Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1132 (2006) 157–164
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Improved method for the determination of zinc pyrithione in environmental water samples incorporating on-line extraction and preconcentration coupled with liquid chromatography atmospheric pressure chemical ionisation mass spectrometry

Jonathan Bones [a], Kevin V. Thomas [b], Brett Paull [a,*]

[a] *National Centre for Sensor Research, School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland*
[b] *Norwegian Intitute for Water Research, Kjelsås, NO-0411 Oslo, Norway*

Received 1 June 2006; received in revised form 24 July 2006; accepted 28 July 2006
Available online 24 August 2006

Abstract

A method has been developed for the determination of zinc pyrithione (ZnPT) in environmental water samples using monolithic reversed-phase silica columns for rapid on-line large volume solid phase extraction in tandem with on-line matrix removal using sacrificial strong anion exchange (SAX) columns. This is coupled with reversed-phase liquid chromatography with atmospheric pressure chemical ionisation mass spectrometric detection. Limits of detection in spiked river water samples, using a 200 mL preconcentration volume, were determined as 18 ng L^{-1}, with a limit of quantitation of 62 ng L^{-1}. The percentage recovery from spiked river water was found to be 72 ± 9 ($n = 3$ extractions), whilst overall method precision, following 10 repeat complete analyses was found to be 27% RSD at 1 μg L^{-1}. Linearity was determined over the concentration range of 0.25–10 μg L^{-1} and the calculated regression coefficient was $R^2 = 0.9802$. The method was used to investigate the environmental fate of zinc pyrithione in waters and its partition coefficient between sediment and water phases.

Keywords: Zinc pyrithione; Environmental waters; On-line extraction; LC-APCI-MS

1. Introduction

From 1 January 2008 the use of antifouling paints containing organotin biocides will be completely banned in accordance with the International Marine Organisation (IMO), convention on the control of harmful anti-fouling systems [1]. As replacements for organotin compounds a number of organic booster biocides have appeared on the market. These organic booster biocides have attracted increased analytical attention due to their inherent environmental toxicity and the ability of some chemicals such as Irgarol 1051 and Diuron to persist in the environment and perhaps bioaccumulate [2].

However, one particular organic booster biocide, zinc pyrithione (ZnPT) (bis-(*N*-oxopyridine-2-thionato) zinc(II)) has received little attention due to the lack of sufficient analytical methods for its determination, despite concerns of considerable environmental toxicity at ultra-trace concentrations [3–7]. The use of zinc pyrithione as an organic booster biocide is relatively new. To date the majority of ZnPT produced has been used as either the active ingredient in anti-dandruff shampoo or as an additive in cosmetics and dermatitis treatments. For example, in Sweden it was estimated that at least 10 tonnes of ZnPT were consumed in the anti-dandruff shampoo sold in 2003, while only 2.4 tonnes of ZnPT were used as an organic booster biocide in marine anti-fouling paints [8]. The permitted levels of ZnPT usage are a maximum of 4% (w/w) in marine paints and a maximum of 1% (w/w) in anti-dandruff shampoo [9].

Therefore, two distinct routes into the aquatic environment exist for ZnPT that depend principally upon the mode of usage. In case of marine antifouling paints, ZnPT may leach directly from a painted ship surface into the surrounding water. The rate of ZnPT leaching has been estimated by Turley et al. to be 1–3 μg cm^{-2} d^{-1} for long lasting paint formulations or 3–11 μg cm^{-2} d^{-1} for self-polishing paints [10]. Using this

* Corresponding author. Tel.: +353 1 7005060; fax: +353 1 7005503.
E-mail address: Brett.paull@dcu.ie (B. Paull).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.07.068

information further, Turley et al. modelled a predicted environmental concentration (PEC) of 0.04 $\mu g\,L^{-1}$ for ZnPT. In the case of anti-dandruff shampoo and other personal care products ZnPT and free pyrithione are introduced into the aquatic environment via municipal household wastewater. Galvin et al. estimate that based upon a rate of production of 100–200 L wastewater per person per day, possible concentrations of pyrithione being introduced into the aquatic environment are in the region of 1×10^{-7} M, ($\sim$0.32 $\mu g\,L^{-1}$) and consequently household wastewater is a significant source of pyrithione with possible pollution of receiving waters [11]. With a reported log K_{ow} of 9.33 it would be expected that ZnPT would accumulate in sediments due to very low aqueous solubility [12], however, studies to determine the extent of the adsorption of ZnPT onto sediments are scarce. In contrast to what would be expected, Turley et al. proposed that free or complexed pyrithione will not persist or accumulate in sediment due partly to rapid photochemical attenuation in the water column but also through anaerobic degradation of any pyrithione adsorbed to the sediment via reduction of the *N*-oxide group [10].

In many of the above studies authors note the absence of suitable analytical methods for the determination of ZnPT in environmental matrices. Chromatographic techniques for pyrithione and ZnPT determination are rare, although the use of electrochemical techniques such as polarography, voltammetry and amperometry have been investigated [13–17]. Difficulties have been reported concerning the chromatographic analysis of pyrithione complexes due to problematic unwanted interactions with the silica stationary phase. Researchers have reported that ZnPT readily transchelates with metallic impurities present in the silica stationary phase such as Fe(II)/(III), leading to severe peak tailing [18]. However, the effect of silanol activity on the chromatography of pyrithione complexes would also be expected to be of high significance due to the presence of the pyridyl moieties on the ligand. Attempts to avoid such unwanted interactions have focused upon the derivatisation of ZnPT with fluorescent reagents such as 5-dimethylaminonaphthalene-1-sulphonylaziridine (DNS-A) or 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), which stabilises the pyrithione complex and aids detection [19]. More commonly, ZnPT is converted to the more stable CuPT by the addition of Cu(II) that thereby facilitates more reproducible chromatography [18,20]. Other problems noted with the chromatographic analysis of pyrithione complexes have included the oxidation of pyrithione by the silica stationary phase yielding a pyrithione dimmer (PT_2) over the course of the chromatographic run [21].

To date only one method has appeared in the literature with the desired levels of sensitivity for the determination of ZnPT in the aquatic environment. Thomas [22] surveyed the levels of ZnPT in marinas in the southern United Kingdom using liquid chromatography coupled to atmospheric pressure chemical ionisation mass spectrometry (LC-APCI-MS) [22]. However, in all instances no ZnPT was detected above the limit of detection (20 $ng\,L^{-1}$). Sample preparation was performed using copper chelation liquid–liquid extraction (LLE) to extract any ZnPT as CuPT. In this paper an improved method for zinc pyrithione in environmental water samples is described, based upon on-line solid phase extraction and matrix elimination followed by LC-APCI-MS. The sensitivity and selectivity of the developed method is appropriate for the desired environmental application and the method has been used for evaluation of the environmental fate of ZnPT in natural waters.

2. Experimental

2.1. Chemicals and reagents

Reagent water used throughout this study was obtained from a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA) and was 18.2 MΩ or greater. Methanol, acetonitrile and dichloromethane were received from Labscan, (Dublin, Ireland) all solvents used were HPLC grade. Zinc pyrithione (95%) was received from Sigma–Aldrich (Steinheim, Germany), as was copper sulphate pentahydrate (99%), copper(II) acetate (98%), 2,6-pyridine dicarboxylic acid (99%), sodium chloride (ACS reagent grade), formic acid (95–97%), ammonium formate (99.995%), ammonium acetate (99%), disodium hydrogen phosphate (99%), pyridine (99%) and phenol (ACS reagent grade). Ferric nitrate nonahydrate, hydrochloric acid, nitric acid, sulphuric acid, glacial acetic acid and ammonia solution (33%) all AnalaR grade were received from BDH Chemicals Ltd. (Poole, UK). Copper(II) nitrate trihydrate and anhydrous sodium sulphate were both received from Riedel de Haen, (Seelze, Germany). Potassium nitrate was purchased from Merck KGaA, (Darmstadt, Germany). Stock 1000 $mg\,L^{-1}$ solutions of ZnPT were prepared in dichloromethane and stored in the refrigerator in darkness and replaced monthly.

2.2. LC-APCI-MS analysis

For method development a Hewlett Packard HP 1050 series HPLC with a Model 78953C variable wavelength detector was used. Agilent ChemStation version A.09.03 was used for instrument control and data analysis (Agilent Technologies, Palo Alto, CA, USA). LC–MS was performed using an Agilent 1100 series HPLC with a Model G1315B photodiode array detector. The LC system was coupled to a Bruker Daltonics esquireLC ion trap mass spectrometer complete with an atmospheric pressure chemical ionisation source operated under positive polarity. Agilent ChemStation version A.06.01 (Agilent Technologies, Palo Alto, CA, USA) and Bruker Daltonics esquire control version 6.08 (Bruker Daltonics, Coventry, UK), were used for LC–MS system control, Bruker Daltonics Data Analysis version 2.0 was used for data processing. For APCI optimisation, solutions of the analyte in dichloromethane were infused using a Cole Parmer 74900 series syringe pump at a rate of 600 $\mu L\,h^{-1}$ (Cole Parmer, Vernon Hills, IL, USA) into a flow of methanol at a rate of 190 $\mu L\,min^{-1}$ from the LC pump through a mixing tee and then into the APCI source. The optimisation was performed using a nebuliser pressure of 50.0 psi, a dry gas flow of 10.0 $L\,min^{-1}$, a drying temperature of 325 °C, a corona voltage of +3200 V and an APCI temperature of 500 °C. Gradient separations were performed on a Merck Chromolith Performance RP-18e, 100.0 mm × 4.6 mm I.D. monolithic silica

column (Merck KGaA, Darmstadt, Germany) with a mobile phase of methanol and 10 mM ammonium acetate. Quantitation was performed upon extracted ion chromatogram (EIC) *m/z* 316.0 traces while the ratio of EIC *m/z* 316.0 to EIC *m/z* 318.0, (∼100:45) was used for qualitative confirmation.

2.3. On-line SPE

On-line SPE was performed using a Rheodyne 7000 six-port column-switching valve (Cotati, CA, USA). The extraction column used was a Phenomenex Onyx C_{18} 10.0 mm × 4.6 mm I.D. monolithic silica guard cartridge (Phenomenex, Macclesfield, UK). A Merck Hitachi LaChrom L-7100 isocratic pump was used for sample delivery at flow rates of 10 mL min^{-1}. The C_{18} monolithic column was conditioned with 20 mL acetonitrile and 20 mL of water, respectively prior to use. Environmental samples were filtered through Whatman GF/C 0.45 μm glass fibre filters to remove particulate matter and adjusted to pH 7.0 prior to extraction. A 200 mL portion of sample was extracted using the C_{18} monolithic column and elution was performed using mobile phase back flushing onto the analytical monolithic column. Removal of matrix interference was achieved by the incorporation of sacrificial strong anion exchange (SAX) column prior to the monolithic concentrator column along with a solvent wash step. The sorbent used was Vydac 301SC anion exchange silica (Si-SAX) packed into a 33.0 mm × 4.6 mm stainless steel column housing. The Si-SAX column was conditioned with 0.2 M ammonium acetate and water before use. The clean-up column was switched off-line prior to elution of ZnPT from the concentrator column onto the analytical column. It was noted that to increase the lifetime of the sacrificial clean-up column for multiple analysis, matrix rich samples required 10-fold dilution.

2.4. Assessment of environmental fate

In order to examine the effect of contact with common inorganic anions solutions of 5 mg L^{-1} ZnPT were prepared in water containing varying concentrations of chloride, sulphate, nitrate and phosphate in the range of 5–20 mg L^{-1} and 5 mg L^{-1} Cu(II). The solutions were then analysed using LC–UV and then reanalysed after both 24 and 48 h, respectively in order to determine the effect of contact time.

The adsorption of ZnPT onto the sediment phase was investigated using two certified reference sediments obtained from the National Research Council of Canada. To an amber glass sample vial, 5 mL of 5 mg L^{-1} ZnPT in water was added to 0.5 g of sediment and repeatedly shaken by hand several times over the course of 2 h. For each sediment sample the behaviour of a control and two ZnPT solutions were examined. An aliquot of the aqueous phase was then withdrawn and placed in an amber autosampler vial for subsequent LC-APCI-MS analysis. The remaining aqueous phase was removed via filtration and the sediment sample was air dried over night. The sediment was then transferred to another amber sample vial and extracted with 5 mL of 50:50, dichloromethane:methanol. The solvent solution was filtered through nylon filters, reduced in volume under N_2 and finally reconstituted with 200 μL of methanol before LC-APCI-MS analysis. Both the nylon and paper filters used were also analysed by LC-APCI-MS in order to examine any possible sources of cross contamination. The concentrations of CuPT were determined from a prepared five-point calibration curve in the range of 0–50 mg L^{-1}.

In order to examine photochemical attenuation, a 10 mg L^{-1} solution of CuPT in river water, along with a control sample was placed in a Duran bottle an exposed to natural sunlight. Sacrificial samples were withdrawn from the Duran bottle into amber vials and immediately frozen until LC-APCI-MS analysis.

3. Results and discussion

3.1. APCI-MS study of transition metal pyrithione complexes

In order to determine the optimum ionisation and ion focusing parameters a solution of 1000 mg L^{-1} ZnPT in dichloromethane was diluted 1/20 with methanol from the LC pump and infused into the mass spectrometer. These parameters were automatically fine tuned using the Bruker esquire software for the ZnPT pseudomolecular ion (*m/z* 317.0) using positive polarity APCI. Under optimised conditions the resultant APCI-MS spectrum for ZnPT is depicted in Fig. 1, which depicts two significant ions, the pseudomolecular $[M+H]^+$ ion at *m/z* 317.0 and another ion at *m/z* 221.2.

The expanded isotopic pattern for the pseudomolecular ion at *m/z* 317.0 is depicted as Inset (A) in Fig. 1 and clearly shows the isotope pattern of elemental zinc with $[M+H]^+$, $[M+H+2]^+$ and $[M+H+4]^+$ ions in the approximate ratio of 100:60:40. It can also be seen that the recorded experimental spectrum correlates well with the expected theoretical spectrum, depicted as Inset (B) in Fig. 1 [23], which again shows an isotope pattern characteristic of the central zinc atom. The ion at *m/z* 221.2 was also observed by Thomas [22], although no definitive molecular structure was assigned. In an attempt to elucidate the identity of the species at *m/z* 221.2 tandem mass spectrometry was per-

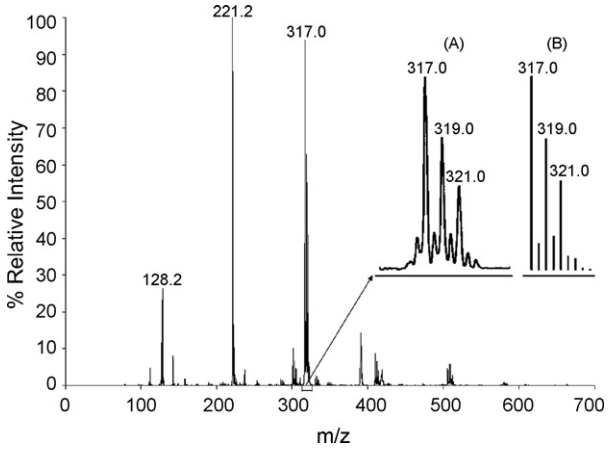

Fig. 1. APCI-MS spectrum of ZnPT showing the $[M+H]^+$ pseudomolecular ion at *m/z* 317.0 and a decomposition product ion at *m/z* 221.1. Inset (A) shows the expanded isotopic pattern of the pseudomolecular ion and Inset (B) depicts the theoretical isotopic pattern.

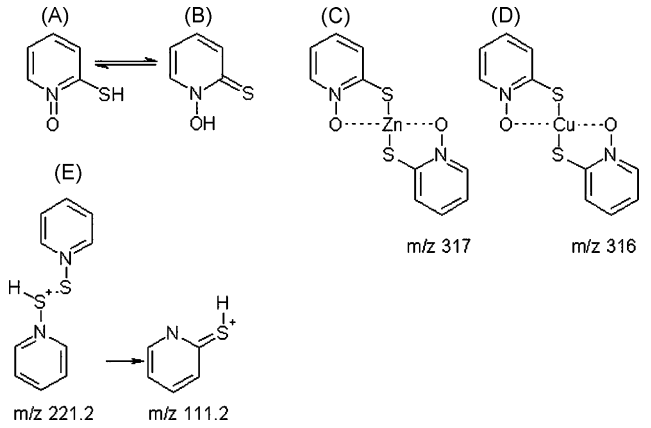

Fig. 2. The structures of the pyrithione ligand tautomeric pair: (A) 2-mercaptopyridine-*N*-oxide and (B) *N*-hydroxypyridine-2-thione; (C) the structure of zinc pyrithione (ZnPT); (D) the structure copper pyrithione (CuPT); and (E) proposed structure the ion at *m/z* 221.2 and the MS/MS daughter ion at *m/z* 111.2.

formed that yielded MS/MS daughter ions at *m/z* 111.2 and 187.1, with the ion at *m/z* 111.2 being significantly more intense. Upon studying the isotope patterns for all the ions it was concluded that the complex at *m/z* 221.2 did not contain a metallic element and the daughter ion at *m/z* 111.2 suggests that the molecule fragments by splitting in half. It is proposed that the species at *m/z* 221.2 is pyridine disulfide and the daughter ion at *m/z* 221.2 arises from α-cleavage of the disulfide bond as shown in Fig. 2(E).

Other metal pyrithione complexes were prepared by LLE of ZnPT with solutions of Cu(II), Fe(III) and Mn(II) and dichloromethane, the APCI-MS spectra of the formed complexes, of which CuPT (golden, Fig. 2(D)) and FePT (violet) were strongly coloured, were then recorded in a similar manner as described for ZnPT. Fig. 3 shows the recorded spectrum of CuPT. Again, as was observed for ZnPT, the only significant ion present is the pseudomolecular CuPT $[M+H]^+$ ion at *m/z* 316.0, the decomposition product at *m/z* 221.1 can also be observed although at a much lower intensity than was present in the ZnPT APCI-MS spectrum. Inserted into Fig. 3 are the experimentally

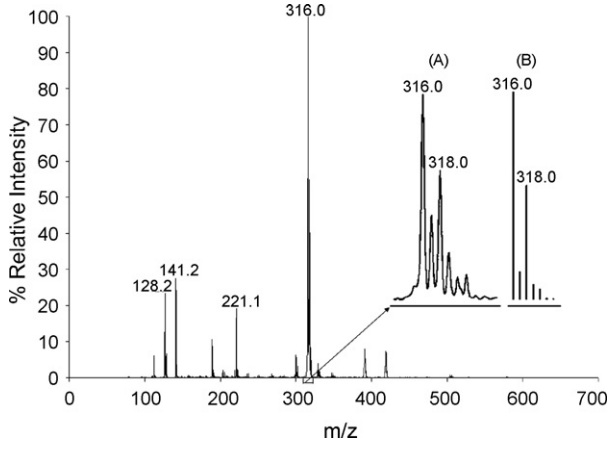

Fig. 3. APCI-MS spectrum of CuPT showing the $[M+H]^+$ pseudomolecular ion at *m/z* 316.0. Inset (A) shows the expanded isotopic pattern of the pseudomolecular ion and Inset (B) depicts the theoretical isotopic pattern.

recorded and theoretical isotopic pattern of the pseudomolecular ion, (depicted as Inset (A) and Inset (B), respectively), both of which clearly show the characteristic copper isotopes with $[M+H]^+$ and $[M+H+2]^+$ ions in the approximate ratio of 100:45. In the case of FePT no pseudomolecular ion was observed in the resulting spectrum (data not shown), the only significant ion present was at *m/z* 127.2 that corresponds to $[\text{pyrithione}+H]^+$, therefore, suggesting that the FePT complex is quite labile and readily decomposes within the APCI source. A similar observation was observed for MnPT.

3.2. Liquid chromatography

Initial work investigated a range of reversed-phase columns for the determination of ZnPT, in an attempt to identify a stationary phase that was essentially free from metallic impurities and also exhibited very low silanol activity. In order to assess the silanol activity of possible analytical columns a pyridine phenol test was performed according to accepted procedures [24]. Ideally, pyridine should elute from the column before phenol, symmetrically with minimal tailing. Active silanols cause excessive retention and tailing of the pyridine peak. Coelution of pyridine and phenol, although undesirable indicates an acceptable level of silanol activity. Fig. 4 shows the resulting traces for the pyridine phenol test performed upon a Chromolith Performance RP-18e 100.0 mm × 4.6 mm monolithic silica column, a Waters Symmetry 50.0 × 2.1, 3.5 μm ODS column, a Hypersil ODS 50.0 × 2.1, 3 μm column and a Hypersil BDS 250.0 × 3.0, 5 μm column. As can be seen, the Chromolith Performance RP-18e monolithic column out performs all the particulate columns with a narrow co-eluting peak observed for pyridine and phenol.

The effect of metal contamination within the silica stationary phase upon the elution of the ZnPT complex was investigated using the Waters Symmetry 50.0 × 2.1, 3.5 μm ODS column and the Chromolith Performance RP-18e monolithic column. Fig. 5(a) demonstrates the effect of metallic impurities within the silica substrate upon the ZnPT peak. Using a simple methanol gradient as depicted in Fig. 5 (with 10 mM acetic acid/ammonium acetate buffer pH 4.7) the peak for ZnPT on the Waters column eluted as a small fronted peak. In an attempt to improve both peak shape and recovery, the column was washed

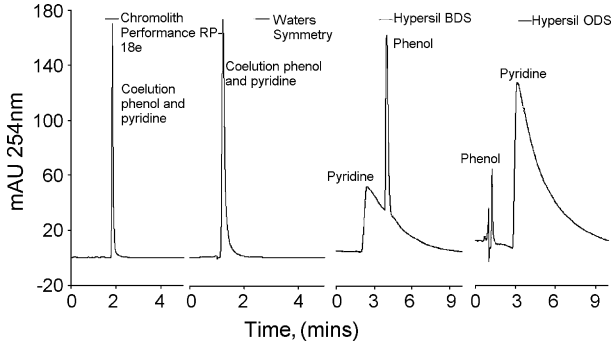

Fig. 4. Pyridine/phenol test chromatograms to examine the silanol activity of perspective analytical columns. Test conditions: mobile phase 50% MeCN in water with UV detection at 254 nm, column temperature 40 °C, concentrations 0.5 μL mL^{-1} pyridine, and 4 mg mL^{-1} phenol.

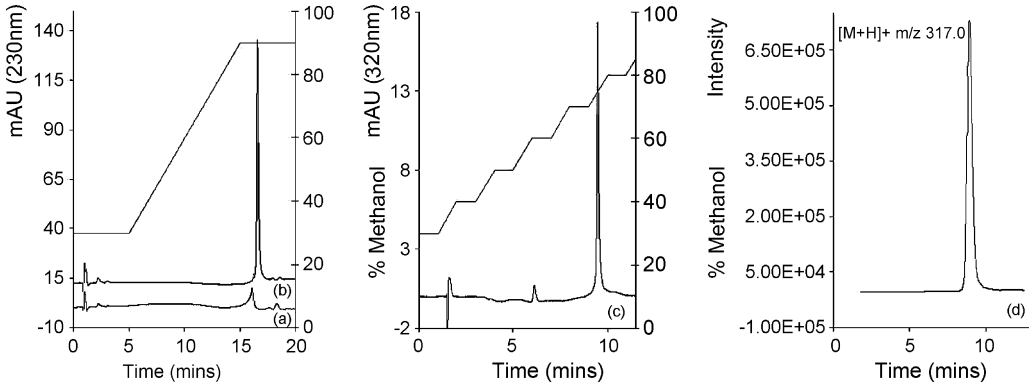

Fig. 5. The effect of stationary phase metal contamination upon ZnPT: (a) a 20 mg L^{-1} ZnPT injection prior to washing the stationary phase with dipicolinic acid; (b) the same 20 mg L^{-1} standard after washing the stationary phase with dipicolinic acid; (c) a 5 mg L^{-1} injection of ZnPT on the Chromolith Performance RP-18e monolithic column using the multi step gradient; (d) a 5 mg L^{-1} ZnPT standard injection on the Chromolith Performance RP-18e monolithic column with APCI-MS detection, methanol gradient as (c), buffer = 10 mM ammonium acetate pH 6.5. The calculated USP asymmetry value was 0.95.

with a selective chelating agent, in this case a solution of 10 mM 2,6-pyridine dicarboxylic acid (dipicolinic acid) pH 4.0, according to [25]. Fig. 5(b) shows the chromatogram obtained from the same standard solution after this washing step, where ZnPT now elutes as a large sharp symmetrical peak at 16.6 min. No such effects were observed when using the Chromolith column, presumably due to the high purity monolithic silica substrate and also the superior level of endcapping. For this reason the Chromolith column was used for all further studies.

Initial investigations into the LC analysis of ZnPT showed that a rather high proportion of organic solvent (>70% methanol) was required to elute the complex from the column, corresponding well with the reported log K_{ow} of ZnPT = 9.33 [12]. Of the gradients examined a rapid linear sweep changing from 30% methanol to 90% methanol in 1 min was initially chosen as it produced a sharp ZnPT peak with a retention time of approximately 8.3 min. However, it was later found when using mass spectrometric detection that the rapid linear gradient was a significant source of ion suppression, (data not shown) as the sharp increase in methanol caused a focusing effect of retained matrix components and therefore, a shallower multi step gradient was developed as shown in Fig. 5(c). Some fronting of the ZnPT peak was seen with the use of the acetic acid/ammonium acetate buffer at pH 4.6, however, this was eliminated through switching to an ammonium acetate buffer at pH 6.5.

To couple the optimised LC method with APCI-MS detection the MS source ionisation parameters were optimised, namely corona voltage and APCI reaction temperature. The corona voltage was optimised in the region of +1000 to +3400 V and a sigmoidal relationship was observed between the resulting intensity and the applied voltage. The optimum applied voltage was deemed to be +2600 V, above which no significant increase in intensity was recorded. The APCI temperature was optimised in the range of 200–500 °C using 25 °C intervals, with a linear increase in intensity with increasing APCI temperature observed up to ~325 °C, followed by a linear decrease in intensity with further increase in the APCI temperature, suggesting that at temperatures exceeding 325 °C ZnPT may begin to decompose. Fig. 5(d) shows the LC-APCI-MS chromatogram for a 5 mg L^{-1} ZnPT standard under the final optimised LC and APCI-MS conditions (calculated USP asymmetry for peak = 0.95).

3.3. On-line extraction and matrix removal

Methods in the literature concerning the determination of ZnPT in environmental samples are limited in number. The method of Thomas [22] appears to be the primary reference for those wishing to examine the presence of ZnPT, however, the method uses large sample volumes and LLE for sample enrichment with dichloromethane. Little or no work has to be focused upon the development of preconcentration using SPE for the trace enrichment of ZnPT prior to instrumental analysis.

Based upon a recent study [26] a short reversed-phase monolithic silica column (10.0 mm × 4.6 mm I.D.) was investigated for the rapid on-line preconcentration of the neutral ZnPT and CuPT complexes. Initial experiments showed that when analysed effluents from the short monolithic concentrator column, during sample loading of both ZnPT and CuPT showed no traces of either complex, whilst the complete elution of the complexes using methanol was possible. Due to the monolithic nature of the concentrator column it was possible to load the sample at flow rates of up to 10 mL min^{-1}, thereby reducing analysis times for trace analysis requiring large sample volumes. An optimisation of the extraction pH and the ionic strength of the sample were then performed. The sample pH was optimised in the working range of the monolithic silica column, i.e. pH 2–7, by extracting solutions of 1 μg L^{-1} ZnPT prepared in either pH adjusted water or buffer solutions. The resulting percentage recovery, calculated by peak area comparison with a 5 mg L^{-1} standard injection, was relatively low below pH 6 (~20–30%), but increased rapidly between pH 6 and 7 (up to ~50%). The addition of NaCl, to the sample to improve recoveries resulted in an almost linear improvement in the recovery of ZnPT from 50% (in water, pH 7) to ~95% (1 M NaCl, pH 7).

Primary on-line SPE investigations with spiked tap water and river water matrices yielded the problem of excessive natural organic matter, (NOM) extraction along with ZnPT and consequent APCI-MS ion suppression. The co-extracted NOM was

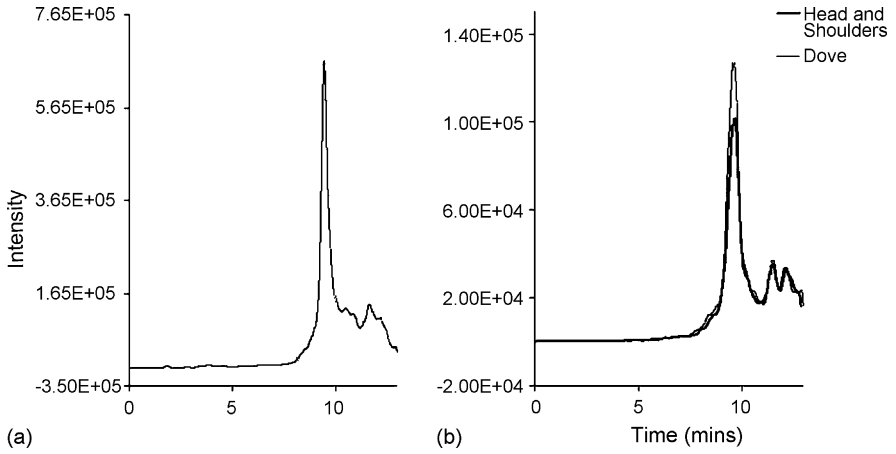

Fig. 6. (a) EIC *m/z* 316 chromatogram depicting the extraction of 1 μg L^{-1} ZnPT from river water. (b) The determination of ZnPT as CuPT in anti-dandruff shampoo samples diluted in river water matrix. The Dove anti-dandruff shampoo contains 0.37% (w/w) ZnPT, the concentration corresponding to chromatogram shown = ∼76 ng L^{-1}. Head and Shoulders Classic Clean shampoo conatins 0.80% (w/w) ZnPT, the concentration corresponding to chromatogram shown = ∼80 ng L^{-1}.

observed to elute from the analytical column in the same region as the ZnPT peak with large intensity due to the presence of a significant ion at each *m/z* value. Attempts to selectively wash retained NOM from the concentrator column proved unsuccessful. Therefore, a sacrificial clean-up column, containing a strong anion exchange sorbent, was introduced prior to the concentrator column, for the retention of acidic NOM was used [27–29]. The dual column clean-up and preconcentration approach was applied to a 1 μg L^{-1} ZnPT spike prepared in river water and extracted under optimum conditions. The resultant LC-APCI-MS chromatogram, shown as Fig. 6(a) showed a clear sharp intense peak with minimal matrix interference. To evaluate sample carryover a 10 μg L^{-1} ZnPT spike was prepared in river water, diluted 10-fold and extracted. Possible sample-to-sample carryover was then evaluated by checking each component of the analytical system after elution and reconditioning, with no traces of ZnPT detected on either the clean-up and preconcentration columns, or the analytical column.

3.4. Speciation and transchelation

It was noted that when using APCI-MS detection that the ZnPT complex underwent rapid transchelation during the developed analytical procedure, such that often a more intense ion at *m/z* 316.0 was observed rather than the expected 317.0. From the observed spectra it was clear that transchelation from ZnPT, ($\log K = 5.3$, [30]) to CuPT ($\log K > 8.5$ [30]), complex was occurring within the chromatographic system. A similar effect was also observed by Doose et al. who noted that ZnPT injected using LC/ESI-MS was detected only as PT_2 and FePT [21]. In environmental samples it would be expected that ZnPT would also rapidly transchelate to its most stable metal complex, this being CuPT. To take this into account, forced transchelation to form CuPT through the addition of Cu(II) salts to the sample was carried out by Thomas [22] for the LC/APCI-MS analysis of ZnPT, and this approach was noted by Doose et. al. as a reasonable option for the chromatographic analysis of ZnPT [21]. Therefore, to ensure unavoidable but quantitative transchelation to CuPT, it was deemed necessary to add a small excess of Cu(II) ions to all solutions after dilution prior to extraction. It was found that a small excess (∼20-fold) of copper(II) acetate to the sample solution allowed for the quantitative transchelation of ZnPT–CuPT without affecting the APCI-MS response.

3.5. Method performance

The performance of the on-line SPE LC/APCI-MS method was evaluated using river water as a real sample matrix. Before usage, the river water was filtered to remove suspended material. Spike solutions were prepared in the filtered river water, diluted 10-fold with reagent water, with the addition of 20 μg L^{-1} Cu(II) and pH adjusted prior to extraction. Method performance data is presented in Table 1. The limits of detection and quantitation were calculated as three and ten times the standard deviation of the baseline noise, respectively for blank extractions of 200 mL aliquots of diluted river water. The signal-to-noise ratio was calculated by peak height comparison with a 1 μg L^{-1} ZnPT spike (as CuPT) also prepared in river water. The calculated LOD also compares well with that obtained by Thomas [22] who achieved a LOD of 20 ng L^{-1} ZnPT, however, the sample volume extracted was 2 L as opposed to the 200 mL (after dilution) extracted using the on-line SPE LC/APCI-MS method. The calculated levels of precision and recovery also compare well with the method of Thomas where overall method precision and recovery were 17% and 77%, respectively [22], whereas using the on-line SPE LC/APCI-MS method precision and recovery

Table 1
On-line SPE LC/APCI-MS method performance data

Parameter	Result
Limit of detection (ng L^{-1})	18
Limit of quantitation (ng L^{-1})	62
Method precision (%RSD, $n = 10$)	27
Linearity (R^2)	0.9802
Recovery (%, $n = 3$)	72 ± 9

were found to be 27% and 72%, respectively. Given the complexity of the sample matrix and the trace concentration of the complex these values were deemed satisfactory. Linearity was determined in the region of 0.25–10 μg L^{-1} prior to dilution and the calculated regression coefficient was $R^2 = 0.9802$.

3.6. Sample validation

Two brands of anti-dandruff shampoo known to contain ZnPT as the active ingredient were diluted in river water matrix and analysed using the developed method. Dilutions of the shampoo samples of 1000-fold (Head and Shoulders) and 500-fold (Dove) were first prepared, with an aliquot of each further diluted 10^4-fold in the river water. The spiked river water samples were finally diluted 1/10 with reagent water prior to analysis. As was previous 20 μg L^{-1} Cu(II) had been added to each sample in order to ensure transchelation to CuPT. Resulting chromatograms for the on-line SPE LC-APCI-MS analysis of the shampoo samples in river water can be seen overlaid in Fig. 6(b). The larger peak corresponds to the sample spiked with Dove anti-dandruff shampoo which contained 0.37% (w/w) ZnPT, therefore, the concentration of ZnTP in the diluted sample was equal to ∼76 ng L^{-1}. The smaller peak corresponds to the sample containing Head and Shoulders Classic Clean shampoo, which contained 0.80% (w/w) ZnPT, therefore, a diluted concentration equal to ∼80 ng L^{-1} [8]. The similarity in peak areas found was highly encouraging, given the 10^8-fold dilution of the original samples, the ultra-trace concentration and the sample matrix involved.

3.7. Environmental fate of ZnPT and CuPT

Utilising the developed LC–UV and LC-APCI-MS methods an investigation into the environmental fate of the pyrithione complexes was performed. The effect of the concentration of common anions, sorption to suspended materials/sediment partitioning and photochemical attenuation were investigated.

Of the anions tested only sulphate had an immediate effect upon CuPT, with a reduction of the resulting CuPT peak area seen with increasing sulphate concentration. Nitrate, chloride and phosphate had no pronounced effect upon immediate contact. Contact time also appeared to have no effect upon the formed CuPT, with a general reduction in peak area seen with time in both control and anion containing solutions. Therefore, ZnPT as CuPT in the environment appears to be unaffected by the presence of most inorganic anions, even though some ions such as nitrates have been previously observed to act as photosensitisers to organic micropollutants no such effects were observed in this study [31].

Using two certified reference sediments obtained from the National Research Council of Canada, the adsorption of ZnPT onto the sediment phase was determined. The two sediment reference materials varied in their total organic carbon content (TOC) and also their metal content, of particular importance were the concentrations of copper and zinc, values for which are inserted in Table 2. Table 3 lists the determined concentrations in the sediment and aqueous phases after a 2 h mixing period. It can be seen that the measured concentrations of CuPT in each phase differ for the two sediment samples. The reason for the difference is attributable to the TOC content of the sediments with a higher proportion of ZnPT adsorbed and transformed on the PACS-1 sediment that contains larger quantities TOC and metals. Therefore, based upon the above findings it can be estimated that sediment aqueous distribution ratio for the BCSS-1 sediment is ∼4:1 and thus, the sediment is likely to preferentially concentrate the pyrithione metal complex from the water. Such an effect is illustrated more clearly with the PACS-1 sediment whereby all of the detected CuPT was found solely on the sediment phase. Upon conversion of the measured data to a percentage of the initial dose, it can be seen that approximately, 97% of ZnPT introduced into the experimental vial disappeared over the course of the experiment. Similar observations were also noted by Turley et al. [33] who found that ZnPT rapidly converted into 2-pyridine sulphonic acid when also performing sorption experiments as a result of light exposure. From such data it can be deduced that ZnPT and CuPT present in the environment are unlikely to accumulate on sediment due to the existence of a more important removal mechanism despite their high partitioning behaviour. However, the need therefore, still exists for further research, to determine the effect of pyrithione metal complexes on sediment biota and to also determine the actual fate of pyrithione metal complexes adsorbed onto sediment.

As mentioned previously, photochemical attenuation appears to be the most important removal mechanism of pyrithione metal complexes introduced into the aquatic environment. However, of the photochemical attenuation studies reported in the literature, the majority were performed using bioassays and primarily in a marine water matrix. As observed in this study, anti-dandruff shampoo appears to be a more significant source than marine antifouling paints regarding the introduction of ZnPT into the aquatic environment, especially into fresh water systems and

Table 2
TOC and metal content of the two certified sediment samples

Component	BCSS-1	PACS-1
C (%)	2.19 ± 0.09	3.69 ± 0.11
Zn (μg g^{-1})	119 ± 12	824 ± 22
Cu (μg g^{-1})	18.5 ± 2.7	452 ± 16
Sampling location	Baie des Chaleurs, Gulf of St. Lawrence	Esquimalt Harbour, British Colombia

Table 3
Determined concentrations of CuPT in the aqueous and sediment phases after a 2 h mixing period

	BCSS-1	PACS-1
Aqueous phase (mg L^{-1})	0.11	0.00
Sediment phase (mg kg^{-1})	0.43	0.86
Sediment:aqueous phase ratio	3.91	
Amount of residue in aqueous phase (μg/vial)	0.55	0.00
Amount of residue in sediment phase (μg/vial)	0.22	0.43
% Initial dose in aqueous phase	2.20	0.00
% Initial dose in sediment phase	0.88	1.72

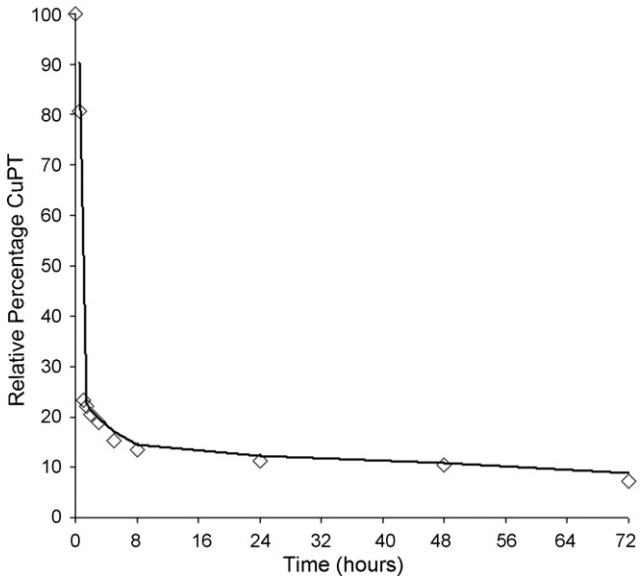

Fig. 7. Degradation of CuPT in filtered river water upon exposure to natural sunlight under laboratory conditions.

ZnPT introduced into the environment will transchelate to form CuPT. Therefore, a brief study was performed to examine the photochemical attenuation of CuPT in filtered river water. The relative presence of CuPT was plotted against time as in Fig. 7. The resulting plot correlates well with previously published data [10,22,32,33] with a relatively rapid decline of CuPT observed, the estimated half life from the graph is ∼45 min.

4. Conclusions

An on-line sample clean-up and preconcentration procedure coupled with LC-APCI-MS detection has been developed for the analysis of ZnPT (as CuPT) in aquatic environmental samples. The method was validated in a real sample matrix and showed high sensitivity with acceptable analyte recovery and reproducibility. Based upon results of a brief environmental fate study it appears that photochemical attenuation appears to be the most important removal mechanism of CuPT, however, sediment adsorption has also been shown to occur, particularly in sediments with a high proportion of TOC.

Acknowledgement

This work is funded by the Irish Research Council for Science, Engineering and Technology under the Embark Initiative.

References

[1] Activities of the European Union, Maritime safety: prohibition of organotin compounds on ships, viewed February 13th 2006. http://europa.eu.int/scadplus/leg/en/lvb/124256.htm.
[2] S.M. Evans, A.C. Birchenough, M.S. Brancato, Mar. Pollut. Bull. 40 (2000) 204.
[3] F. Sanchez-Bayo, K. Goka, Aquat. Toxicol. 74 (2005) 285.
[4] I.K. Konstantinou, T.A. Albanis, Environ. Int. 30 (2004) 235.
[5] N. Kobayashi, H. Okamura, Mar. Pollut. Bull. 44 (2002) 748.
[6] J. Bellas, A. Granmo, R. Beiras, Mar. Pollut. Bull. 50 (2005) 1382.
[7] K. Goka, Environ. Res. 81 (1999) 81.
[8] The Swedish Society for Nature Conservation, The Investigation of Zinc Pyrithione in Dandruff Shampoo (2004), viewed March 30th 2006. http://www.snf.se/pdf/rap-hmv-dandruffeng.pdf.
[9] N. Voulvoulis, M.D. Scrimshaw, J.N. Lester, Appl. Organomet. Chem. 13 (1999) 135.
[10] P.A. Turley, R.J. Fenn, C.J. Ritter, Biofouling 15 (2000) 175.
[11] R.M. Galvin, J.M. Rodiguez Mellado, M. Ruiz Montoya, Eur. Water Man. 1 (1998) 61.
[12] F. Sanchez-Bayo, K. Goka, Aquat. Toxicol. 74 (2005) 285.
[13] A.F. Krivis, E.S. Gazda, G.R. Supp, M.A. Robinson, Anal. Chem. 35 (1963) 966.
[14] L.H. Wang, Electroanalysis 12 (2000) 227.
[15] D.S. Mackie, C.M.G. van den Berg, J.W. Readman, Anal. Chim. Acta 511 (2004) 47.
[16] M. Ruiz Montoya, R.M. Galvin, J.M. Rodriguez Mellado, Electroanalysis 10 (1998) 1030.
[17] Y. Shih, J.-M. Zen, A.S. Kumar, P.-Y. Chen, Talanta 62 (2004) 912.
[18] R.J. Fenn, M.T. Alexander, J. Liq. Chromatogr. 11 (1988) 3403.
[19] N. Voulvoulis, M.D. Scrimshaw, J.N. Lester, Chemosphere 38 (1999) 3503.
[20] K. Nakajima, T. Yasuda, I. Nakazawa, J. Chromatogr. 502 (1990) 379.
[21] C.A. Doose, M. Szaleniec, P. Behrend, A. Muller, B. Jastorff, J. Chromatogr. A 1052 (2004) 103.
[22] K.V. Thomas, J. Chromatogr. A 833 (1999) 105.
[23] The University of Sheffield Chemputer isotope calculator, http://winter.group.shef.ac.uk/chemputer, viewed April 29th 2006.
[24] Intersil Quality Control Manual Chromatographic tests, www.intersil.com/technical_data/inertness.pdf, viewed April 29th 2006.
[25] A.I. Elefterov, S.N. Nosal, P.N. Nesterenko, O.A. Shpigun, Analyst 110 (1994) 1329.
[26] J. Bones, P.N. Nesterenko, K.V. Thomas, B. Paull, Talanta, doi:10.1016/j.talanta.2006.02.026, in press.
[27] J.E. Renew, C.-H. Huang, J. Chromatogr. A 1042 (2004) 113.
[28] I. Ferrer, D. Barcelo, E.M. Thurman, Anal. Chem. 71 (1999) 1009.
[29] R.J.C.A. Steen, A.C. Hogenboom, P.E.G. Leonards, R.A.L. Peerboom, W.P. Confino, U.A.Th. Brinkman, J. Chromatogr. A 857 (1999) 157.
[30] P.J. Sun, Q. Fernando, H. Freiser, Anal. Chem. 36 (1964) 2485.
[31] R. Andreozzi, M. Raffaele, P. Nicklas, Chemosphere 50 (2003) 1319.
[32] K. Maraldo, I. Dahllof, Mar. Pollut. Bull. 48 (2004) 894.
[33] P.A. Turley, R.J. Fenn, J.C. Ritter, M.E. Callow, Biofouling 21 (2005) 31.