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Short communication

Determination of the antifouling agent zinc pyrithione in water samples by copper chelate formation and high-performance liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry

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Abstract

Zinc pyrithione has recently been incorporated into antifouling paints as a booster biocide, which is slowly released into the water as the paint ages. In order to determine concentrations of zinc pyrithione (ZPT) in aqueous samples, a liquid chromatographic method has been developed. Since ZPT interacts with certain reversed-phase packing materials or stainless steel components of the HPLC system, the method uses transchelation of the ZPT into the stable copper(II) complex before analysis by liquid chromatography coupled to atmospheric pressure chemical ionisation mass spectrometry. ZPT was extracted as copper pyrithione using dichloromethane with adequate recovery (77%±17%, $n=6$) from 2-l water samples. The limit of detection was calculated to be 20 ng/l, using selected ion monitoring. The analysis of samples collected from various UK marinas showed no detectable concentrations to be present, whilst a laboratory-based study confirmed that this is probably due to the rapid photodegradation of ZPT in seawater.

Keywords: Zinc pyrithione; Copper chelate

1. Introduction

Zinc pyrithione (ZPT) is the zinc chelate of 2-pyridinethiol 1-oxide (Fig. 1), and has long been known as an effective bactericide, fungicide and algicide. Such properties have led to ZPT being extensively used as an antidandruff agent in hair care products [1], whilst more recently it has been used as a booster biocide in modern antifouling coatings [2,3]. A recent survey of UK chandlers and boat yards showed ZPT-based antifouling products to be amongst the most commonly used in the UK on small boats (<25 m) [4], whilst it has also been reported as widely used on yachts and large ocean-going vessels in Europe and Japan [5]. This scale of usage, combined with ZPT release rates of 7–18 $\mu g/cm^2$ per day (data acquired using ASTM D5 108–90) [2], imply that a significant amount of ZPT is released into the immediate aquatic environment in areas of high boating activity. ZPT has been shown

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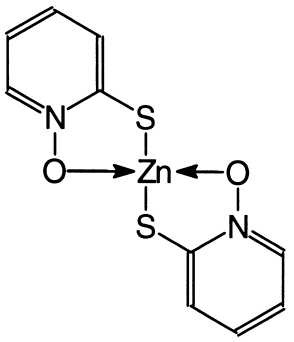

Fig. 1. Zinc pyrithione (ZPT).

to undergo rapid (~15 mins) degradation in sunlight, to form less toxic photo-products (mainly to pyridine sulfonic acid) [2,6]. Any assessment of the ecological risk associated with the antifoulant use of ZPT has been based on predicted environmental concentrations. Before the 'real' risk associated with the antifoulant use of ZPT could be assessed, an analytical method for determining environmental concentrations of ZPT is necessary.

The use of ZPT in antidandruff preparations has required that analytical techniques are available for quality control purposes during manufacture [1,7,8]. The majority of these techniques use reversed-phase HPLC, determining the analyte either as ZPT [9], a fluorescent derivative [10] or by transchelation to the Cu^{II} complex [7,8]. The direct analysis of ZPT is difficult owing to interactions with reversed-phase packing materials or stainless steel components of the HPLC system [11]. Transchelation to the copper complex is quantitative, easily achieved and produces a very stable complex suitable for reversed-phase HPLC [7]. Copper transchelation was therefore chosen as the basis for the present method. ZPT in haircare products is present at concentrations of ~2%; however, it is desirable that any analytical method developed to determine environmental concentrations of ZPT should do so at much lower concentrations and with greater specificity than previous methods. In this paper we describe the application of copper transchelation followed by HPLC–APCI-MS using selected ion monitoring (SIM) to determine concentrations of ZPT in aqueous samples.

2. Experimental

2.1. Materials

HPLC-grade methanol, dichloromethane (DCM) and water were obtained from Rathburn Chemicals (Walkerburn, UK). Analytical-grade copper(II) nitrate hemipentahydrate and polyethylene glycol (mixture of PEG 300, 600 and 1000) were obtained from Aldrich (Gillingham, Dorset, UK), whilst zinc pyrithione, certified to be of a purity >95%, was also obtained from Aldrich. Copper pyrithione was prepared from ZPT and recrystallised to >95% purity [1].

2.2. Preparation of standards

Copper pyrithione standards were made up at concentrations of 0.25, 2.5, 12.5, 25 and 125 ng/μl in DCM saturated with water. ZPT calibration standards were freshly made up at the start of each batch of analyses at concentrations of 1–500 ng/l in 0.43-μm filtered natural sea water (River Crouch, Essex, UK). Typically, ZPT (25 mg) was suspended in reagent-grade water (100 ml) and stirred in the dark for 24 h. The mixture was then passed through a pre-weighed GFC filter (1 μm) and the weight in solution calculated. This stock was then used to prepare sample spikes and the matrix matched calibration standards.

2.3. Sample extraction

Zinc pyrithione was extracted by the addition of $Cu(NO_3)_2$ solution (1 *M*, 10 ml) and DCM (25 ml). Samples (2 l) were shaken on a mechanical shaker for 15 min and the phases allowed to separate for a further 10 min before the DCM was transferred into a Teflon Soveril tube. This was repeated twice more. The Soveril tubes were then sealed and centrifuged at 3000 rpm to expedite phase separation. Any residual water was then drawn off with a glass Pasteur pipette and the organic layer decanted into a Pyrex round bottomed flask. The DCM was then reduced in volume (~2 ml) by rotary evaporation (Bucchi, 25°C), transferred to a graduated centrifuge tube (15 ml) and the volume further 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, a PEEK analytical column (15 cm×4.6 mm) and guard column (4 cm×4.6 mm) packed with Prodigy ODS3 (particle size 5 μm; Phenomenex, Macclesfield, UK). The mobile phase was methanol–water run over a gradient (50:50%, methanol–water linear to 100% methanol for 10 min and held for 5 min). Column temperature was maintained at 30°C. Injections (25 μl) 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 copper pyrithione $[M+H]^+$ ion (m/z 316) and mass calibrated in the positive ion mode on a mixture of PEG 200, 600 and 1000 as per the manufacturer's instructions. The instrument was used in the positive ion mode using the following operating conditions: corona, 3.2 kV; high voltage lens, 0 kV; ion energy, 2.0 V; source temperature, 150°C; probe temperature, 300°C; low mass resolution, 12.5; high mass resolution, 12.5; ion energy, 2.0 V; multiplier, 650.

Full scan acquisitions were made over a mass range of 100–350 AMU. Single ion monitoring (SIM) was performed at m/z 221 and 316, at a cone voltage of 10 V, a dwell time of 0.25 s, an inter-channel delay of 0.02 s and a mass span of 0.2 AMU. The summed peak area of the m/z 221 and 316 ions was used for quantification, at a retention time of approximately 7.5 min.

3. Results

3.1. Extraction of zinc pyrithione

The extraction procedure used was modified for use with sea water from a published method [6]. Initial extraction of ZPT was performed with copper sulphate as the source of Cu^{II} ions; however, CPT recovery was poor. It was suspected that this was due to the formation of a precipitate, thought to be due to the presence of insoluble sulphate salts. Copper nitrate was substituted for copper sulphate and gave very little precipitate. As previous studies [6] have adjusted the pH of the sample to facilitate the transchelation reaction, the effect of reducing the pH on yield was examined. Triplicate extractions with the addition of a pH 5 buffer solution (1 *M* citric acid–2 *M* disodium hydrogen phosphate (97:103); ca. ~10 ml) [7] had very little effect on analyte recovery; less than 5% more.

3.2. Selection of ionisation mode

Both positive and negative ionisation modes were evaluated using a 1 ng/μl solution of copper pyrithione (CPT), which was injected directly into the mass spectrometer and mass spectral data were acquired in full scan mode (ca 100–350 AMU). Poor ionisation of CPT was observed in the negative ion mode, whilst positive APCI produced both a molecular ion (m/z 316) and a characteristic fragment (m/z 221; possibly M+1-ZnNO). The cone voltage and probe temperature were then optimised to minimise fragmentation and maximise sensitivity [12]. The optimum settings were found to be a cone voltage of 10 V and probe temperature of 300°C; however, m/z 221 remained the base peak in the spectrum obtained when the protonated molecular ion was optimised to 50% base peak (Fig. 2).

3.3. Chromatography, validation and analysis of marina samples

A chromatogram of ZPT as the copper chelate (CPT) in sea water, obtained using HPLC–APCI-MS with SIM is shown in Fig. 2. Copper pyrithione is resolved to baseline with good peak shape and remains so after 100+ injections. The calibration graph was linear in the range 2.5–125 ng of CPT, with linear regression producing an equation of $y=837x+1219$ with a correlation coefficient of 0.999 (where y=peak height and x=concentration of CPT (ng/l)). Repeated injections ($n=8$) at 1 ng/μl demonstrated that the method also yields good precision (R.S.D.=11%).

In order to validate the method, sea water samples

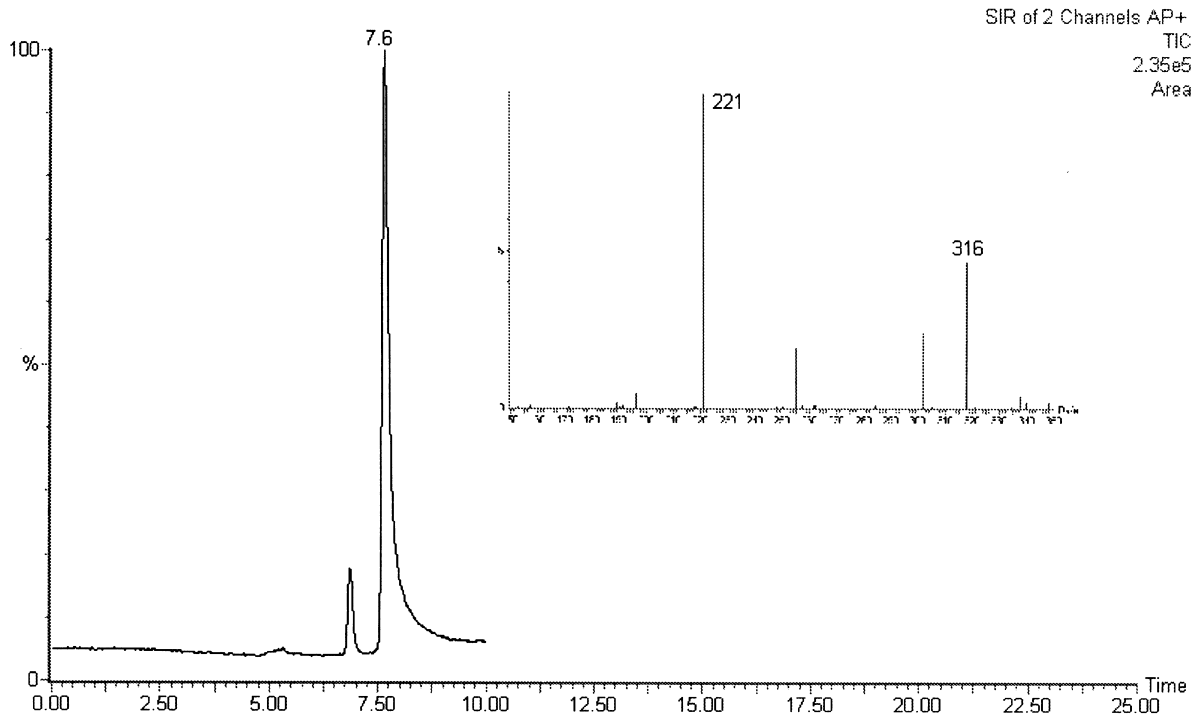

Fig. 2. SIM (m/z 221+316) chromatogram of copper pyrithione showing full scan mass spectrum inset.

spiked with ZPT were prepared (100 ng/l). The mean recovery was good (77%) whilst precision was acceptable (R.S.D. 17%, $n=6$). By using replicate extractions and a signal-to-noise ratio of 10, a limit of detection of 20 ng/l was obtained. This was considered sufficiently sensitive to enable the use of HPLC–APCI-MS for the determination of ZPT in environmental waters. CPT may be used as a booster biocide in the future [5]; however, any concerns over the specificity of the assay are without cause since ZPT does not interfere with the determination of CPT; allowing pre- and post-transchelation samples to be analysed in order to calculate the concentration of ZPT and CPT in the sample should it be needed.

Water samples from several marinas in Southern England were collected in January and May of 1998. The samples collected in January were to provide a winter 'baseline', whilst those collected in May were collected at the start of the UK yachting season when antifouling paint applications are fresh and biocide release rates at their maximum. Using the extraction procedure outlined above, none of the samples was found to contain concentrations of ZPT above 20 ng/l. This limited amount of data suggests that, even within closed marinas where ZPT use is known, rapid removal from the water column is occurring. Published laboratory based data suggest that low water solubility and rapid photodegradation [2,6] may be factors in the removal processes. To confirm this, a simple laboratory degradation experiment was performed. ZPT was dissolved in filtered sea water (33‰) at a concentration of 5 μg/l, and was exposed to room light at 20°C. ZPT concentrations were examined up to 72 h. The ZPT concentration decreased with time (half-life=4 h; Fig. 3) and confirms previous reports that ZPT rapidly photodegrades and the absence of detectable concentrations of ZPT in areas where it is used as an antifouling biocide.

4. Conclusions

A simple method has been developed for the analysis of ZPT using copper transchelation and HPLC coupled to mass spectrometric detection using

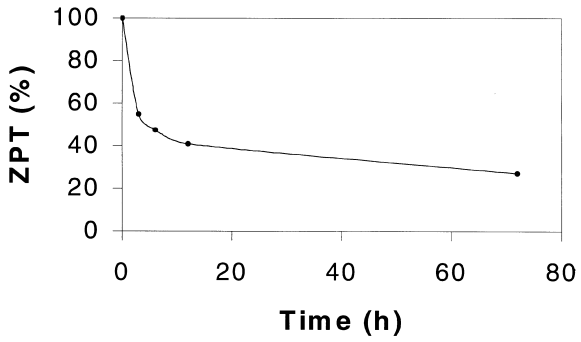

Fig. 3. Degradation of ZPT in filtered sea water under laboratory conditions.

APCI. The method has been shown to have both good precision and accuracy, whilst also yielding a limit of detection that is adequate for the detection of environmental concentrations. The method should be a valuable addition to existing analytical tools available for the determination of booster biocide concentrations in the aqueous samples.

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