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Determination of butyltin and phenyltin species by reversed-phase liquid chromatography and fluorimetric detection

E. González-Toledo, R. Compañó, M. Granados, M.D. Prat*

Martí i Franquès 1-11, E-08028 Barcelona, Spain

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Abstract

Chromatographic separation of monobutyltin (MBT), monophenyltin (MPhT), dibutyltin (DBT), diphenyltin (DPhT), tributyltin (TBT) and triphenyltin (TPhT) was studied using end-capped reversed-phases (RP) and methanol–acetic acid–water or acetronitrile–acetic acid–water mixtures as mobile phases. Several RP columns were evaluated, and the effect of acetic acid, oxalic acid, triethylamine, and organic modifier on peak shape and retention was examined. A method based on gradient elution RPLC and fluorimetric detection is proposed for the determination of DBT, DPhT, TBT and TPhT. The sensitivity of the method makes it suitable for environmental analysis. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Butyltin; Phenyltin

1. Introduction

Butyl and phenyl derivatives of tin are widely used as PVC stabilisers, catalysts and biocides. As a result there is a variety of pathways for their entry into the environment and their presence has been reported in natural systems [1,2]. Owing to their toxicity, environmental regulations which restrict the use of some organotin compounds (OTs), have been enforced in several countries. During the last decade, several methods have been developed to separate and determine these species in environmental samples [3]. Gas chromatography (GC) in combination with selective and sensitive detectors, e.g. atomic spectrometers or mass spectrometers, is a powerful tool for OT analysis. Unfortunately, as most OTs are not volatile, it is necessary to introduce a time consuming and potentially problematic derivatization step. LC methods have also been proposed but they are not widely used [3,4]. Their main limitations arise from the lower resolution of the separation and from detection issues, such as the sensitivity or the compatibility with the chromatographic system. Inductively coupled plasma mass spectrometry (ICP-MS) [5–11] and fluorimetry [6,12–15], which provide the required sensitivity for environmental applications, are the most promising detection techniques. With regard to the separation, the most used LC mode is ion-exchange. Other alternatives have not been fully explored and require more research.

In previous work we developed an LC method for the determination of tributyltin (TBT) and triphenyltin (TPhT) based on ion-exchange chromatography and fluorimetric detection by post-column derivatization with a flavone derivative [13]. The method proved to be suitable for the analysis of water, sediments and marine biological materials

*Corresponding author. Fax: +34-93-402-1233.

E-mail address: prat@zeus.qui.ub.es (M.D. Prat)

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[13,16,17]. Further studies showed that it is possible to extend the post-column fluorimetric detection to mono- and di-OTs, provided that two derivatization solutions are used [14], since chemical conditions for fluorimetric detection of tri-OTs are rather different that those required for mono- and di-OTs [18]. However, separations of butyl- and phenyltin series based on ion-exchange chromatography present some difficulties, due to the strong interaction that mono- and di-OTs experience with the stationary phase. The use of complexing mobile phases [5,14,19,20], or pH-gradient elution [21] have been proposed to facilitate their elution.

Reversed-phase liquid chromatography (RPLC) is another approach to the analysis of OTs species. Most of the published methods using RPLC deal with tri- and tetra-OTs [4], but it has been demonstrated that RPLC can also be suitable for the separation of mono- and di-substituted species.

The aim of this study was to develop an RPLC method for butyl- and phenyltin species, compatible with sensitive fluorimetric detection, and thus suitable for environmental analysis, that could become a cheaper and more convenient alternative to the reported methods based on ICP-MS detection.

2. Experimental

2.1. Reagents

Triphenyltin chloride, diphenyltin chloride, monophenyltin chloride, tributyltin chloride, dibutyltin chloride and monobutyltin chloride were purchased from Fluka (Buchs, Switzerland) or Aldrich (Steinheim, Germany) (purity>95%) and were used without further purification. Morin (Merck, Darmstadt, Germany) and fisetin (Aldrich) were also used as received. Methanol HPLC grade (Merck) and ultrapure water MilliQ-plus (Millipore, Molheim, France) 18.2 MΩ cm^{-1} was used throughout. All other reagents were of analytical reagent grade.

Disposable solid-phase extraction cartridges (average particle diameter 40 μm) containing 100 mg of C_{18} bonded silica (Bond Elut; Varian; Harbor City, CA, USA) were used for seawater analysis.

Glassware used for experiments was previously soaked in 10% nitric acid for 24 h and rinsed with doubly-deionized water.

2.1.1. Stock solutions

Stock solutions (0.5 g l^{-1} Sn) of TBT, DBT, MBT, TPhT, DPhT, and MPhT were prepared by dissolving the corresponding chlorides in methanol and stored at 4°C in dark glass bottles. Working solutions (10–20 mg l^{-1} Sn) were prepared weekly by dilution of the stock solutions in methanol, and subsequent dilutions were freshly prepared.

2.1.2. Mobile phase

Mobile phases consisted of acetronitrile–acetic acid–water or methanol–acetic acid–water, containing oxalic acid and triethylamine (TEA). Each phase was separately filtered through a 0.22-μm nylon membrane filter (MSI, Westboro, MA, USA) and the mixture degassed for 10 min with a helium stream before use. See Section 2.3 for the composition of the mobile phases in the proposed method.

2.1.3. Post-column reagents

Solutions (10^{-2} *M*) of morin and fisetin in methanol were prepared freshly. The fluorimetric reagent for DBT, DPhT, MBT and MPhT detection consisted of a solution of 1.26 10^{-4} *M* morin, 2.0 10^{-2} *M* Triton X-100 and 0.17 *M* HCl. The post-column reagent for TBT and TPhT detection consisted of a solution of 1.25 10^{-5} *M* fisetin, 0.5% Brij-35 and 0.3 *M* succinic acid disodium salt.

2.2. Apparatus

The arrangement of LC separation and detection components used is depicted in Fig. 1.

The HPLC equipment consisted of a double piston pump (Model 525, Bio-Tek Kontron Instruments, Milan, Italy) and an injection valve (Gynkotek MSV 6) fitted with a 200-μl injection loop. The following 250×4.6 mm ID reversed-phase columns packed with 5 μm particles were used: Kromasil C_{18} (Phase Separations), Hypurity elite C_{18} (Hypersil), Hypersil Green Env (Hypersil) and Inertsil 5 C_8 (GL Sciences). All chromatographic separations were carried out at room temperature using 1.0 ml min^{-1} mobile phase flow-rate.

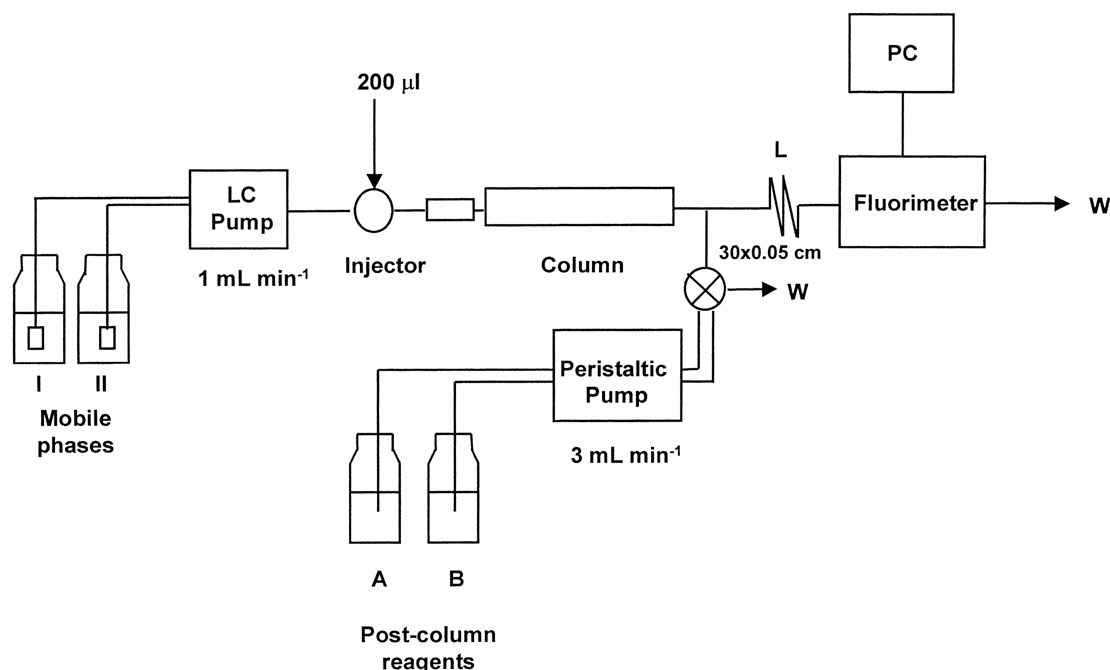

Fig. 1. Scheme of the LC–fluorimetry set-up.

A Minipuls 3 peristaltic pump (Gilson, Villiers-le-Bel, France) was used to deliver the post-column reagents, and a six-port valve, model 5041 (Rheodyne, Cotati, CA, USA), was used to select the appropriate reagent solution. PTFE tubing (0.5 mm I.D.) were used for mixing and reaction coils, together with Tygon tubes for reagents pumping.

An Aminco-Bowman Series 2 spectrofluorimeter (SLM Aminco, Rochester, NY, USA) equipped with a 25-µl flow-cell (Hellma, Mülheim, Germany) was used for detection. The spectrometer operated at 425 nm excitation and 496 nm emission (for mono- and di-OTs) and at 412 nm excitation and 496 nm emission (for tri-OTs).

A radiometer pHM64 equipped with a combined glass electrode (Orion, Boston, MA, USA) was used for pH measurements.

The flow injection manifold used to optimise the post-column derivatization system was the same as in Fig. 1 without the chromatographic set-up. A Rheodyne six-port injection valve with an injection loop of 130 µl was used in these experiments.

2.3. Procedures

2.3.1. LC–fluorimetric method

The separation was performed on a Kromasil-100 C_{18} column (250×4.6 mm I.D., 5 µm, Phase Separations), equipped with a Kromasil C_{18} guard column (10×4.6 mm), using a gradient elution. The mobile phase flow-rate was set at 1 ml min^{-1}. The initial mobile phase consisted of 30 mg l^{-1} oxalic acid and 0.03% (v/v) TEA in methanol–acetic acid–water (57.5:2.5:40). It was changed 2.0 min after injection to a mobile phase consisting of 0.03% TEA in methanol–acetic acid–water (84:1:15). The column effluent merged with post-column reagent (3 ml min^{-1}). The derivatization solution containing morin was delivered while mono- and di-OTs elute, and at 7.0 min the six-port valve was switched to pump the fisetin solution to the T connection, as shown in Fig. 1. Samples and standard solutions of OTs were filtered through a 0.45-µm membrane filter before injection.

2.3.2. Analysis of seawater samples

The disposable C_{18} cartridge was conditioned by rinsing with 5 ml of methanol, followed by 10 ml of water. Up to 0.5 l of sample was pumped through the cartridge (5 ml min^{-1}). After retention, the column was washed with 10 ml of water and dried by pumping air through the cartridge for about 2 min. Elution was performed with 2 ml of 30 mg l^{-1} oxalic acid and 0.03% (v/v) TEA in methanol–acetic acid–water (57.5:2.5:40) i.e. the initial mobile phase of the chromatographic method, at a flow-rate of 0.5 ml min^{-1}. The eluate was filtered and 200 μl were injected into the chromatographic system.

3. Results and discussion

3.1. Fluorimetric detection

The on-line fluorimetric reactions between OTs and flavone derivative reagents in Triton X-100 micellar medium have been described in a previous work [14]. The present study on the fluorimetric detection focused on the compatibility of the mobile phase of the LC system with the post-column derivatization and the most critical point was the adjustment of the experimental conditions for tri-OTs detection.

A flow injection system was used in these experiments. Standard solutions of OTs were injected into a carrier stream, which simulated the chromatographic mobile phase. The carrier solutions consisted of mixtures of water, acetic acid and methanol or acetonitrile, which have been reported as mobile phases for the separation of OTs by RPLC [10,11].

This study pointed out that, in terms of detection, methanol was superior to acetonitrile, since mobile phases containing acetonitrile led to higher baseline noise. This effect was more noticeable in the case of triorganotin species, for which the use of acetonitrile impaired the detection of TBT and TPhT at concentration levels below mg l^{-1}.

Another critical point was the acetic acid content of the mobile phase. Thus, whereas for the chromatographic separation of mono- and di-OTs it is necessary to use an acetic acid concentration higher than 2% (see Section 3.2), these conditions are not favourable for detection of TBT and TPhT, which require pH values above 5.5. This value may be achieved using a post-column reagent containing sodium succinate. However, the salt concentration required to neutralize high acetic acid levels may lead to precipitation when mobile phases rich in organic solvent merge with the post-column reagent. Therefore, the acetic acid content in the mobile phase should be kept low enough to prevent precipitation. Methanol was again a better organic modifier than acetonitrile, since it allowed a higher acetic acid concentration without subsequent precipitation in the derivatization system.

In global terms it was found that the coupling of the RPLC and the fluorimetric derivatization systems imposed some restrictions on the mobile phase composition, but had no disturbing effects on mono- or di-OT detection. On the other hand, it led to some loss of sensitivity in the case of tri-OTs. However, this adverse effect could be overcome by changing the surfactant agent. Brij-35 micellar medium provided better fluorescence signals than Triton X-100, for tri-OTs, and it was used in following studies (see Section 2.1.3 for final detection conditions)

3.2. Chromatographic separation

Initial conditions were taken from Ref. [11] and consisted of a C_{18} Kromasil 100 column and 0.05% TEA in acetonitrile–acetic acid–water (65:10:25) as the mobile phase. Separation of DBT, DPhT, TBT and TPhT was achieved, but mono-OT peaks were not resolved and there was some overlap between MBT and DPhT peaks. Moreover, the high content of acetic acid adversely affected the fluorimetric detection of tri-OTs, and consequently the sensitivity for these species was very poor. Methanol based mobile phases provided similar separations, but decreased retention of tri-OTs, especially of TBT. From the point of view of detection, methanol was more advantageous, as it led to more intense fluorescence signals.

Some experiments with C_{18} (Kromasil 100 and Hypurity elite) and C_8 (Hypersil G.E. and Inertsil) columns were performed using methanol–acetic acid–water mobile phases. Di- and tri-OT retention times were higher with C_{18} than with C_8 columns, but the most significant difference was the behaviour of mono-OTs: no peaks were detected with C_8

columns, even at high acetic acid percentage. Regardless of the column or mobile phase composition, the elution order was (mono-), di- and tri-OTs. Moreover, phenyl-OTs always eluted before than the corresponding butyl-OT species. The best performance was achieved with the Kromasil column and methanol-based mobile phases, and thus this system was used in further experiments.

The acetic acid content was varied between 2.5 and 10%, water between 15 and 25% and TEA between 0 and 0.07%. The water content in the mobile phase was a crucial parameter in the retention of the analytes. An increase in the water percentage led to an increase in the retention times of all compounds (Fig. 2a). Moreover, the water content determined the resolution between DPhT and DBT and between TPhT and TBT.

The acetic acid concentration had no effect on tri-OTs, but strongly affected the peak shape of di-OTs and both retention time and peak shape of mono-OTs. Thus, at acetic acid concentrations below 5% tailing of di-OT peaks occurred. In the case of mono-OTs, mobile phases containing 10% acetic acid eluted MBT and MPhT at the void volume, whereas as the acetic acid content decreased, peaks broadened and retention times increased, and at 2.5% acetic acid no peaks were detected. In contrast, TEA had no effect on mono- and di-OTs, whereas an increase in the percentage of TEA resulted in an increase on retention times of TBT and TPhT (Fig. 2b) as well as in an improvement on TBT peak shape.

The effect of oxalic acid was also studied, since it has been reported that it has a strong influence on the elution of OTs [10,14,19]. The results showed that TPhT and TBT retention times were not significantly affected, but there was a marked reduction of mono- and di-OT retention under the conditions tested (Fig. 2c). The role of oxalic acid was not elucidated, but it may be related to complexation reactions.

This screening study highlights that an isocratic separation of the butyl- and phenyltin species is not possible, as conditions that favour the chromatographic behaviour of some species are not compatible with elution and/or detection of other species.

Therefore it was decided to perform the separation under conditions such that mono-OTs eluted at the void time, whereas DPhT and DBT were completely

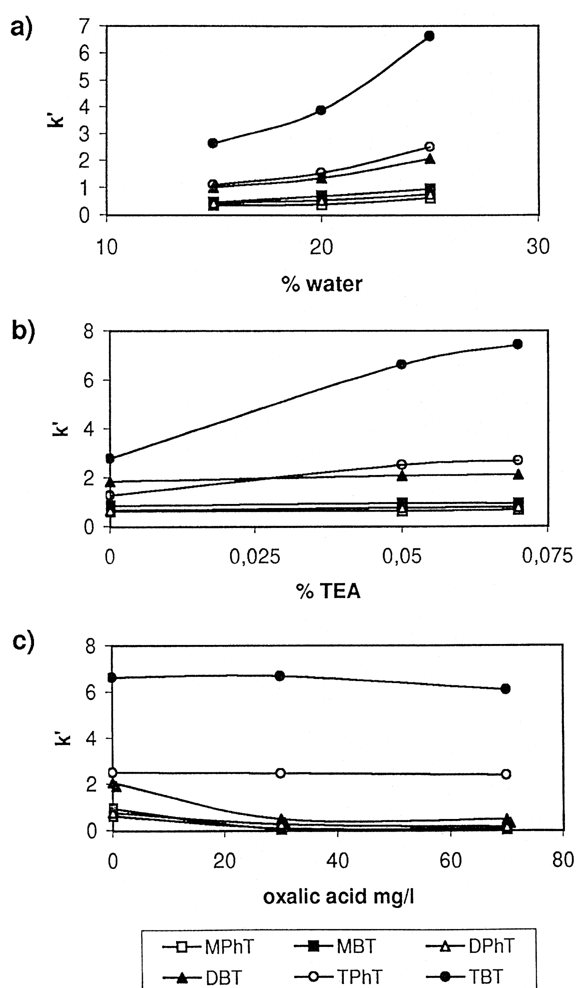

Fig. 2. Effect of mobile phase composition on OT retention times. Column: C_{18} Kromasil 100. Mobile phase system: methanol–acetic acid–water; flow-rate: 1 ml min^{-1}. (a) Effect of water content (b) effect of triethylamine concentration (c) effect of oxalic acid concentration.

resolved, thus allowing quantification of di- and tri-OTs without interference from mono-OTs. This was achieved with methanol–acetic acid–water (57.5:2.5:40) containing 30 mg l^{-1} oxalic acid and 0.03% TEA. With this mobile phase TPhT and TBT were strongly retained, due to the high aqueous content. In order to reduce analysis time, a gradient elution was applied, and 2.0 min after injection the initial phase was replaced by a phase consisting of 0.03% TEA in methanol–acetic acid–water (84:1:15). Under the selected conditions, a run took

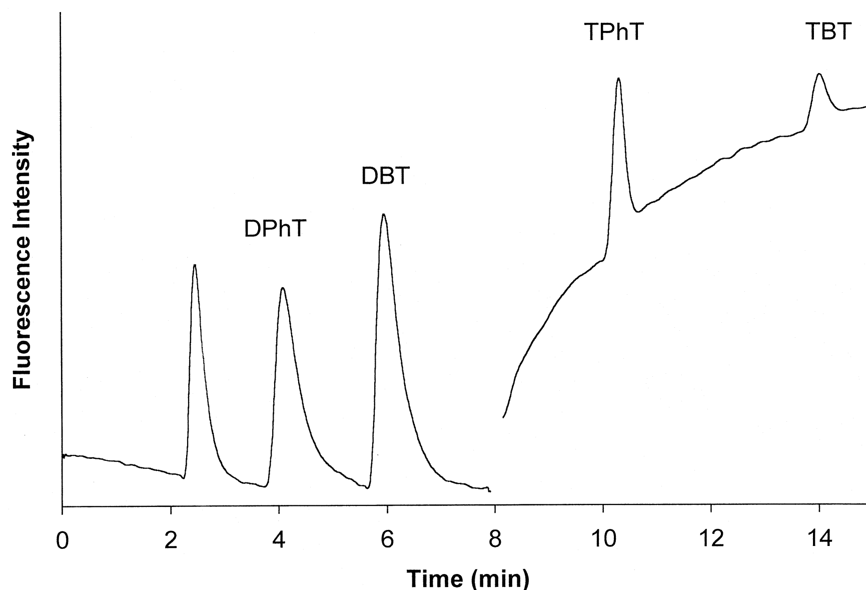

Fig. 3. Chromatogram of a standard mixture of OTs containing 9 μg l^{-1} DPhT, 20 μg l^{-1} DBT, 9 μg l^{-1} TPhT and 128 μg l^{-1} TBT.

less than 14 min (Fig. 3). The shift in the baseline is not due to the change of mobile phase composition but to the change of the derivatizing solution.

3.3. Analytical performance of the method

Figures of merit for DBT, DPhT, TBT and TPhT are summarized in Table 1. Calibration graphs were obtained for both the peak height and the peak area modes, and since no significant differences were found, the former was selected for quantification. Peak height reproducibility was determined from seven replicate measurements. The detection and quantification limits were determined at signal-to-noise ratios of 3 and 10, respectively. The mass detection limits, for a 200-μl injection volume, were in the range 20–100 pg, except for TBT which was 1 ng, all concentrations referring to tin.

The chromatographic method was applied to the analysis of di- and tri-OTs in seawater samples from marinas in Barcelona, on the north-west Mediterranean coast. Samples were collected in 2.5-l glass bottles, preserved by addition of HCl to pH 2, filtered through a 2-μm glass microfibre filter (Whatman, Maidstone, UK) and stored at 4°C. A previously described SPE method, which proved to be suitable for TBT and TPhT, was used (see Section 2.3). The SPE method allows the preconcentration of

Table 1
Figures of merit of the chromatographic method

	DPhT	DBT	TPhT	TBT
Dynamic range[a] (μg Sn l^{-1})	0.5–200[a]	2–200[a]	2–200[a]	10–800[a]
Peak height RSD (%) $n=7$	1.59	1.57	3.26	8.21
LOD[b] (μg Sn l^{-1})	0.1	0.5	0.5	5.0
LOQ[c] (μg Sn l^{-1})	0.5	1.9	2.0	11.0

[a] Higher concentration assayed.
[b] Determined as three times the standard deviation of the base line noise ($V_{inj}=200$ μl).
[c] Determined as ten times the standard deviation of the base line noise ($V_{inj}=200$ μl).

Table 2
OT recovery from spiked seawater samples by the SPE and LC–fluorimetry method

Volume of sample (ml)	DPhT		DBT		TPhT		TBT	
	Added[a]	Recovery (%)[b]	Added[a]	Recovery (%)[b]	Added[a]	Recovery (%)[b]	Added[a]	Recovery (%)[b]
500	46	68±4	99	91±4	47	89±9	641	80±12
250	92	76±4	197	94±6	93	100±6	1282	84±13

[a] Concentration values expressed as ng Sn l^{-1}.
[b] Mean value±standard deviation (n=4).

up to 0.5 l of sample, and the elution is performed with 2 ml of solution. Therefore a preconcentration factor of 250 can be attained. Recoveries obtained from spiked seawater samples are given in Table 2. Chromatograms of SPE extracts were very clean, with no interference from sample matrix. Fig. 4 shows a chromatogram from a 250-ml seawater sample containing 48 ng l^{-1} of DBT, and spiked with 5 ng l^{-1} of DPhT, 10 ng l^{-1} of DBT, 25 ng l^{-1} ng l^{-1} TPhT and 250 ng l^{-1} of TBT.

The combination of SPE and the chromatographic method is a powerful procedure for OT analysis, which allows the detection of low ng l^{-1} levels of the analytes, except in the case of TBT, for which a LOD of 20 ng l^{-1} is attained when 500 ml of sample is processed.

The proposed method has shown to be suitable for environmental monitoring of the most toxic OT species, and a good alternative to the more laborious GC methods or the expensive LC–ICP-MS methods.

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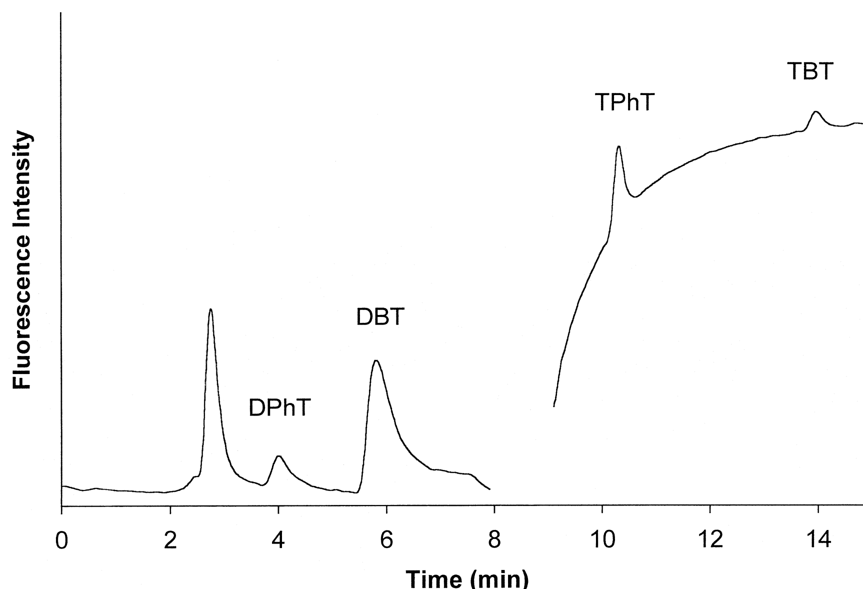

Fig. 4. Chromatogram of an SPE extract from a seawater sample spiked with 5 ng l^{-1} DPhT, 10 ng l^{-1} DBT, 25 ng l^{-1} TPhT and 250 ng l^{-1} TBT. Volume of sample: 250 ml; volume of extract: 2 ml.

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