ENVIRONMENTAL OCCURRENCE AND CONCERNS OF ANTIFOULING BIOCIDES

An overview of the analytical methods to determine the main antifouling paint biocides in marine samples

This paper offers a general overview of the analytical techniques and instruments employed in trace analysis of common booster biocides from antifouling (AF) paints, in seawater and sediment samples. Due to low concentrations and matrix effects, a suitable sample preparation step is usually performed prior to analysis. To identify and quantify AF compounds, gas or liquid chromatography is typically used, with either a selective detector that exploits analyte properties, or a mass spectrometer that allows the analysis of a broader range of compounds

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Introduction

As the International Maritime Organization (IMO) prohibited the presence of highly toxic tributyltin (TBT) on ship and boat hulls, paint manufacturers have developed copper-based antifouling (AF) paints; however these alternative AF paints have to be supplemented with specific organic compounds, the so-called 'booster biocides', in order to achieve protection against copper-resistant fouling organisms. Main booster biocides used in AF paints are Irgarol 1051, Diuron, dichlofluanid, chlorothalonil, Sea-Nine 211, TCMTB (2-(thiocyanatomethylthio)benzothiazole), zinc pyrithione (ZnPT), dithiocarbamates (including maneb, thiram, zineb and ziram), and TCMS-pyridine (2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine).

Some AF biocides are also used as herbicides and fungicides in agriculture. A large number of studies has been performed on the adverse effects of these active compounds to non-target marine organisms, and they showed toxic action at the µg/L and ng/L levels. Due to the harmful behaviour and in some cases environmental persistence, AF biocides raised concern as environmental contaminants, and this has encouraged the development of reliable and sensitive analytical methods able to monitor their occurrence in the marine environment.

Environmental samples are usually characterized by trace levels of organic pollutants, but also a large number of matrix components which may disrupt the analysis. To overcome these problems, the analytical methodologies usually involve a pre-concentration/clean-up step prior to the determination by gas or liquid chromatography (GC or LC). A further step (derivatization) may be required for some AF compounds (e.g., Diuron) not directly amenable to GC analysis. Sample pretreatments are usually labor-intensive and time-consuming tasks, and often constitute the bottleneck of the analytical procedures since they account for more than 75% of the analysis time.

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In this paper we will focus on the main methods aimed at the extraction and analysis of booster biocides in both seawater and sediment matrix, which are reported in the scientific literature. These analytical methodologies are summarized in Table 1.
A recent trend in determining AF biocides is towards the development of multiresidue analytical methods that allow the simultaneous determination of several analytes in a single analysis, thus reducing time and costs. This approach is not feasible for the determination of ZnPT and specific methods have been reported [1, 2].

Compound	Matrix	Extraction method	Analytical system	% Recovery (R.S.D.[a])	LOD (ng/L in seawater, ng/g dw in sediment)	Reference
Chlorotalonil	Seawater	LLE (DCM)	GC-EI-MS	90-92 (4-6)	20.0	3 [b]
	Seawater	SPE (C18)	HPLC–DAD	92 (5.9)	10.0	6 [b]
	Seawater	LLE (toluene)	GC-MS	120.3 (4.9)	5.5	4 [b]
	Seawater	SME (toluene, xylene)	GC-ECD	94 (3.4)	2.5	14 [b]
	Seawater	SPE (C18)	GC-ECD	93 (3-12)	5.0	13 [b]
	Seawater	SPME (PDMS 100 um)	GC-ECD	103 (5-15)	5.0	13 [b]
	Seawater	SBSE (PDMS)	GC-MS	81-120 (6.4)	10.0	15 [b]
	Sediment	Shaking (acetone, DCM)/LLE	GC-EI-MS	81-82 (8-12)	50.0	3 [b]
	Sediment	sonication (acetone)/SPME (PDMS)	GC-MS	74 (11)	6.0	19 [b]
Dichlofluanid	River water	LLE (DCM)	GC-EI-MS	90-91 (5-7)	20.0	3 [b]
	Seawater	SPE (C18)	HPLC–DAD	68 (10.8)	415.0	6 [b]
	Seawater	LLE (toluene)	GC-MS	93.8 (2.3)	1.8	4 [b]
	Seawater	SME (toluene, xylene)	GC-ECD	88 (4.6)	3.0	14 [b]
	Seawater	On line SPE (LiChrolut EN)	HPLC-APCI-MS	87-89 (1-8)	5.0	7 [b]
	Seawater	SPE (EACDc)	GC-ECD	95 (3-12)	9.0	13 [b]
	Seawater	SPME (PDMS 100 um)	GC-ECD	103 (5-15)	2.0	13 [b]
	Seawater	SBSE (PDMS)	GC-MS	76-119 (6.6)	30.0	15 [b]
	Seawater	On line SPE (PLRP-S)	GC-MS	67 (5-19)	20.0	24 [b]
	Seawater	SPE (Envirelut Pesticide)	HPLC–ESI/MS/MS	>72 (<10)	0.1-0.2	31 [b]
	Sediment	Shaking (acetone, DCM)/LLE	GC-EI-MS	81-83 (4-7)	50.0	3 [b]
	Sediment	sonication (acetone)/SPME (PDMS)	GC-MS	84 (7)	1.0	19 [b]
	Sediment	MAE (MeOH) +SPE (Envirelut Pesticide)	HPLC-MS/MS	76.2 (4.4)	0.3	22 [b]
Diuron	River water	LLE (DCM)	GC-EI-MS	92-93 (2-4)	20.0	3 [b]
	Seawater	SPE (C18)	HPLC–DAD	101 (3.5)	38.0	6 [b]
	Seawater	LLE (DCM)	HPLC–ESI/MS/MS	98 (5.2)	0.7	5 [b]
	Seawater	On line SPE (LiChrolut EN)	HPLC–APCI-MS	97-99 (1-8)	10.0	7 [b]
	Seawater	SPE (Excelpak SPE-GLF)	HPLC–ESI/MS/MS	93 (11)	0.7	8 [b]
	Seawater	SPE (C18)	HPLC–APCI-MS	100.3 (12.1)	1.0	9 [b]

	Seawater	SPE (C18)	HPLC-MS/MS	127 (10)	2.0	21 [b]
	Seawater	SPE (Envirelut Pesticide)	HPLC–ESI/MS/MS	>72 (<10)	0.1-0.2	31 [b]
	Sediment	Shaking (acetone,DCM)/LLE	GC-EI-MS	84-85 (4-7)	50.0	3 [b]
	Sediment	Shaking (acetone,DCM)	HPLC–ESI/MS/MS	96 (8)	0.08	5 [b]
	Sediment	Shaking (ACN)+SPE (Excelpak SPE-GLF)	HPLC–ESI/MS/MS	94 (7.5)	0.08	8 [b]
	Sediment	ASE (DCM)	HPLC-MS/MS	91 (13)	0.3	21 [b]
	Sediment	MAE+SPE (Envirelut Pesticide)	HPLC-MS/MS	92.9 (5.1)	0.2	22 [b]
Folpet	Seawater	On line SPE (LiChrolut EN)	HPLC-APCI-MS	85-90 (1-8)	200.0	7 b
	Seawater	SPE (C18)	GC-ECD	82 (3-12)	5.0	13 b
	Seawater	SPME (PDMS 100 um)	GC-ECD	99 (5-15)	10.0	13 b
Irgarol 1051	Seawater	SPE (C18)	HPLC–DAD	93 (3.8)	31.0	6 [b]
	Seawater	LLE (toluene)	GC-MS	73.5 (1.6)	7.7	4 [b]
	Seawater	LLE (DCM)	HPLC–ESI-MS/MS	90 (6.5)	0.8	5 [b]
	Seawater	On line SPE (LiChrolut EN)	HPLC-APCI-MS	91-95 (1-8)	5.0	7 [b]
	Seawater	SPE (Excelpak SPE-GLF)	HPLC–ESI-MS/MS	97(6.5)	0.8	8 [b]
	Seawater	SPE (C18)	GC-ECD	96 (3-12)	2.0	13 [b]
	Seawater	SPME (PDMS 100 um)	GC-FTD, GC-MS	101 (5-15)	5.0	13 b
	Seawater	HS-SPME (PDMS–DVB 65 um)	GC-FTD	118 (5-15)	8.0	16 b
	Seawater	SBSE (PDMS)	GC-MS	97-116 (7.3)	5.0	15 b
	Seawater	SFE-IAC	GC-NPD	87 (8.5)	3.0	17
	Seawater	SPE (C18)	HPLC-MS/MS	102 (18)	1.0	21 b
	Seawater	On line SPE (PLRP-S)	GC-MS	84 (5-19)	10.0	24 b
	River water	SPE (SDB)	GC-MS (ion trap)	101 (8.7)	0.1	25
	Seawater	SPE (Isolute ENV+)	GC-MS (ion trap)	94.6-116 (2.5)	3.1	26
	Seawater	SPE (Envirelut Pesticide)	HPLC–ESI/MS/MS	>72 (<10)	0.1-0.2	31 b
	Sediment	MAE (water)+SPE (C18)	GC-MS	94.1(7.1)	1.7	23
	Sediment	Shaking (acetone,DCM)	HPLC–ESI/MS/MS	85 (7)	0.08	5 b
	Sediment	Shaking (ACN)+SPE (Excelpak SPE-GLF)	HPLC–ESI/MS/MS	80 (10)	0.048	8 b
	Sediment	sonication (acetone)/SPME (PDMS)	GC-MS	91 (4)	0.5	19 b
	Sediment	Soxhlet acetone/SPE(C18)/GPC	GC-AFID, GC-MS	61 (13)	0.55	20
	Sediment	ASE (DCM)	HPLC-MS/MS	89 (16)	0.3	21 b
	Sediment	MAE+SPE (Envirelut Pesticide)	HPLC-MS/MS	91.1 (2.5)	0.1	22 b
	Sediment	MAE (water)	GC-MS (ion trap)	>85 (2.5)	1.7	26
M1	Seawater	LLE (DCM)	HPLC–ESI/MS/MS	90 (8.9)	1.9	5 b
	Seawater	SPE (Excelpak SPE-GLF)	HPLC–ESI/MS/MS	83 (13)	1.9	8 b
	Seawater	SPE (C18)	HPLC-MS/MS	109 (15)	1.0	21 b
	Seawater	SPE (Isolute ENV+)	GC-MS(ion trap)	82-96.4 (2.5)	0.5	26
	Sediment	Shaking (acetone, DCM)	HPLC–ESI-MS/MS	95 (9)	0.18	5 b
	Sediment	Shaking (ACN)+ SPE(Excelpak SPE-GLF)	HPLC–ESI/MS/MS	103 (8.5)	0.18	8 b

	Sediment	ASE (DCM)	HPLC-MS/MS	99 (18)	0.3	21 b
	Sediment	MAE (water) +SPE (C18)	GC-MS	93.1 (2.7)	0.9	23
	Sediment	MAE (water)	GC-MS (ion trap)	>85 (2.5)	0.9	26
Sea-nine 211	Seawater	SME (toluene, xylene)	GC-ECD	91 (9.1)	2.5	14 b
	Seawater	LLE (DCM)	HPLC–ESI-MS/MS	85 (10)	0.3	5 b
	Seawater	SPE (C18)	GC-ECD	94 (3-12)	5.0	13 b
	Seawater	SPME (PA 85 um)	GC-ECD	92 (5-15)	1.0	13 b
	Seawater	SPE (Excelpak SPE-GLF)	HPLC–ESI/MS/MS	75 (12)	0.3	8 b
	Seawater	SPE (C18)	HPLC–APCI-MS	100.4 (10)	1.0	9 b
	Seawater	HS-SPME (PDMS–DVB 65 um)	GC-FTD	96 (5-15)	7.0	16 b
	Seawater	SBSE (PDMS)	GC-MS	72-106 (7.2)	8.0	15 b
	Sediment	Shaking (acetone,DCM)	HPLC–ESI/MS/MS	80 (11)	0.04	5 b
	Sediment	Shaking(ACN)+SPE (Excelpak SPE-GLF)	HPLC–ESI/MS/MS	75 (10)	0.04	8 b
	Sediment	sonication (acetone)/SPME (PDMS)	GC-MS	88 (6)	1.5	19 [b]
TCMS pyridine	Seawater	SPE (C18)	HPLC–APCI-MS	113.1(18.4)	5.0	9 [b]
TCMTB	Seawater	SPE (C18)	HPLC–DAD	85 (4.7)	7.0	6 b
	Seawater	SPE (C18)	HPLC-APCI-MS	91.2 (20.1)	1.0	9 b
	Seawater	SBSE (PDMS)	GC-MS	79-125 (11)	900	15 b
	Seawater	SPE (Envirelut Pesticide)	HPLC–ESI/MS/MS	>72 (<10)	0.1-0.2	31 b
	Sediment	MAE+SPE (Envirelut Pesticide)	HPLC-MS/MS	78.1 (3.3)	0.3	22 [b]
Thiram	Seawater	SPE (C18)	HPLC–DAD	96 (6.6)	22.0	6 [b]
ZnPT, PT	River water	SAX- SPE (monolithic C18)	HPLC–APCI-MS	72 (27)	18.0	1
	Seawater	LLE (DCM)	HPLC–APCI-MS	77 (17)	20.0	2
	Seawater	LLE (DCM)	HPLC–ESI-MS/MS	83 (13)	80.0	5 b
	Sediment	Shaking (acetone, DCM)	HPLC–ESI-MS	90 (13)	8.0	5 [b]

a) Relative Standard Deviation;
b) multiresidue method;
c) EACD: Empore-activated carbon disks;
d) M1: Irgarol 1051 degradation product (2-methylthio-4-t-butylamino-6-amino-s-triazine)

TABLE 1 Methods for the extraction and analysis of common AF biocides in water and sediment matrices

Sample preparation

Seawater

Extraction of booster biocides from aqueous samples can be performed with different techniques.

The traditional Liquid-Liquid Extraction (LLE) has been extensively reported in less recent studies, but it is a simple and popular procedure still used today [3, 4, 5]. LLE involves the use of a water immiscible solvent, such as dichloromethane (DCM), toluene and hexane, to partition AF compounds from seawater into the organic solvent. Despite its low cost and satisfactory recoveries, this technique has severe limitations, namely the use of

large volumes of solvents and being a time-consuming and labor-intensive procedure. These drawbacks have led to the development and spread of faster methods, with the possibility of easy automation and where lower solvent volumes are employed.

In the last decades LLE has been largely replaced by Solid Phase Extraction (SPE). With this approach, the target analytes are removed from the liquid sample due to retentive interactions with a sorbent phase and, subsequently, are selectively eluted with an appropriate solvent. A large variety of sorbent materials – such as octadecylsilane (C18 bonded silica), graphitized carbon black (GCB) and polymeric materials (poly(N-vinylpyrrolidone-divinylbenzene) copolymer, PVP-DVB; polystyrene-divinylbenzene copolymer, PS-DVB; hydroxylated polystyrene-divinylbenzene copolymer, PS-DVB-OH) – is commercially available, and applications to real samples have been described [6, 7, 8, 9, 10]. Main drawbacks for SPE are the use of specific glassware, namely SPE vacuum manifold to simultaneously process many samples, and the need of preventive filtration of seawater so as to avoid the frits of SPE columns can be blocked by particulate matter. Gatidou et al. [11] carried out a study where they compared PS-DVB/ PS-DVB-OH polymeric materials with C18 bonded silica for the extraction of Diuron, Irgarol 1051, and some of their metabolites. For polymer-based SPE columns, a smaller sorbent mass is usually required to achieve extraction than C18-based (200 versus 500-1000 mg). In addition, higher recoveries for polar compounds such as the metabolites were observed due to further interaction mechanisms with target analytes (π–π and dipole-dipole interaction, hydrogen bonds). However satisfactory recoveries (>70%) were obtained for both solid phases with all analytes except 3,4-dichloroaniline (<35%). GCB materials are suitable for the SPE of six common booster biocides (dichlofluanid, chlorothalonil, Diuron, TCMTB, Irgarol 1051 and Sea nine 211) and some degradation products of Diuron and Irgarol 1051 from seawater, but due to the great adsorption power, elution of the analytes is troublesome, demanding the use of 18 mL dichloromethane-methanol (8:2) mixture, followed by 2 mL methanol [12]. Poor batch-to-batch reproducibility is another issue for this material.

Evaporation of the SPE eluate or LLE organic extract to obtain a final extract with an adequate concentration factor is usually a critical step, and procedural loss for some biocides could be observed unless a careful control of key parameters (temperature, very gentle stream of N_2) is realized. Some specific cartridges with low polymeric mass (Envirelut Pesticide) allow to skip this step as elution of the analytes can be carried out with small volume (1 mL) of an organic solvent (methanol) compatible with HPLC analysis [6].

Solvent-free approaches such as Solid-Phase MicroExtraction (SPME) [13], Solvent Micro Extraction (SME) [14], and Stir Bar Sorptive Extraction (SBSE) [15] have also been applied for the determination of AF biocides in coastal waters. SPME is based on an equilibrium process that involves partitioning of analytes from a liquid phase into the polymeric phase according to their distribution coefficients, K_d. A very small amount of polymeric material is used as a fused silica fiber coating, so that SPME process could be considered as a miniaturized, albeit non-exhaustive, extraction. Poly(dimethylsiloxane) (PDMS), polyacrylate (PA), poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB), and Carbowax–DVB are typical examples of SPME coating materials with different ranges of polarity and thickness. SPME is a simple and quick technique and in most cases it is carried out by direct dipping of the coated fiber into the aqueous sample [13]. In Headspace SPME, the fiber is exposed to the headspace of the sample solution that is heated and stirred to increase the volatility of analytes [16]. The main parameters affecting K_d for AF Biocides (i.e., pH, salt additives, stirring rate, and adsorption-time profile) should be carefully optimized during the method development.

With SME, a microdrop of solvent is suspended from the tip of a syringe needle, and then immersed in the sample under investigation for a predefined time. The microdrop is then withdrawn into the syringe to be analysed [14].

In the SBSE enrichment method, the target analytes are absorbed onto a thick film of stationary phase (PDMS) coating a glass magnetic stir bar during its immersion in the aqueous sample. The relatively large volume of PDMS (50 µl) increases absorption capacity so SBSE has shown a greater sensitivity than SPME [15].

Immunoaffinity chromatography (IAC) exploits the specific antibody–antigen interaction for purification

and pre-concentration of target analytes from the sample, and can be considered as a tailored SPE. Specific immunosorbents for selective extraction of Irgarol 1051 were prepared and IAC procedure was applied for the determination in real seawater samples [17].
On the other hand, passive samplers are an example of modern sampling strategy that combines sampling, analyte isolation and preconcentration in a single step. These tools are used to measure time-averaged environmental contamination of surrounding waters, not affected by short-term fluctuations in analyte concentrations, and this avoids some drawbacks of the grab sampling. Recently a type of passive sampler well suited for deployment of polar pollutants (Polar Organic Contaminants Integrative Sampler, POCIS) has been used for a monitoring study of AF biocides among others, in the marine environment [18]. Moreover, the passive sampling technique is the focus of another paper of this Special Issue [37].

Sediment

Booster biocides, mainly those compounds with a log $K_{ow} \geq 3.0$ and half-life > 50 days, should be considered as persistent and bioaccumulative pollutants; they tend to partition onto sediments, where they may be a source of ongoing contamination, thus representing a potential threat to the marine ecosystem. On the occasion of dredging in harbours or other events disturbing the sediment, the trapped biocides can be once more released in the marine environment. Therefore, an investigation of their presence in the sediment is required.
The conventional approach to sample preparation of solid matrices is a labor-intensive procedure that involves a liquid-solid extraction usually ultrasound assisted (USE), or combined with mechanical shaking. Organic solvents most frequently used are acetone, DCM, acetonitrile (ACN), methanol (MeOH), or proper mixtures (e.g., acetone with DCM or *n*-hexane). Raw extracts obtained from sediments are not directly amenable to LC or GC analysis and an additional clean-up step, based on LLE [3], SPE [8] or alternative SPME [19] technique, is often carried out to remove matrix interferences. On the other hand, Biselli et al [20] employed the traditional Soxhlet apparatus to extract Irgarol 1051 from marine sediment, but the recovery was low (61%).
Some new methodologies, with possibility of automation, allow to minimise the solvent usage and extraction time with respect to conventional ones. The systems for Pressurized Liquid Extraction (PLE) operate under high pressure: this allows to perform the extraction of the analytes from the solid matrix at temperatures above the boiling points of conventional organic solvents. At elevated temperature analyte desorption from matrix is faster, and so is the transfer of AF biocides from marine sediment to the bulk of organic solvent. PLE with DCM was used for fast extraction of Irgarol 1051, its major metabolite and Diuron from marine sediment [21].
Likewise, the Supercritical Fluid Extraction (SFE) employs CO_2, at temperature and pressure near or above the critical point, and mixes it with a low percentage of organic solvent (MeOH) to further enhance the solvent power of the supercritical fluid. SFE was employed for the determination of Irgarol 1051 in marine sediments, with a recovery up to 87% [17].
A very promising technique is the Microwave-Assisted Extraction (MAE). It allows to accomplish an extraction of several samples simultaneously, in a few minutes, with reduced amounts of organic solvent, a great reproducibility and high recovery rates. Extraction solvent absorbs the microwave energy and reaches a temperature near the boiling point in a closed vessel. This promotes the diffusion of the target compounds from the sediment into the solvent. Due to the mild temperature conditions achieved, MeOH has been chosen as solvent in the MAE procedure for thermally labile constituents, such as Diuron and dichlofluanid, and good recoveries (> 75%) have been obtained [22]. A drawback of MAE is the co-extraction of interferences, so an additional clean-up step such as SPE is needed. For Irgarol 1051 and its main degradation product, water can be an optimal extraction solvent, making the MAE technique even more convenient and environmentally friendly [23]. The solvent evaporation and/or dilution step is avoided, and the aqueous extract can be directly loaded on the SPE cartridge.

Chromatographic determination

General remarks

The identification and quantification of AF biocides in environmental samples are generally based on the appli-

cation of chromatographic methods, such as Gas Chromatography or Liquid Chromatography, both coupled to mass spectrometer detection (GC–MS or LC–MS), which have been widely used because of their inherent selectivity and sensitivity. In the last decade LC–MS has been effectively applied to the determination of AF biocides, and many analytical methodologies based on this technique were developed. Some recent published LC–MS methods rely on the use of tandem mass spectrometry detection (MS/MS). The MS/MS fragmentation pattern is a powerful tool for obtaining confidence in compound identification as well as structural elucidation. In addition, the use of MS/MS detection allows a great gain in the limits of detection of these micropollutants and quantification to ultra trace level, especially when triple quadrupole mass analyzers are used.

Gas chromatography

Gas chromatography is a suitable technique for the separation and determination of all booster biocides with a GC amenable molecular structure. This includes chlorothalonil, dichlofluanid, Irgarol 1051 and its stable degradation product M1, Sea nine 211 and TCMTB. Diuron is a compound with poor thermal stability and decomposes during GC injection, although it can also be determined using GC after a derivatization procedure, but results are often unsatisfactory.

The chromatographic separation of these compounds can usually be achieved with common GC capillary columns filled with nonpolar stationary phases, such as methylpolysiloxane or phenyl-methylpolysiloxane, and increasing GC oven temperatures from 60-80 °C up to 280-320 °C. Splitless injection mode is a well-established approach because of its robustness, but injection volume is limited to sample volumes as low as 1-2 µl, since band broadening and peak deformation are usually observed when large amount of solvent enters the capillary column. In order to increase sensitivity but avoiding the drawback, GC with large volume injection was developed, where bulk of solvent is separated from analytes before chromatography starts. Some authors used cool on-column interface with partially concurrent solvent evaporation using a solvent vapor exit accessory, and were able to inject 100 µl ethyl acetate SPE extract. This made the development of an online SPE-GC-MS method for the determination of Irgarol 1051 and dichlofluanid [24] feasible. The alternative technique of programmed temperature vaporization (PTV) injection in the solvent vent mode, improved the analytical procedure for the determination of Irgarol 1051 in estuarine samples. A 40 µl sample of the 200 µl final extract could be injected in the capillary GC column with this PTV injector [25].

Conventional GC detection systems, such as electron capture detector (ECD), flame thermionic detector (FTD), flame ionization detector (FID), alkali flame ionization detector (AFID) and nitrogen phosphorous detector (NPD), have been used for the determination of booster biocides. Specifically, ECD is a selective detector for halogenated compounds (i.e., chlorothalonil, dichlofluanid) in environmental samples that offers high sensitivity and good reproducibility. However, interference can be frequently observed and, due to the low identification capability of conventional GC detectors, false positives could be detected. On the other hand, MS detectors provide unambiguous component identification due to the availability of library spectra. Hence GC-MS methods are most frequently used to determine the concentration of these compounds in seawater and marine sediments, and they are progressively replacing classic GC detectors.

A remarkable increase in sensitivity of MS systems can be obtained with selected ion monitoring (SIM) and tandem (MS/MS) operation modes. Sub-to-low ng/L levels (0.1–1 ppt) were the reported detection limits using an ion trap mass spectrometer in MS/MS mode, combined with large volume injection GC [25].

The single quadrupole analyzer with electron impact ion source (EI) is a very common analytical approach to the determination of AF biocides, giving optimal sensitivity especially when the SIM mode is used [26, 27, 28]. An alternative ionization technique, such as chemical ionization (CI) with methane as the reagent gas, has been evaluated in some papers. Negative chemical ionization (NCI) is also suitable for the analysis of chlorinated biocides (chlorothalonil, dichlofluanid, Sea nine 211) as it offers higher sensitivity than EI. However NCI is not the ideal ionization technique for Irgarol 1051 since a great loss in sensitivity with respect to EI was observed, which is a serious limitation to the development of multi-residue methods [29]. The absence of spectral libraries as

well as poor fragmentation are also drawbacks for CI. In this sense, considering the identification power offered by the EI spectrum on the basis of the number of fragment ions and relative abundance, EI has been used by most authors.

Liquid chromatography

Despite the traditional use of GC for booster biocide determination, LC is able to separate these compounds (including Diuron) effectively without tedious derivatization processes, hence several LC methodologies have been developed. Reversed phase high performance liquid chromatography (RP-HPLC) is commonly used for the separation of AF biocides with an octadecyl silica stationary phase (C18), although octyl columns (C8) have also been used [30]. HPLC columns are usually packed with 5 μm particles, whilst in more recent papers the use of smaller particle size (2.4 and 3 μm) have been reported [31, 32]. The mobile phase used for elution consists of either methanol or acetonitrile mixed with water. Some modifiers (e.g., ammonium acetate, formic acid or ammonium formate) are commonly added to HPLC eluents in order to enhance ion production and improve the sensitivity of MS detection. All these buffers are volatile and thus suitable for atmospheric pressure ionization (API) MS techniques. Binary gradients starting from a low percentage of organic solvent and increasing linearly to high percentage are usually adequate to separate mixtures of AF compounds and degradation products, but also isocratic conditions were reported [31]. Injection volumes are typically increased up to 50 μL in order to improve the detectability of the target analytes, but this requires the evaporation to dryness of sample extract and reconstitution in a suitable elution solvent mixture [32].

A more affordable choice than MS is the absorbance detection using a diode array detector (DAD), which has traditionally been employed for analysis of phenylurea pesticides. In some studies, DAD has been used for the simultaneous determination of Irgarol 1051, Diuron and their main degradation products [11]. The identification of analytes by LC-DAD is accomplished by comparing the retention time and UV spectrum obtained for detected peaks in the sample with those of the target compounds in a standard solution. A limited identification capability can be achieved by this detector.

Over the last decade liquid chromatography-mass spectrometry (LC-MS) has advanced dramatically in sensitivity, specificity and reliability; this allowed it to gain acceptance as a routine analytical technique and led to its widespread application in environmental analysis, also for the determination of AF biocides in marine samples. The use of MS detectors coupled to LC enabled a more discriminatory identification of analytes and the obtainment of high quality data on the occurrence of organic contaminants in the environment at very low concentration levels. Moreover LC-MS allows the determination of practically all of AF biocides (except zinc pyrithione) in a single analysis, and this means the development of true multi-residue analytical methods with reduction of time and costs.

The mass analyzers that have been commonly used are single quadrupole [32, 12], triple quadrupole [33, 34] and, more recently, hybrid instruments such as triple stage quadrupole/linear ion trap [35]. Different ionization techniques are usually available in LC-MS: electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Both negative ionization (NI) and positive ionization (PI) modes have been evaluated. Better sensitivity is achieved for chlorothalonil, dichlofluanid and TCMTB using NI mode whereas Irgarol 1051, Diuron and Sea nine 211 are commonly determined using PI [12]. In recent papers, the ionization of molecules of AF biocides is obtained by ESI [31, 32, 33, 34, 35] and this preference was confirmed by performing a comparison of ESI and APCI which showed that the best ionization technique for Irgarol 1051, Diuron and their main degradation products was ESI with PI mode [36]. Ionization of chlorothalonil is only possible using APCI and not by ESI.

One of the limitations of LC-MS is the susceptibility of interfaces to coelution with matrix components of the sample that can result in the suppression or, less frequently, in the enhancement of the analyte signal. However, these matrix effects can be minimized by good sample preparation and improved chromatographic separation, or can be compensated for with the use of isotopically labelled internal standard.

When a single quadrupole analyzer is used, structural information about a particular molecule is produced by increasing cone voltage, which affects the transmission and fragmentation of the molecular ion MH^+. Thus,

with a high voltage, more fragmentation occurs and an in-source collision induced dissociation (CID) of MH^+ is obtained. Determination of target analytes has been usually carried out with selected ion monitoring (SIM) mode in order to increase sensitivity.

In environmental analysis, the confirmation of positive findings should be based on the use of identification points (IPs) proposed by the European Commission Guidelines (EU Commission Decision 2002/657/EC) for the identification and quantification of organic residues and contaminants. The decision proposes a system of IPs, where at least three IPs are required to confirm a positive finding. In addition, the deviation of the relative intensity of the recorded ions must not exceed ±20% with respect to that observed in the reference standard, and the retention time must not deviate more than 2.5%. This means we should acquire at least three ions in single-mass spectrometry instruments (3 IPs), but this is not viable for analytes with poor fragmentation and unequivocal identification is compromised.

The MS/MS fragmentation is a more powerful tool for obtaining confidence in compound identification. This is based on its two stages of mass analysis: the former to pre-select an ion (precursor ion) and the latter to analyze the induced fragments (product ions). Selective precursor-product ion transitions (SRM) are obtained. The setting of the SRM channels for the determination of target analytes is commonly selected considering the signal intensities and structure-specificities of the product ions. MH^+ is generally used as the precursor ion. Two SRM transitions are followed with MS/MS instruments (e.g., triple quadrupole), and are enough for reliable identification since 4 IPs result.

Conclusions

Despite many efforts to develop environmentally friendly alternatives to inhibit biofouling, such as foul-release coatings relying on silicone technology or paints containing natural marine compounds, these novel AF strategies are limited either to fast moving vessels (e.g., large yachts, cruise ships, ferry boats), or to promising AF compounds still in early stages of development. We currently do not have a viable option for the replacement of booster biocides in AF paints, and a long timeline (approximately 10 years) is expected for approval process and widespread use of possible novel AF candidates. Due to the actual large use, and likely for the next years, of AF paints based on organic biocides and potential detrimental effects to the aquatic environment, monitoring data on environmental occurrence of AF biocides is needed and will still be in the future.

This paper overviews the main analytical approaches to the determination of AF biocides in different matrices from the marine environment (coastal waters and sediments) which makes feasible trace level detection of these contaminants in real samples.

Future trends will focus on the improvement in sample preparation, especially in terms of automation and development of online SPE technology, since this reduces sample manipulation and analysis time, and minimises the required amount of sample. In addition, great efforts will be devoted to obtain greener methodologies, involving less consumption of solvent and energy. In this sense, passive samplers are a promising tool since they combine sampling and preconcentration in a single step, but this novel technique has to be still further developed to obtain reliable quantitative results.

As for LC separation, the main advances will concern the application of fast and high separation efficiency approaches using both UHPLC and traditional HPLC systems based on columns packed with sub-2μm and superficially porous particles, respectively.

Future development of generic analytical protocols that will permit the simultaneous determination of AF biocides and other relevant compounds potentially detectable in the coastal marine environment (polar pesticides and emerging contaminants, such as pharmaceuticals and personal care products, alkylphenols) is required. More research devoted to metabolites and transformation products of AF biocides is also needed. In this sense, high resolution MS strategies based on powerful hybrid instruments such as QqTOF and Orbitrap are expected to be applied for the analysis of AF biocides and relevant marine contaminants. These approaches offer the possibility to achieve accurate mass measurements and acquire indispensable qualitative information through full-scan spectra, with the additional advantage of performing a retrospective analysis in order to screen non-target molecules.

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