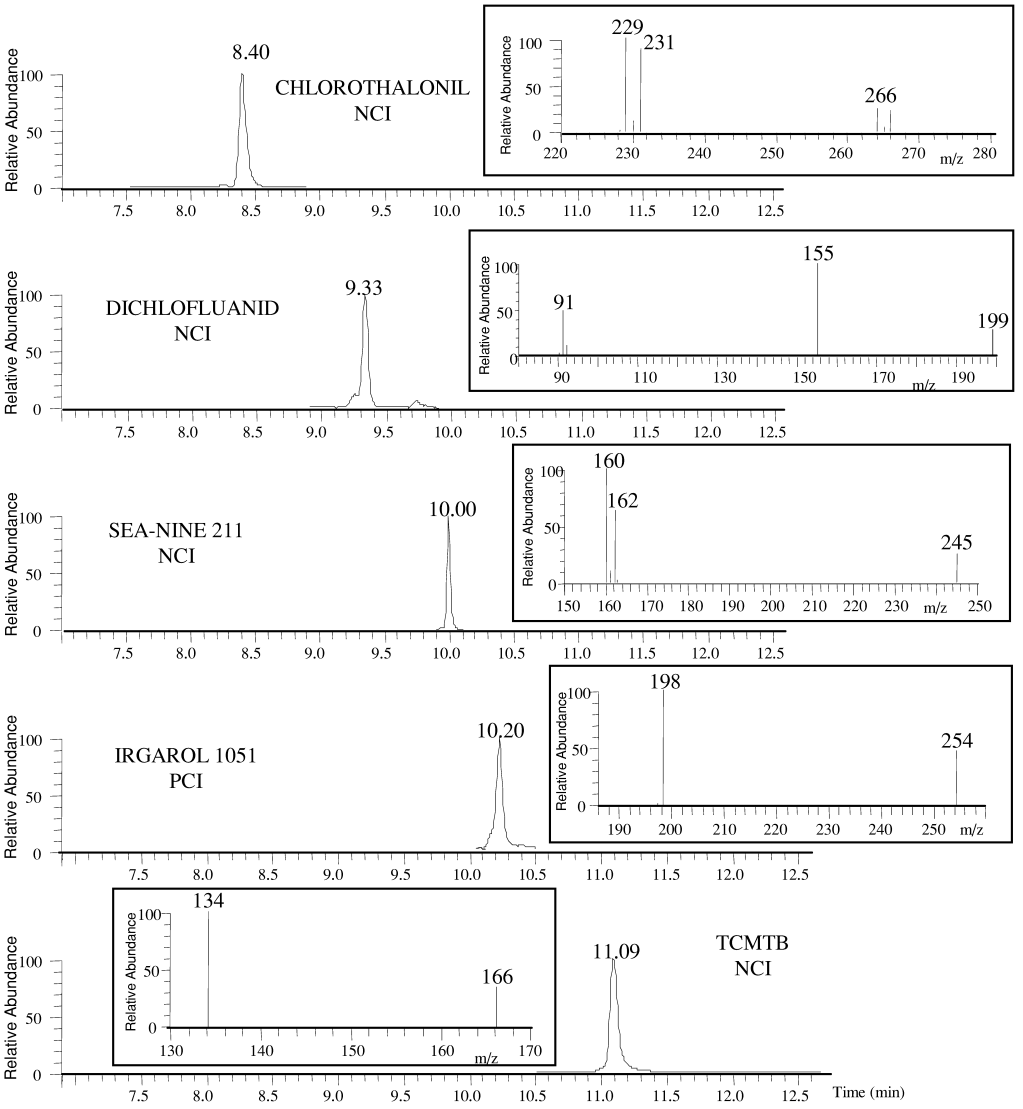

Fig. 2. Selected ions chromatograms and MS–MS spectra correspondent to a GC–PCI/NCI-MS–MS analysis of a seawater extract spiked with the biocides at a concentration of 50 ng l^{-1}.

only based on a slight increase (around two times) in the S/N of the peaks of some compounds. Under the optimised conditions, the NCI and PCI mass spectra obtained were very simple, exhibiting only one or two prominent peaks. Only in two cases (Chlorothalonil and Irgarol 1051), did the base peak correspond with the molecular ion. For the rest of the compounds, an ion fragment was obtained as the base peak: $[M-SCCl_2F]^-$ for Dichlofluanid, $[M-CH_2SCN]^-$ for TCMTB and $[M-ClH]^-$ for Sea-Nine 211. Table 1 shows the fragment ions obtained and their relative abundances. Important variations in the relative abundance of the fragment ions and even higher fragmentation have been observed for some compounds (especially Dichlofluanid and Sea-Nine 211) with respect to the spectra previously reported

[18], probably as a consequence of the different CI conditions selected.

Once the ionisation process has been optimised, the MS–MS procedure has to be established. In tandem MS–MS mode, optimal operation conditions are compound dependent and, for this reason, specific conditions are required in segments for targeted pesticides. These segments include information about the precursor ion(s) to be isolated, the ion isolation window and resonance excitation voltage applied to induce the dissociation of the precursor ion(s) and to obtain the product ion spectrum. Table 1 shows the values assigned to these parameters as well as the main product ions obtained and their relative intensities. As can be seen, the base peak on the CI spectrum has been selected as the precursor ion in all the cases with a narrow mass isolation window, in order to get good sensitivity and selectivity. Only when the precursor ion contains chlorine atoms (Chlorothalonil and Sea-Nine 211), the window is extended to include the isotopic cluster in order to increase the qualitative information of the MS–MS spectra.

MS–MS conditions have to be adjusted to fragment precursor ions to deliver daughter ions, which are indicative of the structure of the analyte. The resonance excitation voltage applied to the fragmentation of the parent ion was adjusted in order to avoid its complete disappearance. So, the parent ion was present in the MS–MS spectrum of each compound with a relative abundance of 30% or lower. In these conditions, very little fragmentation was observed in the spectra, where only one or two fragments were present. In any case, it is generally accepted that in addition to a chromatographic retention time match the match of m/z values and signal intensity ratios of one or two transitions (parent-to-daughter ions) between an unknown peak and that due to the correspondent standard is a conclusive positive identification. The ratios of the signal intensities for the qualifier ions had to agree within 20–30%.

3.2. Validation of the method

A complete validation of a chromatographic method includes not only the evaluation of those aspects such as precision, linearity in the response, etc., that can affect the correct quantification of the analytes in the samples, but also those parameters that have influence in a confident confirmation. For that reason, specific confirmation criteria parameters, such as qualifier ions and retention times have to be studied for a given range of concentrations.

All the validation studies were performed in seawater spiked extracts in order to estimate the influence of matrix interferences in the quantification and identification studies. The extracts were obtained by preconcentration of 200 ml of seawater samples by using an off-line SPE procedure previously reported [18]. This extraction method provided recoveries in the range of 42–98% for the biocides studies with good reproducibility (RSD$<$15%) in all the cases. The use of a sample volume of 200 ml allows an increased sample throughput but requires the application of more selective and sensitive analytical techniques if we want to achieve low ppt concentration levels. The spiked extracts were prepared by taking 200-μl aliquots of a blank extract, evaporating to dryness under a gentle stream of nitrogen and dissolving again, with sonication, in 200 μl of ethyl acetate solutions containing the pesticides studied.

The precision of the method was evaluated in terms of repeatability and reproducibility. In both cases, precision in the peak area of the quantification ions and relative abundance for the qualifier ions was evaluated. For repeatability studies, spiked seawater extracts at two concentration levels, 10 and 100 μg l^{-1} in the extracts (50 and 500 ng l^{-1} in the samples), were analysed ten times during the same day. The reproducibility (that is to say inter-day precision) was evaluated by the analysis of the same spiked extracts during 5 days, over a period of 2 weeks. Precision observed within the data, expressed as relative standard deviation, is showed in Table 2. The repeatability in the response, at the higher concentration (Level 1), was in the range of 4–13%. At the lower concentration, coefficients of variation obtained were in a similar range of 5–9%, except for Dichlofluanid (22%). The reproducibility showed coefficients of variation between 6 and 17% in all the cases. Taking into account the low concentration levels analysed, the results obtained are, in all cases, within the established tolerances.

An acceptable repeatability was also observed in

Table 2
Precision studies performed at two concentration levels

	Repeatability RSD (%)[a] Level 1 (500 ng l^{-1})/Level 2 (50 ng l^{-1})			Reproducibility RSD (%)[b] Level 1 (500 ng l^{-1})/Level 2 (50 ng l^{-1})		
	Response	RA (Ion-1)	RA (Ion-2)	Response	RA (Ion-1)	RA (Ion-2)
Chlorothalonil	6/9	8/3	26/19	17/6	3/7	7/16
Dichlofluanid	13/22	4/17	13/7	14/17	23/15	17/24
Sea-Nine 211	4/7	8/4	3/17	16/15	21/18	2/11
Irgarol 1051	13/9	3/9	–	9/7	8/15	–
TCMTB	9/5	2/5	–	6/8	7/11	–

[a] $N=10$.
[b] $N=5$.

the relative abundance (RA) of the qualifier ions even at the lower concentration level with RSDs lower than 20%. Only Chlorothalonil showed a higher RSD (26%) for the ion at m/z 266, which can be attributed to the higher variability usually observed for cluster ions. Reproducibility studies showed, in general, higher RSDs but always lower than 25%. Overall, these values indicate a good performance of the method.

The linearity of the response curves for each compound was evaluated in fortified blank extracts, containing known amounts of the target pesticides. The curves were obtained representing, for each compound, peak area values for the quantification ions (those included in Table 1). A concentration range from the limit of determination to 1000 μg l^{-1} (that is 5000 ng l^{-1} in the samples) was studied. In our experience, presence of biocides in seawater samples infrequently exceeds the upper concentration limit considered [15]. The responses observed were not linear in the whole range studied. The observation of the curves allows us to confirm the presence of two different linear ranges, depending on the concentration. In Table 3 the correspondent equations of the calibration curves and correlation coefficients obtained for the lower concentration range are shown. In all the cases, a good linear relationship is observed, with correlation coefficients of >0.9967. When the concentration of biocides in the samples exceeds the linear range, it is necessary to use another level of calibration, i.e., to use standards with concentrations surrounding those of the unknown samples.

The limits of detection (LODs) for the current method were calculated experimentally by decreasing the concentration of the target compounds in the spiked extracts until the moment in which the S/N ratio for the peaks were lower than five or incorrect relative ratios of the diagnostic ions prevented identification. As in most of the cases, a correct identification requires the presence of peaks clearly distinguished from the baseline background ($S/N>$ 10), the LODs calculated can be considered as limits of determination of the method. The LODs obtained, showed in Table 3, are in general lower than those previously reported for these compounds, as a consequence of the enhanced selectivity and sensitivity provided with this methodology, especially for Chlorothalonil, Sea-Nine 211 and TCMTB, that showed values of 0.05 ng/l for the seawater sample. A higher LOD was obtained, however, in the case of Irgarol 1051. Although this concentration is low

Table 3
Calibration data and limits of detection (LODs) obtained for the target biocides in seawater samples by GC–PCI/NCI-MS–MS

Compound	Linear range (ng l^{-1})	Calibration equation	R^2	LOD (ng l^{-1})
Chlorothalonil	0.05–250	$y = 22\,928x + 4960$	0.9997	0.05
Dichlofluanid	5.00–5000	$y = 276.4x - 524.98$	0.9991	5.0
Sea-Nine 211	0.05–50	$y = 69\,438x + 5944.6$	0.9996	0.05
Irgarol 1051	50.00–5000	$y = 3407.8x - 32\,860$	0.9967	50.0
TCMTB	0.05–50	$y = 16\,190x + 3645$	0.9996	0.05

enough to detect Irgarol 1051 at the levels usually present in marinas, it can be too high for other applications, such as estuarine or coastal waters, where the typical concentration range is 1–40 ng/l. An improvement in the sensitivity of Irgarol 1051 could be obtained by using EI instead of PCI because of the higher response obtained in this ionization mode [18], but changes from EI to CI during the analyses are not possible with the present configuration of the instrument and require performing two injections. Other possibilities include the preconcentration of larger volumes of water or the use of LVI techniques.

Selectivity and matrix effects were also evaluated. The application of the proposed chromatographic conditions allowed the complete separation of the compounds, with adequate resolution in all the cases (>1.5). The retention times obtained are shown in Fig. 2. The analysis of real samples, fortified with the target compounds, also showed the absence of coelutions with other compounds that could be present in the matrix. That is a consequence of the high selectivity of the MS–MS technique.

Occurrence of matrix effects was evaluated in those aspects that affect both correct identification of the compounds and adequate evaluation of the concentration present. With this purpose, the effect of matrix interferences in the rations of the abundances for qualifier ions in the mass spectra and in the pesticide responses was evaluated.

In order to determine matrix effects in the analyte responses, calibration curves were obtained in both pure solvent and spiked extracts. Discrepancies observed between the slopes of both curves are indicatives of the presence of matrix effects. In order to evaluate these discrepancies, we plotted peak area data obtained for each concentration level by using as abscissa peak areas in pure solvent and as ordinate peak areas in spiked extracts. It is expected that in the absence of matrix effects the data fit a straight line with a slope equal to one. Curves obtained, presented small variations in the slopes, from 0.89 to 1.23. That indicated the absence of a strong matrix effect, being observed, in general, an enhancement of the signal in the presence of matrix.

The influence of the matrix effect in the confirmation of the target compounds is caused by interferences, which disturb the relative ratios of the diagnostic ions. In our studies, a good concordance in the relative abundances (RA) could be observed between standards in pure solvent and standards in seawater extracts, even at very low concentrations. It is accepted that the ratios in the signal intensities for the samples and standards have to be agree within 20–30%.

3.3. Application to real samples

The applicability of the method was tested on real seawater samples coming from the monitoring study that our research group has been carrying out in different marinas located in the southeast of Spain. The presence of Irgarol 1051 was detected in all the samples analysed in a concentration range from 50 to 1000 ng/l, while other biocides (chlorotahlonil, Sea-nine 211 and TCMTB) were only detected very occasionally and in very low amounts (<10 ng/l). Fig. 3 shows the GC–CI-MS–MS selected ions chromatograms obtained for one of the samples where Irgarol 1051 and Sea-nine 211 were detected at concentrations of 150 and 5 ng/l, respectively. The MS–MS spectra of Irgarol 1051 and Sea-nine 211 in the sample that is shown in the inset were in agreement with those obtained in the standard within an acceptable margin of error ($\pm 20\%$, as was established in the identification criteria), thus, confirming the identity of the peak.

4. Conclusions

After performing the validation study, it can be concluded that the proposed GC–PCI–NCI-MS–MS method complies with the performance characteristics requested for the analysis of this group of biocides in seawater samples. This means good selectivity, good precision in the response (RSD$<$ 22%), good sensitivity (LOD$<$50 ng/l) and confident and robust identification criteria. The combined use of NCI and PCI during the same analysis allows us to select the best sensitivity conditions for each compound, while MS–MS fragmentation provides an improved selectivity and characteristic product ion spectra, enough for a suitable pesticide confirmation. The applicability of the method to routine analysis was tested in real seawater samples with good results.

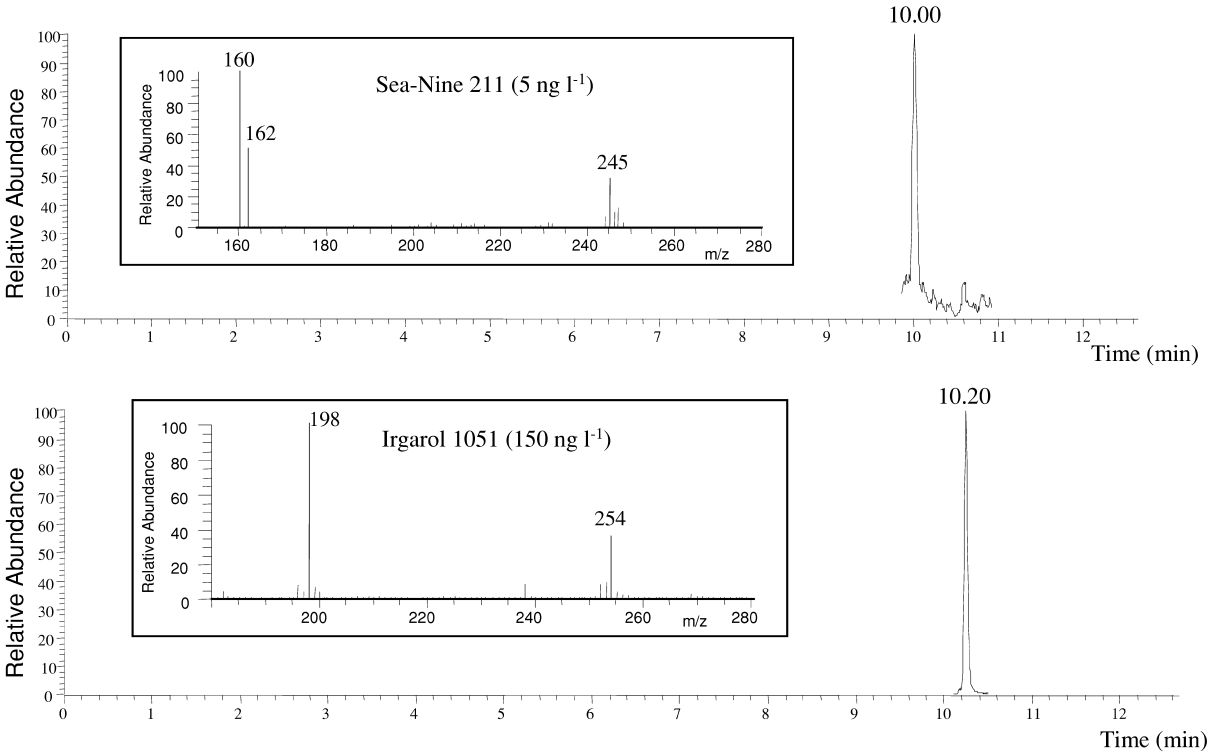

Fig. 3. Chromatogram and MS–MS spectrum of a real seawater sample where Sea-Nine 211 and Irgarol 1051 have been confirmed at concentrations of 5 and 150 ng l^{-1}.

Acknowledgements

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