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Determination of selected antifouling booster biocides by high-performance liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry

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Abstract

A simple and rapid technique is described for the quantitative determination of four antifouling booster biocides [diuron, 4,5-dichloro-2-*n*-octyl-4-isothazolin-3-one (Kathon 5287), (2-thiocyanomethylthio)benzothiazole (TCMTB) and (2,3,5,6-tetrachloro-4-methylsulphonyl) (TCMS pyridine) in aqueous samples. The analytes were extracted with high recoveries (ca. 100±~15%) from 2.7-l water samples, using C_{18} solid-phase extraction. Sample extracts were quantitatively analysed by reversed-phase HPLC and polarity switching atmospheric pressure chemical ionisation (APCI) MS using selective ion monitoring. Limits of detection for the four compounds were: diuron, 1 ng/l, Kathon 5287, 1 ng/l, TCMTB, 1 ng/l and TCMS pyridine, 5 ng/l. Analysis of samples collected from various UK marinas showed detectable concentrations of diuron to be present, however, concentrations of other three booster biocides were below their respective limit of detection. Crown Copyright © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Water analysis; Environmental analysis; Pesticides; Booster biocides; Diuron; Kathon 5287; (2-Thiocyanomethylthio) benzothiazole; (2,3,5,6-Tetrachloro-4-methylsulphonyl) pyridine

1. Introduction

Antifouling coatings are used to prevent marine life from colonising the bottoms of boats. As a consequence of the ban imposed by many countries in the late eighties of the use of tributyltin (TBT) in antifouling coatings on small boats [1], there has been a return to the use of copper-based antifouling paint formulations. In general, these products contain booster biocides to improve the efficacy of the formulation by inhibiting primary growth of copper resistant fouling organisms such as algal slimes. Since these alternatives to TBT are also toxic, there is concern over the potential effect of booster biocides on the ecology of the aquatic environment and the UK Health and Safety Executive (HSE) are currently reviewing all organic booster biocides. Before the risk to the environment posed by these compounds can be assessed robust methodology is required to measure their concentrations in aquatic systems.

At present, nine actives are supported for use as booster biocides in the UK (Table 1). A recent review emphasised a lack of established analytical methodology for many of these compounds [2]. Of

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Table 1
Actives approved for use in antifouling coatings by the UK HSE

Biocide (IUPAC name)	Alternative trade name	CAS no.	Structure
Chlorothalonil (2,4,5,6-Tetrachloro-isophthalonitrile)		1897-45-6	
Dichlofluanid (N′-dimethyl-N-phenylsulphamide)		1085-98-9	
Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea)		330-54-1	
Irgarol 1051 (2-methylthio-4-tertiary-butylamino-6-cyclopropylamino-s-triazine)		28159-98-0	
Kathon 5287 (4,5-dichloro-2-n-octyl-4-isothazolin-3-one)	Sea-Nine 211	64359-81-5	
TCMS Pyridine (2,3,5,6-tetrachloro-4-methylsulfonyl)pyridine	Densil 100	13108-52-6	
TCMTB (2-thiocyanomethylthio) benzothiazole	Busan	21564-17-0	
Zinc pyrithione (bis(1-hydroxy-2(1H)-pyridethionato-o,s)-(T-4) zinc)		13463-41-7	
Zineb (zinc ethylenebis-(dithiocarbamate)		12122-67-7	

the compounds listed (Table 1) methods have been reported for the analysis of Irgarol 1051 [3–7], dichlofluanid, chlorothalonil [8–10], diuron [11,12] and Kathon 5287 [13] in aqueous samples. These methods typically use liquid–liquid extraction or solid-phase extraction (SPE) followed by gas chromatography–mass spectrometry (GC–MS), or, in the case of diuron, reversed-phase high-performance liquid chromatography (HPLC) coupled to MS. As suitably robust methods exist for the simultaneous analysis of chlorothalonil, dichlofluanid and Irgarol 1051 [10], methods are required for the analysis of the remaining compounds in aqueous samples.

Over the past five years several reports have highlighted the suitability of HPLC–atmospheric pressure chemical ionisation (APCI) MS to the routine analysis of various pesticide classes and their residues in environmental samples [14,15]. This is mainly linked to the relatively inexpensive instrumentation required and the ''soft'' ionisation which yields relatively simple spectra often consisting of protonated $[M+H]^+$ or deprotonated molecules $[M-H]^-$. It has more recently been reported that HPLC–APCI-MS has been used for the analysis of diuron and Irgarol 1051 contributions to the aquatic environment from antifouling paints [16]. In this paper we describe the application of SPE followed by HPLC–APCI-MS using ionisation polarity switching to simultaneously determine concentrations of diuron, 4,5-dichloro-2-*n*-octyl-4-isothazolin-3-one (Kathon 5287), (2-thiocyanomethylthio)benzothiazole (TCMTB) and (2,3,5,6-tetrachloro-4-methylsulphonyl) (TCMS) pyridine in aqueous samples.

2. Experimental

2.1. Materials

HPLC-grade methanol and water were obtained from Rathburn (Walkerburn, UK). Polyethylene glycol (mixture of PEG 300, 600 and 1000) was obtained from Aldrich (Gillingham, Dorset, UK). $[^2H_6]$Dimethyl chlorotoluron (dimethyl-d_6 chlorotoluron) and TCMTB were obtained from Promochem (Welwyn Garden City, UK). Diuron was obtained from Aldrich whilst TCMS pyridine and Kathon 5287 were kindly supplied by Zeneca Specialities (Manchester, UK) and Rohm and Haas (Philadelphia, PA, USA), respectively. Each compound was certified to be of a purity >95%.

2.2. Preparation of standards and spiked samples

Calibration standards were made up at concentrations of 0.025, 0.05, 0.10, 0.5, 1.0 and 5 ng/μl in methanol. Spiked sea water samples for recovery determination were prepared by the addition of a known amount of standard (diuron 19.4 ng/l, TCMTB 10.0 ng/l, Kathon 5287 21.5 ng/l and TCMS pyridine 20.9 ng/l) in methanol solution to 2.7 l of natural sea water (River Crouch, Essex, UK) which was shaken vigorously for 10 min prior to extraction.

2.3. Sample extraction

Typically, water samples (2.7 l) were collected in clean glass winchesters by means of a stainless steel water sampler [17]. All samples were stored at <4°C and extracted within 48 h of collection. Sample extraction was carried out on a non-endcapped octadecyl silane SPE columns (1 g/6 ml volume; International Sorbent Technology, Hengoed, UK). To aid column solvation during extraction, methanol (2 ml) was added to each sample and shaken thoroughly. SPE columns were solvated with acetone (10 ml), dried under vacuum aspiration for 5 min, solvated with methanol (10 ml) followed by deionised water (10 ml). These procedures were carried out at a flow-rate of approximately 5 ml/min. The sample was then introduced to the column directly from the sampling bottle by means of a PTFE tube (2 mm I.D.) at a flow-rate of approximately 10 ml/min. When extraction was complete the column was dried thoroughly using vacuum aspiration for 20 min. Salts were removed by elution with water (10 ml) whilst the biocides were extracted from the column by elution with methanol (5 ml). The column was allowed to become saturated with methanol and soaked for 5 min. The procedure was repeated and all 10 ml was collected in a centrifuge tube (15 ml) containing internal standard solution (200 μl; dimethyl-d_6 chlorotoluron; 1 ng/μl) and the volume

reduced to ~200 μl using a TurboVap (Zymark, USA; 37°C).

2.4. HPLC

HPLC was carried out using a Hewlett-Packard 1050 system fitted with a quaternary pump and a Bakerbond ENV reversed-phase column (150×4.6 mm I.D.) fitted with a guard (Mallinckrodt Baker, Milton Keynes, UK). The mobile phase was acetonitrile–water run over a gradient [acetonitrile–water (10:90) for 5 min; linear to 100% acetonitrile for 15 min and held for 5 min]. Column temperature was maintained at 50°C. Twenty-five μl injections were made onto the column following a 5-min post-run equilibration period.

2.5. Mass spectrometry

Mass spectra were obtained on a VG Platform bench-top mass spectrometer (VG, Altrincham, UK). The mass spectrometer was initially tuned on background solvent ions (CH_3CNH^+, *m/z* 42). Tuning was then optimised on the diuron $[M+H]^+$ ion (*m/z* 233) and mass calibrated in the positive ion mode on a mixture of PEG 200, 600 and 1000 as per manufacturer's instruction. Following preliminary evaluation of negative and positive modes, ionisation polarity switching was used for all determinations. Typical operating conditions were: positive ionisation, corona 3.2 kV, high voltage lens 0 kV, ion energy 2.0 V; negative ionisation, corona 1.8 kV, high voltage lens 0 kV, ion energy 2.0; source temperature 150°C, probe temperature 400°C, low mass resolution 12.5, high mass resolution 12.5, ion energy 2.0 V, multiplier 650.

Full scan acquisitions were made over specific mass ranges for individual compounds to determine the optimum mode of ionisation. Single ion monitoring (SIM) was performed as follows: polarity switching (±); positive ionisation at *m/z* 219.2, 233.2, 282.0 and 284.0, cone voltages 20 V, 20 V, 15 V, 15 V, respectively, dwell time 0.25 s, interchannel delay, 0.02 s and mass span 0.2 u from 2.5–15 min and 18–25 min; negative ionisation at *m/z* 166.1, 230.0, 232.0, 234.0 and 236.0, cone voltages 32 V, 20 V, 20 V, 20 V, respectively, dwell time 0.2 s, interchannel delay, 0.02 s and mass span 0.2 u from 15–18 min. Peak area of the following ions was used for quantitation: dimethyl-d_6 chlorotoluron, *m/z* 219.2; diuron; *m/z* 233.2; Kathon 5287, *m/z* 282.0+284.0; TCMTB, *m/z* 166.1 and TCMS pyridine *m/z* 230.0+232.0+234.0+236.0. Retention times were approximately; dimethyl-d_6 chlorotoluron, 16.4 min; diuron; 14.4 min; Kathon 5287, 19.0 min; TCMTB, 16.0 min and TCMS pyridine 17.6 min.

3. Results

3.1. Selection of ionisation mode

Preliminary evaluations were carried out for the selected biocides and the internal standard. Typically a 1 ng/μl solution of the biocide of interest was injected directly into the mass spectrometer and data acquired in full scan mode (ca. 100–350 μ). As discussed in previous reports, adjustment of the cone voltage and APCI probe temperature can be used to optimise the formation of ions of interest (e.g., $[M+H]^+$) in order to obtain minimum fragmentation and maximum sensitivity [13].

3.1.1. Diuron

Poor ionisation of diuron was observed in the negative mode whilst positive APCI produced an intense protonated molecule $[M+H]^+$ (*m/z* 233; Table 2). The cone voltage and probe temperature

Table 2
Mass spectral data for the four booster biocides selected

Analyte	APCI mode	Cone voltage (V)	*m/z*
Diuron	+ve	20	233 (100%), 235 (75%)
Kathon 5287	+ve	15	282 (100%), 284 (70%)
TCMTB	−ve	32	166 (100%)
TCMS pyridine	−ve	20	230 (90%), 232 (100%), 234 (50%), 236 (20%)
Dimethyl-d_6 chlorotoluron	+ve	20	219 (100%)

were optimised to 20 V and 400°C, respectively. The isotopic ratio is as expected for a molecule containing two chlorine atoms.

3.1.2. Dimethyl-d_6 chlorotoluron

As with diuron, positive ionisation produced an intense protonated molecule (*m/z* 219; Table 2) when the cone voltage was set to 20 V and the probe temperature to 400°C.

3.1.3. Kathon 5287

Once again, positive ionisation produced an intense protonated molecule (*m/z* 282/284; Table 2). Optimisation of the cone voltage and APCI probe temperature showed 15 V and 400°C to be the most sensitive.

3.1.4. TCMTB

Positive ionisation of TCMTB produced a protonated molecule (*m/z* 239), however, further analysis showed negative ionisation to be much more sensitive. Negative ionisation did not yield a deprotonated molecule $[M-H]^-$. Simple fragmentation was obtained which yielded an intense characteristic ion (*m/z* 166; Table 2). It is suggested that this fragment is formed due to the loss of the thiocyanomethylthio substituent. Conditions for the formation of this ion were optimised to a cone voltage of 32 V and a APCI probe temperature of 400°C.

3.1.5. TCMS pyridine

It was not possible to form a de-protonised molecule for TCMS pyridine when using negative ionisation, whilst positive ionisation gave poor sensitivity. The formation of a characteristic cluster of ions was possible using negative ionisation (*m/z* 230, 232, 234, 236; Table 2). The isotopic ratio indicates that the fragment contains four chlorine atoms which therefore suggests that the fragment may be formed by cleavage of the methylsulphone group which is then replaced by an oxygen atom or via re-arrangement with an oxygen atom from the methylsulphone substituent (Fig. 1). Similar fragmentation schemes have been observed in the past using negative APCI-MS to analyse chlorinated pesticides [14]. The optimised conditions for this fragmentation to occur were for the cone voltage to be set at 20 V and the APCI probe temperature set to 400°C.

Fig. 1. Proposed fragmentation scheme for TCMS pyridine using negative APCI. M.W.=Molecular mass.

3.1.6. Detector response

Using polarity switching APCI in the SIM mode the calibration data (ca. 0.01 ng/μl–5 ng/μl) for standards in methanol is shown in Table 3 with the system linear in all cases. Repeat injections ($n=8$) of each analyte and the internal standard (dimethyl-d_6 chlorotoluron) at 1 ng/μl concentrations demonstrated that the detection method showed good precision for all four analytes (Table 3).

3.2. HPLC

Chromatograms typical of spiked estuary water (ca. 30‰ salinity), obtained using HPLC–APCI-MS with polarity switching in SIM mode, are shown in

Table 3
Calibration and precision data for the APCI-MS detection of selected booster biocides

Analyte	Calibration equation	R^2	Linear range (ng)	R.S.D. (%) (n=8)[a]
Diuron	y=14 849x+352.54	0.999	0.25–250	2.8
Kathon 5287	y=2262.4x+83.598	0.999	0.35–350	5.6
TCMTB	y=727.89x+61.036	0.999	0.25–150	7.7
TCMS pyridine	y=73.728x+218.23	0.993	0.5–100	9.8

[a] One ng injected into the mass spectrometer.

Fig. 2. Each compound is resolved to baseline with good peak shape.

3.3. SPE

SPE was based on published methods for diuron and Kathon 5287 [12]. By using replicate extractions of all four standards in sea water, limits of detection (LODs) were obtained for each compound. LODs for each biocide are in the low ng/l concentration which is sufficient for environmental monitoring (Table 4).

3.4. Application

As an application, we investigated concentrations of the four biocides in five marinas in the UK during January 1998. Diuron was found to be the only analyte which was present at concentrations greater than the limits of detection (3–117 ng/l). The concentrations of diuron observed are similar to those reported to be found in ports and marinas in the Mediterranean [16]. The absence of any the other biocides was not surprising since the samples were collected at a period when boating activity in the UK is infrequent and hence the main inputs of these compounds into the water phase is at its lowest but episodic pulses of actives occur due to hosing down and repainting operations.

It is also not clear to what extent these alternative biocides are used in modern antifouling coatings and whether their use causes significant contamination. A clearer insight into the impact of such compounds will come with more detailed investigations into their environmental concentrations, utilisation rate and

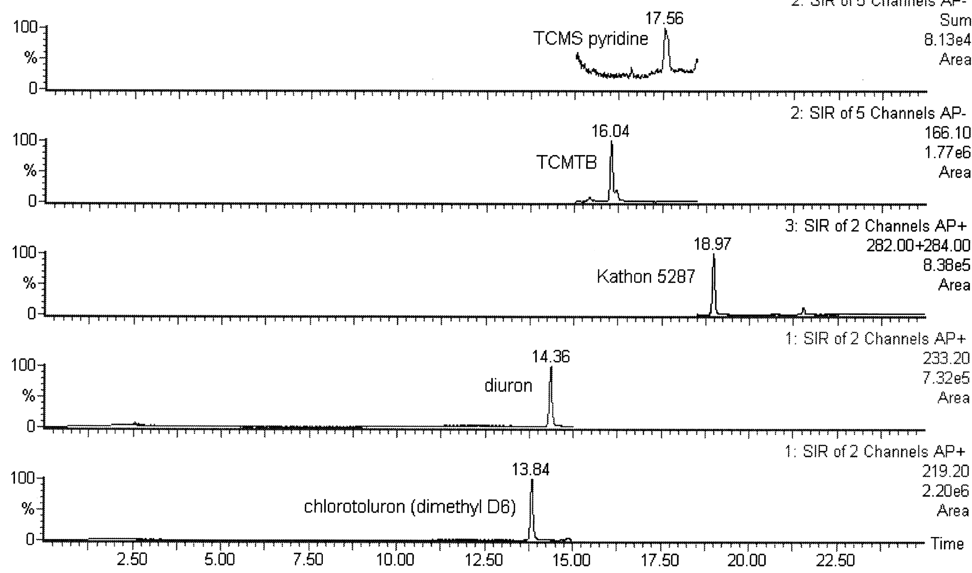

Fig. 2. Chromatograms showing the analysis of a mixture of biocide standards spiked into sea water and extracted by SPE. Retention times (min): TCMS pyridine 17.6, TCMTB 16.0, Kathon 5287 19.0, diuron 14.4 and dimethyl-d_6 chlorotoluron 13.8.

Table 4
Recovery of selected booster biocides from spiked samples

Analyte	Mean Recovery (%)[a]	R.S.D. (%) (n=8)	LOD[b] (ng/l)
Diuron	100.3	12.1	1
Kathon 5287	100.4	10.0	1
TCMTB	91.2	20.1	1
TCMS pyridine	113.1	18.4	5

[a] Calculated using: recovery=$100(X_S - X_U)/K$, where X_S=concentration measured in spiked sample, X_U=concentration measured in unspiked sample and K=known value of the spike in the sample. Twenty ng spiked into each sample.
[b] Calculated with a signal-to-noise ratio of 10.

rates of release from antifouling coatings under environmental conditions. Data to this effect will soon be available from a study funded by the UK HSE.

4. Conclusions

A simple and robust methodology has been established which allows the simultaneous and rapid quantitative analysis of four antifouling booster biocides in aquatic samples. The method relies on polarity switching to obtain protonated molecules during positive ionisation and characteristic fragments during negative ionisation which are detected using SIM. The method has been shown to have both good precision and accuracy whilst also giving LODs which are sufficiently low to detect environmental concentrations. The method should be a valuable analytical tool in the determination of booster biocide concentrations in the aquatic environment.

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