APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 12, 1–12 (1998)

Determination of Phenyltin Compounds by Reversed-phase Liquid Chromatography

C. Kalambaheti,[1]* P. Wilairat,[1] N. Sakulpaisith[1] and P. Visoottiviseth[2]
[1] Department of Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand
[2] Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

An analytical procedure for the determination of phenyltin compounds in environmental sample waters was studied. Chromatography of mono-, di- tri-phenyltin (MPT, DPT and TPT) was performed on a reversed-phase C_{18} column with the mobile phase comprising methanol/10^{-2} M H_3PO_4 (80:20 v/v) at pH 3 and UV detection at 214 nm. To enhance the sensitivity of the detection system, the post-column reaction between morin or 3-hydroxyflavone and phenyltin compounds was formed before fluorescence detection. Several parameters affecting the fluorescence intensity were studied systematically, including the optimum condition for the post-column reagent that was also compatible with the eluent. The parameters concerned in this study were the pH, the percentage of Triton X-100, the ratio of fluorigenic reagent to phenyltin compounds and the amount of methanol in the eluent. Detection limits before the preconcentration process were in the region of 1.5 ppb for TPT and 150–250 ppb for MPT and DPT, respectively. Utilizing solid-phase extraction on a C_{18} cartridge for sample clean-up as well as preconcentration successfully reduced the detection limit of TPT to the level of ng dm^{-3} and can be applied to seawater analysis. Recovery in the range 95.0–98.0% was obtained by developing the optimum elution profile in the preconcentration step.

Appl. Organometal. Chem. **11**, 1–12 (1998)

Keywords: phenyltin; analysis; liquid chromatography; reversed-phase; post-column reaction; morin; hydroxyflavone; preconcentration; sample clean-up

Received 8 March 1996; revised 9 January 1997; accepted 22 April 1997

INTRODUCTION

Triorganotin distribution in marine and freshwater environments has caused concern because of the detrimental effects of such compounds on many marine organisms. Organotin compounds have been widely used not only as plastic stabilizers or catalytic agents in industry but also as biocidal compounds, i.e. bactericides, fungicides and insecticides in agriculture and medicine as well as antifouling agents for ships. There have been restrictions on the uses of antifouling paints in order to control and to reduce the magnitude and extent of pollution by them. In the USA, a level of 10 ng dm^{-3} has been adopted as a quality standard water.[1] Very sensitive and accurate laboratory-based analytical methods are required for assessing and monitoring purposes.

Several approaches to organotin speciation have been suggested.[2, 3] One of the more promising approaches is to couple the separatory power of chromatography with selectivity and sensitivity detection. Gas and liquid chromatography are suitable techniques for the analysis of complexes of the interesting analytes. Although gas chromatography (GC) has been coupled to atomic absorption spectrometry (AAS) for organotin speciation, it is limited to volatile and thermally stable organometallic compounds. The use of high-performance liquid chromatography (HPLC) has considerably expanded the analysis

---

* Correspondence to: C. Kalambaheti, Department of Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

Contract grant sponsor: National Science Technology Development Agency

CCC 0268–2605/98/010001–12$17.50

of many chemical and physical species. Unfortunately, the usual HPLC detectors did not offer the required sensitivity. Many authors have used HPLC combined with specific detection techniques, such as graphite furnace AAS,[4–7] hydride formation and quartz AAS,[8] or even ICP–MS.[9] A drawback of these techniques is that they required complex interfacing of the HPLC effluent to the detector. Another approach is to use post-column detection coupled with an HPLC system. The most common reagents that yield fluorescent complexes with phenyltin compounds were oxine (8-hydroxyquinoline),[10] morin[11, 12] and 3-hydroxyflavone.[13] In this work, reversed-phase liquid chromatography was used for separation and was then coupled with a post-column reagent containing 3-hydroxyflavone in micellar media to enhance detectability. The selection of the mobile phase and the composition of the post-column reagent were performed systematically, based on a study of the parameters affecting the fluorescence intensity. The validation of this method has been proved by application to the analysis of seawater and pond waters in the environment. Preconcentration and sample clean-up by solid-phase extraction (SPE) was shown to be a very useful approach.

EXPERIMENTAL

Instrumentation

The HPLC equipment consisted of a Waters model 510 pump, a Rheodyne 7025 injector fitted with a 20 μl sample loop and a Jasco FP-210 spectrofluorimeter. The output was displayed on an *x–t* Venture chart recorder; alternatively, peak area measurement for quantification was monitored on a Waters Datamodule 745B. The two columns selected for the separation were Waters Nova-Pak C_{18} (4 μm particle size; 150 mm × 3.9 mm i.d.) and Waters Nova-Pak Phenyl (4 μm particle size; 150 mm × 3.9 mm i.d.). The post-column reagent was delivered by another high-pressure pump (Waters model 510 pump) to merge with the chromatographic effluent in a mixing T-joint before its introduction into the fluorimetric detector.

A Jasco Uvidec-650 double-beam spectrophotometer and a Jasco FP 770 spectrofluorimeter were employed for scanning the absorption spectrum andthe fluorescence spectrum.

Reagents and procedure

Stock solutions of each phenyltin compound (1000 ppm) were prepared by dissolving 50 mg of compound in methanol in a 50 cm^3 volumetric flask. The phenyltin compounds employed in this experiment were monophenyltin trichloride (98% purity), diphenyltin dichloride (96% purity) purchased from Aldrich, WI, USA, and triphenyltin chloride (97% purity) purchased from Fluka, Buchs, Switzerland. These solutions were stored at 4 °C in dark glass bottles, and appropriate solutions were freshly prepared by dilution with methanol.

Mobile phases
The mobile phase was prepared by measuring 400 cm^3 of methanol and pipetting 0.34 cm^3 of 85% H_3PO_4 (Carlo Erba, Italy) and making up the volume to 500 cm^3 with doubly deionized water from a Milli-Q Plus water purification system (Milli-Q, MA) to 18.2 MΩ cm resistivity. The eluent consisted of methanol/10^{-2} M phosphoric acid (80 : 20, v/v), pH 3.

Post-column reagent
Morin solution was prepared freshly before use by dissolving 0.00169 g of morin in methanol and diluting to 500 cm^3 with methanol in a volumetric flask to give a concentration of 1.0×10^{-5} M.

Hydroxyflavone solution (FlOH) for post-column reaction was prepared by dissolving 0.04765 g of 3-hydroxyflavone (99% purity; Aldrich, WI, USA) and 50 cm^3 of Triton X-100 (BDH Chemicals, Poole, UK), diluting to 500 cm^3 in a volumetric flask with succinate buffer, pH 5.5. A mixture with the final concentration of 10^{-4} M FlOH and 0.7% w/v Triton X-100 in 0.05 M succinate buffer, pH 5.5, was employed as a post-column reagent.

Preparation of water samples
The environmental water samples, both from a fish pond and seawater, were prepared to perform recovery experiments by spiking with 50 and 100 μl of 10 ppm standard solutions of TPT to obtain the final concentrations of 50 and 100 ppb, respectively. To investigate the effect of suspended particles in water, two kinds of samples were prepared. They were in filtered and non-filtered water. The former was prepared by filtering samples through a 0.45 μm membrane before addition of the standard. The latter was obtained by addition of standard before filtering

the sample through a 0.45 μm membrane.

For preconcentration and sample clean-up, the water samples were passed through a C_{18}-bonded phase cartridge (Chromabond Macherey–Nagel, Germany) which had been conditioned with 5 cm^3 of methanol followed by doubly deionized water. Low levels of TPT standard solution and spiked TPT water samples were passed through the C_{18} cartridge with the help of a vacuum manifold (Alltech, USA) combined with a vacuum/pressure station (Bernant, IL, USA). TPT (25 cm^3, 8 ppb) was passed through the pre-column and then eluted with 2 cm^3 of the solution that had the same composition of mobile phase to obtain the final concentration of 100 ppb. Before eluting, the pre-column should be washed with 10 cm^3 of water to eliminate the interfering ions.

To decrease the detection limit of the technique, increased volumes of the samples in the range 25–1000 cm^3 were passed through the concentrator column. The amount of TPT was calculated and was used as an original concentration of organotin before loading. The organotin trapped on the column was eluted with 2 cm^3 of methanol and was then introduced to the HPLC. The signal obtained was then compared with the expected standard solution that would leave the concentrator column. This practice was performed to derive the percentage recovery.

RESULTS

Optimization of liquid-chromatography conditions

In the beginning, it was attempted in this work to use a universal liquid-chromatographic system which consisted of a reversed-phase column and UV detection to study the possibility of the separation of MPT, DPT and TPT compounds simultaneously, without perturbing the sensitivity of detection. Usually the mobile phase employed with the reversed-phase column consisted of organic modifiers such as methanol and acetonotrile. For this work it was also necessary to add phosphoric acid to adjust the charge on the phenyltin species, which could affect the retention of phenyltin compounds in the chromatographic process. The charges on the phenyltin compounds were changed when varying the pH of the mobile phase, as a consequence giving a different interaction in the C_{18} column. Figure 1(a) and (b) shows the retention behaviour of TPT and DPT obtained when varying the phosphoric acid and methanol concentrations. The retention of MPT was absent in these figures due to its coelution with DPT.

Chromatograms of phenyltin species obtained with optimum conditions are shown in Figs 2 and 3. The mobile phase was 80% (v/v) MeOH in 10^{-2} M H_3PO_4. Separation of TPT and DPT can be achieved successfully. It was shown that tailed-shape peaks of mono- and di-phenyl compounds occurred, which made them overlap with the triphenyl compound. The retention times of MPT and DPT were 2.85 min; they came out before TPT (R_T = 3.70 min). By employing a Phenyl Nova-Pak column whose functional groups have similar structures to phenyltin compounds, the peak shapes were

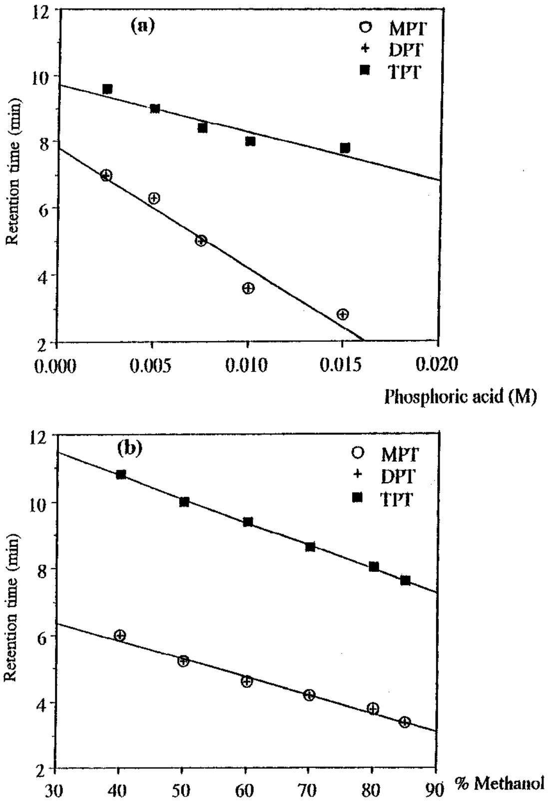

Figure 1 Effect of the modifiers in the mobile phase on the retention times of DPT and TPT. (a) With variation of phosphoric acid, and constant methanol concentration (80% v/v). (b) With variation of MeOH, and constant phosphoric acid concentration (0.01 M). Other chromatographic conditions: phenyl Nova-Pak column, injection volume 20 μl, UV detection at 214 nm, flow rate 1 cm^3 min^{-1}.

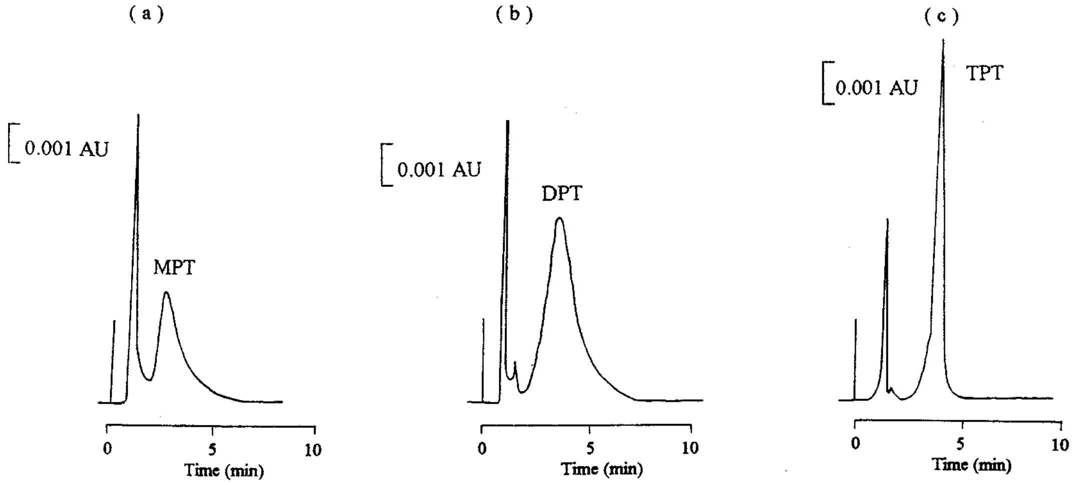

Figure 2 Chromatograms of standard phenyltin compounds: (a) 50 ppm MPT; (b) 50 ppm DPT; (c) 10 ppm TPT, obtained with injection of 20 μl of standard solutions on a Nova-Pak C_{18} column using methanol/10^{-2} M H_3PO_4 (80 : 20 v/v) as mobile phase, flow rate 1 cm^3 min^{-1}, UV detection at 214 nm.

improved and the selectivity was altered. Mono- and di-phenyls were eluted before triphenyls and the resolution was better due to more symmetrical peaks being obtained, as shown in Fig. 3. Nevertheless, the detection limits of these three compounds were too high to use for environmental sample analysis. They were 400, 100 and 40 ppb of MPT, DPT and TPT, respectively. Therefore, it was necessary to couple this technique with fluorescence detection in order to gain better sensitivity.

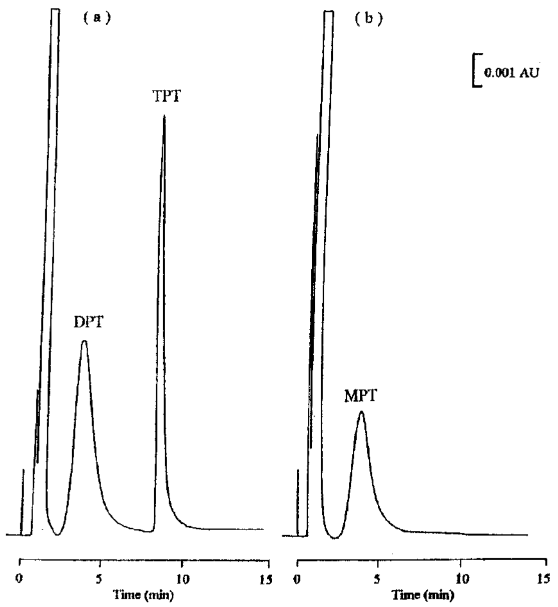

Figure 3 Chromatograms of standard phenyltin compounds: (a) mixtures of DPT and TPT at 20 ppm and 5 ppm, respectively; (b) 20 ppm MPT. Chromatographic conditions as in Fig. 2, except a phenyl Nova-Pak column was used.

Fluorescence spectral characterization of phenyltin complexes

Morin (2',3',4',5,7–pentahydroxyflavone) and 3-hydroxyflavone are useful fluorescence reagents for organotin analysis, especially for measuring the intensity in spectrofluorimetry. Fluorescence spectra characterization of phenyltin complexes forming with both fluorescent reagent, was studied in different media. Two kinds of media were selected according to their effects on the fluorescence intensity; they were micellar media and organic solvents. The excitation and emission spectra of each phenyltin are shown in Fig. 4(a–d). Figure 4(a, b) shows the hydroxyflavone complex of each phenyltin species (TPT, DPT and MPT) as well as a blank. Figure 4(c, d) also shows the fluorescence intensity of those species complexing with morin in the different media mentioned previously. The fluorescence intensities of the complexes in both media have been observed and compared. It is shown that for TPT a suitable post-column reagent was 3-hydroxyflavone in micellar media, whereas the complexes of morin in methanol solution were preferable reagents for determination of DPT and MPT.

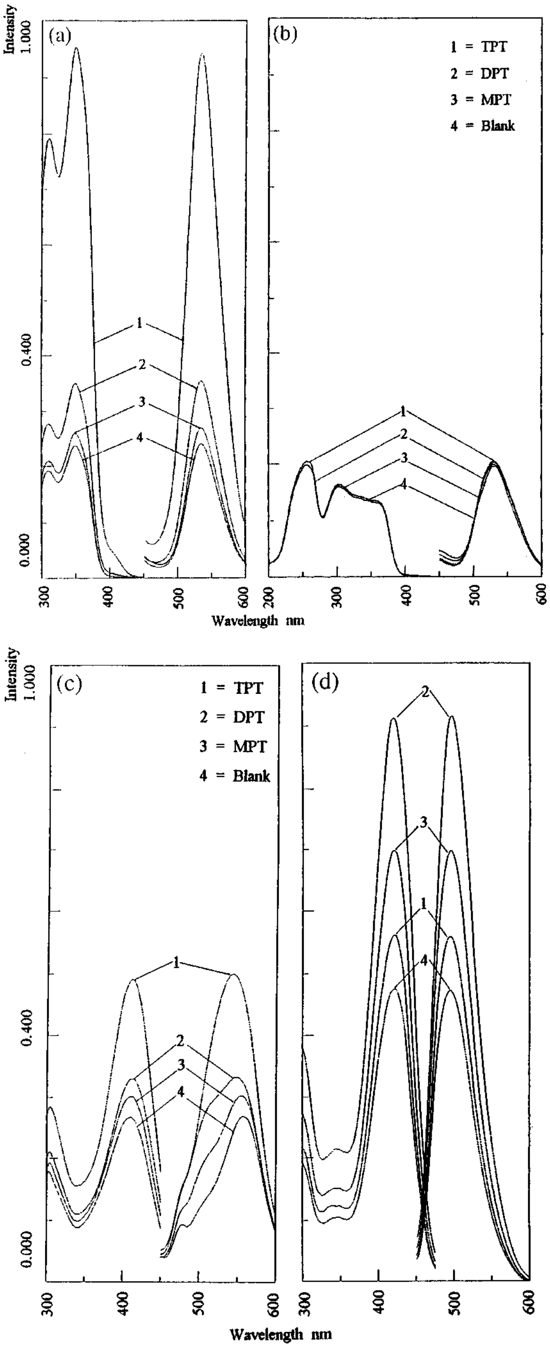

Figure 4 Excitation and emission spectra of each phenyltin species at 10^{-6} M (1 = TPT, 2 = DPT, 3 = MPT, 4 = Blank). (a) Hydroxyflavone complex in micellar medium. (b) Hydroxyflavone complex in organic solvent. (c) Morin complex in micellar medium. (d) Morin complex in organic solvent. The compositions of the solutions for measurement were prepared in similar ratios of mobile phase and post-column reagents, in chromatographic conditions as described in the Experimental section.

As described in the Experimental section, the volume ratio of phenyltin compounds and fluorescence reagent ws kept at 1 : 2.5, similar to the composition used in the post-column chromatographic process. Various species of phenyltin–FlOH complexes were formed in both organic solvent and micellar media. Methanol was chosen as an organic solvent medium due to it being commonly used in HPLC systems. Triton X-100, which was varied in the concentration range from 0.6 to 0.7%, was also selected for experimental use. It was found that the different reagents and different phenyltin compounds needed specific media to enhance their fluorescent intensities. For example, TPT gave the highest intensity in micellar solution at the same concentration level. On the contrary, if using an organic solvent (i.e. methanol) for the preparation of phenyltin complexes, TPT gave the lowest fluorescence intensity among all the phenyltin species, as shown in Fig. 4(a, b). According to these results, it is indicated that the organic solvent could not give enhanced intensity for fluorescence detection. Therefore, micellar medium is a preferable solution in post-column reaction with 3-hydroxyflavone.

Morin is another fluorescence reagent that has quite different properties. Figure 4(c, d) shows the excitation and emission spectra of phenyltin–morin complexes in methanol and Triton X-100. The ranking of fluorescence intensities of phenyltin species was slightly different from that of flavone complexes. Diphenyltin species prepared in micellar media gave higher intensities than TPT whereas the intensity ranking of morin–phenyltin complexes in methanol was similar to that of hydroxyflavone complexes, except that morin complexes gave lower intensities. Therefore, the preferred system for continued studies was the formation of phenyltin–flavone complexes in micellar media, especially for the triphenyltin compound, which is the pollutant that predominates in the environment. The fluorescence spectra being scanned on a spectrofluorimeter had been examined and the optimum excitation and emission wavelengths were selected to be 385 and 500 nm, respectively. However, too high a concentration of organic solvent can lower the sensitivity of the detection. In such a system, the characteristics of a fluorescence reagent such as its concentration and pH, and the percentage of Triton X-100, should be optimized, to achieve an effective post-column reaction in HPLC analysis.

Effect of composition of fluorescence reagent

As mentioned earlier, the aim of the study was to find the most appropriate composition of post-column reagent to produce the highest fluorescence intensity. The parameters concerned with this subject are pH, concentration of Triton X-100 and also the concentration of hydroxyflavone. To study this, the conditions of the variables were varied in solutions that had similar ratios of the solutions in the post-column reaction, and then measured for comparison of fluorescence intensity.

The effect of pH on fluorescence intensities of the post-column reagent is shown in Fig. 5. Triton X-100 was used as a surfactant at 0.7% and [FlOH] was 10^{-4} M. In pH range 2–9, the fluorescence intensity increased with increasing pH and reached a maximum at pH 4–9. A similar trend was also observed when varying Triton X-100 concentrations from 0.01 to 1.0% (w/v), as shown in Fig. 6. The last important parameter is the concentration of FlOH which is necessary in excess to form the complexes completely. The experiment was performed by varying FlOH from 1×10^{-4} to 3.4×10^{-4} M to obtain various mole ratios between *ca* 3 and 9, and measuring their intensities. No differences were observed in such solutions; therefore 1×10^{-4} M was selected for the analysis.

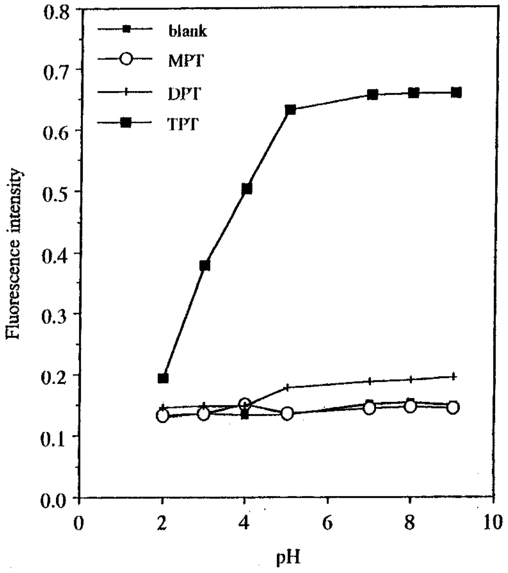

Figure 5 Relationship between pH of post-column reagent and fluorescence intensity of phenyltin–FlOH complexes in micellar media obtained when varying pH in the range 2–9. [FlOH] = 10^{-4} M; [Triton X-100] = 0.7%; [phenyltin] = 10 ppm.

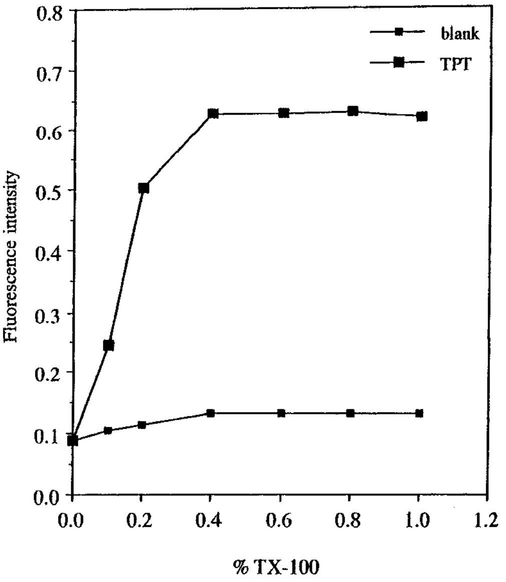

Figure 6 Relationship between concentration of Triton X-100 used as micellar medium for the post-column reagent and the fluorescence intensity obtained when varying the percentage of Triton X-100 from 0.1 to 1%. [TPT] = 10 ppm.

Another important characteristic of fluorescent reagent that has an effect on the intensity is the composition of the organic solvent. As mentioned previously, the preparation of phenyltin–complexes in methanol gave a lower sensitivity of detection. In our cases the mobile phase employed in the experiment was 80% (v/v) MeOH in 0.01 M H_3PO_4, pumped constantly at a flow rate of 1.0 cm^3 min^{-1} while the second pump could deliver the reagent at a variable flow rate. If the amount of methanol in the mixture was properly adjusted, it was possible to obtain high sensitivity. Therefore the flow rate of the post-column reagent was varied in the range

Table 1 Ratios between eluent and FlOH in micellar medium corresponding to concentrations of MeOH in final solution

Eluent (cm^3)	FlOH (cm^3)	MeOH (% v/v)
1.00	1.00	40
1.00	1.50	32
1.00	2.00	27
1.00	2.50	23
1.00	3.00	20

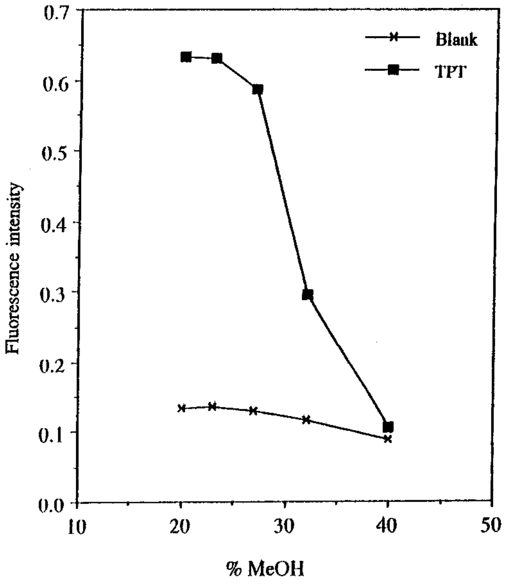

Figure 7 Relationship between amount of methanol in post-column reaction and fluorescence intensity.

1–3 cm^3 min^{-1}. The experiment was conducted at various flow rates, as shown in Table 1, which led to variable amounts of MeOH being transferred to the reaction. The fluorescence intensities were compared and it was found that the higher the amount of MeOH delivered, the lower was the signal obtained, as in Fig. 7. The optimum range should be in the range 20–27% MeOH, which corresponded to a flow rate of around 2.5 cm^3 min^{-1}. In this last stage, the optimum composition and conditions used for post-column reaction consisted of 10^{-4} M FlOH in 0.7% Triton X-100 at pH 5.5 and the flow rate delivered by second pump was 2.5 cm^3 min^{-1}.

The results from the above study gave the optimum conditions for the separation and detection of phenyltin compounds as shown in Fig. 8. It was noticed that unlike the chromatogram of phenyltin obtained by using UV detection, no solvent peak in front of the chromatogram was observed with fluorescence detection. As different phenyltin species require different fluorescence reagents, two systems of post-column reagent were selected for the analysis. For triphenyltin compounds FlOH in micellar media was used, and for mono- and di-phenyltin a morin solution was selected. The chromatographic conditions and the analytical results are shown in Tables 2 and 3.

Preliminary studies on interfering effects in real samples

To validate the proposed method, this approach was applied to real samples. Two kinds of samples that had different matrices were selected to perform the analysis: water from a fish pond

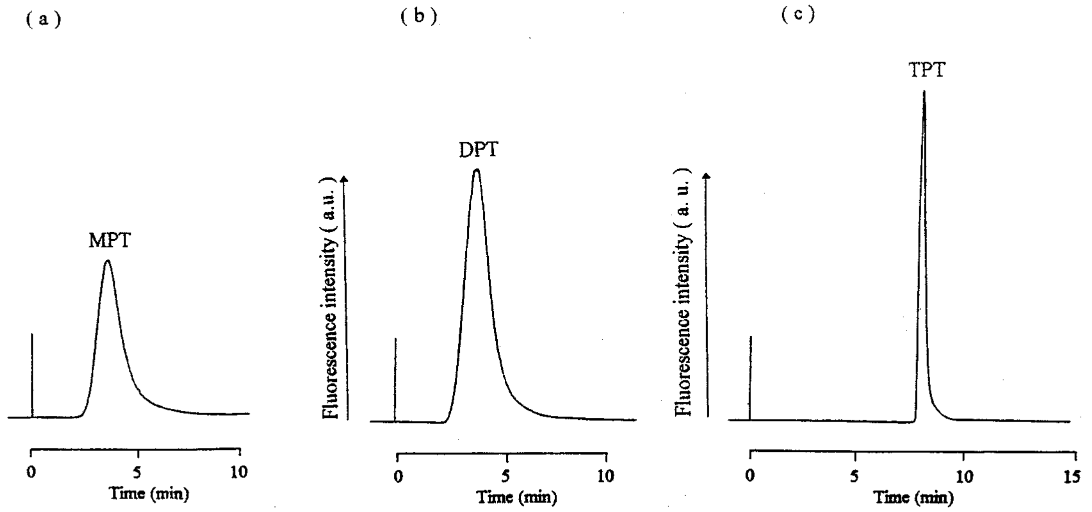

Figure 8 Chromatograms of standard (a) MPT and (b) DPT (5 ppm each) and (c) 0.5 ppm TPT obtained on reversed-phase phenyl Nova-Pak column with fluorescence detection. Mobile phase: 20:80 (v/v) methanol/10^{-3} M H_3PO_4 at flow rate 1.0 cm^3 min^{-1}; injection volume 20 μl; post-column reagent 10^{-4} M morin solution in methanol for MPT and DPT, and 10^{-4} M FlOHin 0.7% Triton X-100 prepared in succinate buffer, pH 5.5 for TPT, delivered at a flow rate of 2.5 cm^3 min^{-1}. Fluorimetric detection: excitation wavelength 500 nm and emission wavelength 385 nm.

Table 2 Analytical results for TPT obtained when using FlOH in micellar media as a post-column reagent

Detection limit (ppm)[a]	TPT concentration (ppb)	Peak height ± S.D. (cm)[b]
1.5	50	1.26 ± 0.10
	100	2.28 ± 0.08
	200	4.28 ± 0.14
	400	8.20 ± 0.13

[a] *S/N* ratio = 3. [b] Average of three runs; S.D. = standard deviation ($n=3$).

Regression line: $y=0.01982x+0.2922$. Correlation coefficient: $r=1.0000$. Slope = 0.0198. Intercept = 0.2922.

and seawater. These kinds of samples, which were surface water, had various loads of suspended matter which might affect the extraction efficiency of triorganotin due to strong sorption to particulate matter. The results obtained from another author[14] had shown that non-filtered water (obtained when spiking the standard before filtering) gave slightly lower recoveries than filtered water (obtained when spiking the standard after filtering) at the same spiking level. Their work used n-hexane to extract the compounds of interest. Instead of using liquid–liquid extraction as mentioned here, the technique, namely solid-phase extraction, was performed by passing the sample through a pre-column. It was found that the recovery of either sample from spiked standards before or after filtering suspended matter was similar. However, it was noticed that the reproducibilities of the filtered sample were superior. Standard phenyltin compounds at the level of 50 and 100 ppb were spiked in such samples and the recoveries obtained by comparing with the standard in aqueous solution are shown in Table 4.

Table 3 Analytical results for MPT and DPT obtained when using morin in methanol as a post-column reagent

Phenyltin species	Detection limit (ppm)[a]	Concentration (ppm)	Peak height ± S.D. cm)[b]
MPT	0.25	1.25	0.90 ± 0.08
		2.50	1.80 ± 0.14
		5.00	3.30 ± 0.20
		10.00	7.80 ± 0.25
DPT	0.15	1.00	0.80 ± 0.07
		2.50	2.18 ± 0.11
		5.00	3.90 ± 0.18
		10.00	9.50 ± 0.10

[a] *S/N* ratio = 3. [b] Average of three runs; S.D. = standard deviation (n=3).

For MPT, regression line: $y=0.7889x+0.2478$. Correlation coefficient: $r=0.9962$. Slope = 0.7889. Intercept = + 0.2478.

For DPT, regression line: $y=0.9637x-0.3620$. Correlation coefficient: r=0.9952. Slope = 0.9637. Intercept = − 0.3621.

It was found that the experiment performed by direct injection without a clean-up step gave a low recovery in seawater and higher than 100% if performed in pond water. It was also found that there was an interfering peak that eluted before the triphenyltin peak. At this stage this peak had not yet been identified. It was assumed that this interfering species would have similar chromatographic behaviour to the compounds of interest and that it was therefore retained on this column. Alternatively, it might be some other organometallic compound such as organolead or organomercury. The retention time of TPT in the real samples also decreased dramatically as well as giving a distorted and wider peak shape in the chromatogram, as shown in Fig. 9. This leads to imprecise peak-area integration when performing the analysis.

At this stage the analytical results obtained from direct injection of the real samples did not give the desired results; even the R.S.D. values were unacceptable. Two kinds of interfering effects could be classified according to this work: firstly, interferences that gave higher recoveries which were found in pond water; and secondly interfering species (e.g. Cl^-, SO_4^{2-}) giving a competing effect on the active site of the column and making the solute bind loosely on the column, so that lower recoveries were obtained. Therefore, it was necessary to explore a suitable approach for the sample preparation step. The solid-phase extraction (SPE) technique was proposed to fulfil this requirement.

Optimization of preconcentration parameters

The preconcentration process involves selective binding between the analyte and the pre-column or concentrator column quantitatively, and an ability of elution of the solute without influence from interferents. Preconcentration steps consisted of the following: ‘conditioning’, ‘loading’, ‘washing’ and ‘elution’. Usually the process began by conditioning the pre-column with the appropriate eluent to provide the active surfaces on the column ready for binding with the analyte. The next step, ‘loading’, represented taking the

Table 4 Recoveries (%) obtained when spiking TPT in water from fish pond and in seawater

Sample	Recovery (%)[a]	R.S.D. (%)[b]	Recovery (%)[a]	R.S.D. (%)[b]
	Spiked at 50 ppb		Spiked at 100 ppb	
Fish pond water				
Spiked before filtration	111.25 ± 5.11	4.59	104.62 ± 1.19	1.14
Spiked after filtration	108.20 ± 0.69	0.64	102.36 ± 0.32	0.31
Seawater				
Spiked before filtration	82.62 ± 24.79	22.38	70.50 ± 8.37	8.86
Spiked after filtration	81.14 ± 3.58	3.29	68.75 ± 7.33	7.59

[a] Mean ± S.D. for triplicate determinations.

[b] Relative standard deviation.

sample solution onto the top of the column. For the ‘washing’ step a weaker-strength eluent or water was passed through the cartridge to remove any interferents. The last step, namely ‘elution’, was performed to strip the analyte from the pre-column for further analysis. Every step mentioned here was a critical factor that led to a successful preconcentration process; therefore the volume and the properties of the eluent need to be optimized.

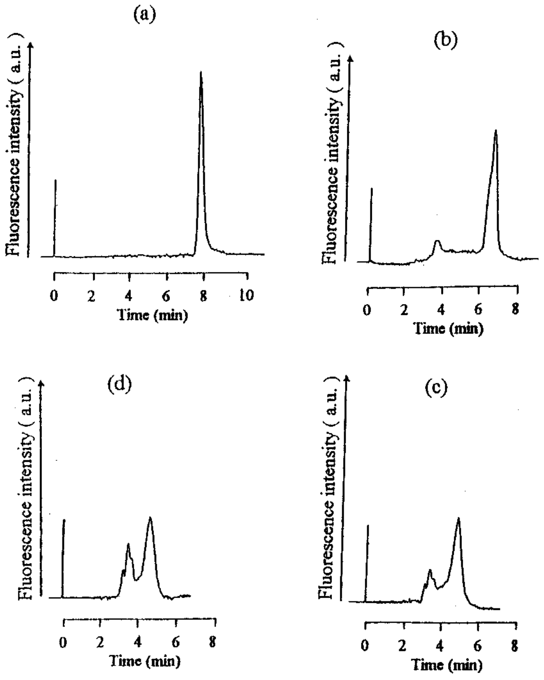

Figure 9 Chromatograms of standard 100 ppb TPT in different matrices obtained by direct injection: (a) deionized water; (b) filtered pond water; (c) aqueous solution of 35 g dm^{-3} NaCl; (d) seawater Chromatographic conditions were as in Fig. 8.

This work was intended to explore suitable eluents and to optimize the volume of eluent used in the ‘washing’ and ‘elution’ steps. For loading volume another author[15] proposed that with this kind of concentrator column (i.e. a C_{18} cartridge), the load sample volume can reach 100–1000 cm^3 without breakthrough or overloading. To optimize the sample clean-up process, a fixed sample volume of 25 cm^3 was employed for loading onto the pre-column. A standard solution of TPT at 4 ppb of phenyltin was loaded onto the C_{18} cartridge mounted on the vacuum manifold. After leaving to dry for 5 min, the selected solution was used for eluting TPT from the pre-column by varying the volume. The two kinds of eluent were methanol and 80% MeOH in 10^{-2} M H_3PO_4. (The latter eluent was used as the mobile phase in HPLC.) It was found that methanol was not suitable, due to its lower strength in elution, and unreproducible results were obtained, even on increasing the volume of methanol to 10 cm^3. Acceptable values can be obtained by employing the liquid-chromatographic eluent as shown in Table 5. This indicates that methanol did not have enough strength to elute TPT from the concentrator column quantitatively. The results in Table 5

Table 5 Influence of volume of mobile phase on TPT recovery, employing for elution the analyte from the concentrator column

Mobile phase (cm^3)	Recovery[a] (%)	R.S.D. (%)
1	97.57 ± 0.03	1.04
2	97.92 ± 0.05	1.41
5	93.34 ± 0.05	1.53
10	92.26 ± 0.06	1.86

[a] Mean ± S.D. for $n = 4$.

Table 6 Recovery of TPT in seawater obtained after addition of a washing step with various volumes of water for wasing the pre-column in the sample clean-up step

Volume of water (cm^3)	Recovery (%)[a]	R.S.D. (%)[a]
3	44.88 ± 0.61	1.35
7	89.89 ± 2.07	2.30
10	96.85 ± 1.96	2.02
15	98.96 ± 2.68	2.71

[a] Mean ± S.D. for n=4.

show that the recovery of TPT was nearly 100% when a 2 cm^3 elution volume was used. Therefore in the following experiment, 2 cm^3 of eluent will be used instead of pure methanol.

Modification of preconcentration step for sample clean-up

Environmental water samples chosen for analysis were spiked with small amounts of TPT and the preconcentration steps were peformed as described previously. Not only were low recoveries still obtained, but a shorter retention time of TPT was also observed, as in Fig. 19. A low detection limit for TPT (1.5 ppb) can be obtained without the need for preconcentration, but low recoveries could cause unreliable results. A sample clean-up step was proposed by modifying the preconcentration step by passing the spiked seawater and water from a fish pond through the pre-column. After drying the cartridge by drawing air through it by vacuum for 1 min, a washing step with various volumes of water was introduced, and the elution step with the eluent mixture still remained in the process. The purpose of the washing with water was to eliminate co-anions such as Cl^- in seawater from active sites in the pre-column. These interferents can make the TPT move faster, as resulted in the direct-injection method above. It was found that recoveries improved as well as the retention of TPT behaving normally, as obtained from standard solutions, after modifying the sample clean-up step. Table 6 shows that the optimum washing solution was in the range of 10–15 cm^3. Satisfactory analytical data and statistical values are shown in Table 7, which demonstrates a suitable analytical method for phenyltin compounds in fish pond water.

Although the modified sample preconcentration step was able to overcome the influence of interfering species and satisfactory results could be obtained, the detection limits of this method were still too high for real sample analysis. Organotin concentrations in the nanogram/litre region would be expected for seawater. To fulfil this requirement, an increased volume of sample loading should be used for the analysis. In Table 8 the sample volume was increased to 1000 cm^3 and the concentration of organotin before loading could be decreased to as little as 25 ng dm^{-3} or 0.025 ppb, which was low enough for the analysis. The high percentage recoveries in Table 8 indicated that triphenyltin species could be concentrated on the column without loss caused by breakthrough. The proposed method was applied to seawaters around the eastern coast of the Gulf of Thailand, as shown in Table 9. This table shows the concentrations of tributyltin (TBT) and triphenyltin (TPT) compounds. As this work used only the HPLC approach for TPT analysis, TBT was found by gas chromatography with flame photometric detection,[16] which is not described here in detail. The results show that TBT is commonly found in these areas, whereas

Table 7 Analytical data, statistics and recoveries of TPT from spiked water from a fish pond

Concentration (ppb)	Average peak (cm)[a] ± S.D.	Recovery (%)[b]	R.S.D. (%)
50.0	2.59 ± 0.06	95.48 ± 2.32	2.43
100.0	4.85 ± 0.06	90.66 ± 1.08	1.19
150.0	7.36 ± 0.08	96.20 ± 0.65	0.68
200.0	9.74 ± 0.14	95.34 ± 1.02	1.07

[a] Average of four runs (n = 4); S.D. = standard deviation.

[b] Mean ± S.D. for n=4.

Regression line: $y = 0.0479x + 0.1450$. Correlation coefficient: $r = 0.9998$. Slope = 4.792×10^{-2}. Intecept = 0.1450.

Table 8 Recovery of TPT in seawater obtained after addition of a washing step with various volumes of water for washing the pre-column in the sample clean-up step

Volume of water (cm^3)	Concentration of TPT (ppb)	Recovery[a] (%)	R.S.D. (%)
25	1	98.51 ± 0.89	3.19
100	0.25	95.11 ± 1.07	2.91
500	0.05	97.22 ± 1.51	2.25
1000	0.025	96.31 ± 2.01	4.51

[a] Mean ± S.D. for $n = 3$.

TPT, which is not commonly used, is seldom found in most areas.

DISCUSSION

Separations of phenyltin species were performed on a Nova-Pak C_{18} column using an eluent comprising 80 : 20 methanol/10^{-2} M H_3PO_4 at pH 3. Initially it was necessary to vary the concentration of phosphoric acid to optimize ionic suppression and consequent separation of phenyltin compounds. Unfortunately, mono- and di- species could not be separated using these conditions. Nevertheless, the prominent species in environmental samples were triphenyltin compounds, which are quite persistent. A mobile-phase pH higher than 2 is advisable in order to avoid the destruction of the links existing between the octadecylsilicylic groups and the silica surface leading to inactivation of the column. It was also found that when the C_{18} column was replaced with a phenyl column, it gave a better peak shape, although the lifetime of the column was not so long (*ca* 1000 injections). Different selectivities were also obtained when using a column that had octadodecyl sulphonate as a substrate. That TPT came out after the tailing peaks of MPT and DPT made the quantitation of TPT difficult in this situation. The chromatograms obtained from the phenyl column had a more symmetrical peak shape and therefore better quantitative measurements could be obtained. The latter chromatographic conditions were selected for separation and were coupled with fluorimetric detection.

To optimize the composition of the post-column reagent, a study of several parameters was performed to show the relationship between such factors and the fluorescence intensity of each phenyltin species. Morin and 3-hydroxyflavone were suitable with different phenyltin compounds in different media. Both fluorigenic reagents were used for complexing with organotin in two different kinds of media (i.e. organic solvents and micellar media). TPT gave higher fluorescence intensities when forming complexes with 3-hydroxyflavone in micellar media, whilst MPT and DPT formed effectively with morin in methanol solution. The post-column reagent for

Table 9 TBT and TPT levels analysed in seawater around the eastern coast of the Gulf of Thailand[a]

Volume of water (cm^3)	TBT ± S.D. (ppt)[b]	TPT ± S.D. (ppt)[b]
Mab Ta Put	62.24 ± 5.59	ND
Kao Loi	93.95 ± 14.90	ND
Samed	75.57 ± 10.14	ND
Laem Chabang	71.15 ± 2.22	44.98 ± 4.24
Had Mae Rum Poong	ND[c]	ND

[a] TBT was deterined by GC–FPD and TPT was analysed by the proposed method.
[b] S.D. = standard deviation for $n = 3$.
[c] ND = cannot be detected.

TPT was 10^{-4} M FlOH and 0.7% Triton X-100 prepared in succinate buffer, pH 5.5, delivered at $2.5\ \mathrm{cm^3\ min^{-1}}$. However, for the analysis of triphenyltin species, preconcentration and sample clean-up studies were also selected for optimization due to the stability of these species in the environment.

It was found that this technique was applied to real sample analysis, not only a low recovery (*ca* 40%) was obtained but a shifting of retention time from the standard and a distorted peak shape were found. This might be due to the effect of interfering anions in the real samples (e.g. Cl^- and SO_4^{2-} in seawater), acting as competing ions at the active sites on the column surface. These could prevent the phenyltin species from binding properly onto the surface of the column, leading to low recovery. To overcome this, a preconcentration process was used. Usually this comprised 'loading' and 'elution', steps. 'Loading' meant introduction of the water sample containing a trace level of analyte onto the pre-column, after the phenyltin species were trapped, a suitable eluent with minimum volume was chosen for the 'elution' step or the analyte was transferred for preconcentration analysis. A modification step was also necessary to give an efficient method for clean-up purposes, therefore 'washing' step was inserted before the 'elution' step. For optimization, wash volume was varied and recovery was then compared. It was found that 10–15 $\mathrm{cm^3}$ of water was enough for washing and recovery could reach 96–98%.

The proposed method including a preconcentration step on a C_{18} cartridge was selective and sensitive for various phenyltin species in environmental samples. To exploit the usefulness of this work, one could study the reactions of other organometallic compounds with different complexing agents to give fluorescence intensities, or even UV-visible absorption, with a view to developing further analytical methods for trace-level compounds.

Acknowledgements The authors are grateful to the National Science and Technology Development Agency for partial financial support of this work.

REFERENCES

1. A. M. Ure and C. M. Davidson, *Chemical Speciation in the Environment*, Blackie, Glasgow, 1995.
2. L. Ebdon, S. Hill and R. W. Ward, *Analyst (London)* **111**, 1113 (1986).
3. L. Ebdon, S. Hill and R. W. Ward, *Analyst (London)* **112**, 1 (1987).
4. K. L. Jewet and F. E. Brinckman, *J. Chromatogr. Sci.* **19**, 583 (1981).
5. F. E. Brinckman, W. R. Blair, K. L. Jewet and W. P. Iverson, *J. Chromatogr. Sci.* **15**, 493 (1977).
6. T. M. Vickrey, H. E. Howell, G. V. Harrison and G. J. Ramelow, *Anal. Chem.* **52**, 1743 (1980).
7. L. Ebdon and S. Hill, *Analyst (London)* **110**, 515 (1985).
8. D. Thorburn Burns, F. Glockling and M. Hariot, *Analyst (London)* **110**, 515 (1985).
9. U. T. Kumar, J. G. Dorsey and J. A. Caruso, *J. Chromatogr. Part A* **654**, 261 (1993).
10. W. G. Lakata, E. P. Lankmayr and K. Muller, *Fresenius Z. Anal. Chem.* **319**, 563 (1984).
11. W. Langseth, *Inorg. Chim. Acta* **87**, 47 (1984).
12. T. Yu and Y. Arakawa, *J. Chromatogr.* **315**, 351 (1984).
13. R. Compañó, M. Granados, C. Leal and M. D. Prat, *Anal. Chim. Acta* **302**, 185 (1994).
14. J. A. Stäb, J. M. Rozing, B. Van Hattum, P. Cofino and UA. TH. Brinckman, *J. Chromatogr.* **609**, 195 (1992).
15. R. Compano, M. Granados, C. Leal and M. D. Prat, *Anal. Chim. Acta* **283**, 272 (1993).
16. C. Kalambaheti and T. Tiensing, Abstract, 22th Congress on Science and Technology of Thailand, Bangkok, October, 1996. No. A72.