Reversed-phase ion-pair chromatography with inductively coupled plasma–mass spectrometry detection for the determination of organo-tin compounds in waters

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Abstract

A method for the determination of tributyltin (TBT) and triphenyltin (TPhT) in waters has been developed using high-performance liquid chromatography–inductively coupled plasma–mass spectrometry. After evaluation of different column packing materials and mobile phases, a system using a 25 cm×4.6 mm I.D. column packed with Kromasil-100 5C$_{18}$ and a mobile phase of 82% (v/v) methanol, 2.5% (v/v) acetic acid, 0.3% (v/v) tetraethylamine and 10 mg l^{-1} oxalic acid was developed. After preconcentration on a Sep-Pak C$_{18}$ mini-column a detection limit of 2 ng l^{-1} for TBTCl was obtainable. It was found that ion-pairing agents were not necessary for the separation of the tri-substituted compounds. The reported method ensures the separation of the tri-substituted tins from the mono- and di-substituted forms.

1. Introduction

Over the last decade what is known as speciation analysis has become one of the major growth disciplines in analytical chemistry [1]. This derives from the realization that to accurately assess the environmental impact or toxicity of an element information had to be obtained about its chemical form. In many cases total elemental determinations are inadequate and indeed legislation is fast being implemented citing controls and levels for specific species. One analytical determination at the forefront of this changing awareness has been the determination of organo-tin compounds in environmental

matrices. Several of these compounds have become prevalent in the environment due to their use in antifouling paints, e.g. tributyltin (TBT) [2], and as pesticides, e.g., triphenyltin (TPhT) [3]. These tri-substituted compounds are highly toxic to marine organisms and have very different toxicological impacts on the environment compared to the mono- and di-substituted members of these families [4]. Therefore, there is a need for accurate and robust methodologies for the separation and subsequent determination of these molecules.

Traditionally, the two main separation techniques have been gas chromatography (GC) and high-performance liquid chromatography (HPLC). For GC the end detector has typically been flame photometry [5] or atomic absorption spectrometry (AAS) [6]. In

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the case of HPLC the choice of end detector has included hydride generation-AAS (HG–AAS) [7], inductively coupled plasma–optical emisson spectrometry (ICP–OES) [8] and inductively coupled plasma–mass spectrometry (ICP–MS) [9,10]. In recent years, due in part to superior detection limits, ICP–MS has become increasingly popular as an element specific detector for speciation studies [11]. In the case of organo-tin compounds, various HPLC strategies have been developed for a wide variety of tin species. These include micellar HPLC [10], ion-exchange [12], and reversed-phase chromatography [9]. However, with particular respect to reversed-phase methodologies, little or no comment has been made as to the mode of separation, or how possible improvements could be made to peak shape and chromatographic resolution. Most recent research has studied improvements in the extraction procedure or detection devices.

In this paper we will discuss investigations into optimizing a HPLC–ICP–MS method for the determination of TBT and TPhT in water samples using reversed phase chromatography. The effect of different column packing materials, along with various mobile phases and counter ions, will be discussed and conclusions drawn as to the possible mechanisms of the separation process. Details about the modification of the ICP–MS instrumentation and coupling interface to the HPLC system will also be described.

2. Experimental

2.1. Reagents

Organo-tin compounds, butyltin trichloride (BT), dibutyltin dichloride (DBT), tributyltin chloride (TBT), phenyltin trichloride (PhT), diphenyltin dichloride (DPhT), triphenyltin chloride (TPhT) and triethyltin bromide (TET) were obtained from Aldrich Chemicals (Gillingham, UK) and were used as received. Stock solutions (1000 mg l^{-1}) were prepared by dissolving 0.1 g in 100 ml of HPLC grade methanol (Fisons, Loughborough, UK). Working standards were prepared by serial dilution with methanol. All standard solutions were kept in pre-cleaned glassware at 5°C in the dark.

Mobile phases were prepared from HPLC grade methanol, deionised water (>17.8 MOhm, Elgar, High Wycombe, UK) and glacial acetic acid (Analar, BDH, Poole, UK). Ion-pairing reagents 1-penta-nesulfonic acid sodium monohydrate and triethyl-amine (Fluka, Gillingham, UK) were dissolved directly into the mobile phases as required.

2.2. ICP–MS instrumentation

An Elan 5000A ICP–MS (Perkin-Elmer, Beacons-field, UK) was used throughout these studies. The normal Ryton™ corrosion-resistant spray chamber was replaced by a double-skinned, silvered glass spray chamber cooled by a RTE-110 chiller unit (NESLAB Instruments, Nottingham, UK) with a mixture of ethyleneglycol–water (50:50, v/v). Typical ICP–MS operating conditions are given in Table 1. Before coupling the ICP–MS to the HPLC system the ICP–MS was optimised for maximum signal for ^{120}Sn isotope by the aspiration of a 10 μg l^{-1} Sn solution in a methanol–water (80:20) mixture.

All chromatographic data were assessed using the Chromafile-MS™ (Perkin-Elmer, Beaconsfield, UK) software package.

Table 1
ICP–MS operating conditions

ICP		
Power		1250 W
Plasma gas		15.0 l min^{-1}
Auxilary gas		0.8 l min^{-1}
Nebuliser gas		0.95 l min^{-1}
Oxygen		25 ml min^{-1}
Cones		Pt
Lenses	P	44 (switch settings)
	B	48
	S	45
	E	25
Chiller unit		−15°C
Data acquisition		
Mass		120
Dwell time		1000 ms
Sweep/reading		1
Readings/replicates		1
Number of replicates		300
Points across peak		1
Resolution		Normal

2.3. HPLC system

A Waters Model 510 HPLC pump fitted with a Rheodyne injector and a 100-μl sample loop was used throughout. Analytical columns, packed with either Spherisorb ODS2, Spherisorb ODSB or Kromasil-100 (Hichrom, Reading, UK), were all 25 cm$\times$4.6 mm I.D. with 5-μm particle size. Mobile phase flow-rate was kept constant at 1 ml min^{-1} for all experiments. All standards and sample solutions were brought up to room temperature before analysis.

The HPLC columns were connected directly to the nebulizer of the ICP–MS by a 30 cm$\times$0.8 mm I.D. length of teflon tubing.

2.4. Preparation of glassware

All glassware was washed with Crawley's reagent (30% v/v HNO_3, 5% v/v HF and 2% v/v Teepol) for 12 h and thoroughly rinsed with deionised water before use (care should be taken over glassware consistently re-used for organo-tin work, as in our experience contamination from organo-tin compounds can readily occur, resulting in unidentified chromatographic peaks).

2.5. Sample preparation and preconcentration

For the analysis of real samples a preconcentration procedure based on the one reported by Garcia Alonso et al. [13] for TBT was adapted to include TPhT. The organo-tin compounds were preconcentrated onto Sep-Pak C_{18} mini-columns (Waters, Watford, UK). Each mini-column was conditioned by passing through 10 ml of HPLC grade methanol followed by 10 ml of deionised water at a flow-rate of 3.5 ml min^{-1}. This was carried out with a peristaltic pump, using organic solvent resistant pump tubing (Perkin-Elmer, Beaconsfield, UK). Sample aliquots of 100–200 ml (although more could be accommodated) were loaded onto the columns at the same flow-rate. The retained compounds were recovered by passing through 5 ml of a 80% (v/v) methanol–3% (v/v) acetic acid solution at 0.5 ml min^{-1}. Each 5-ml fraction was collected in 5-ml volumetric flasks and stored at 5°C in the dark until use.

3. Results and discussion

3.1. ICP–MS optimization

Before coupling the HPLC to the ICP–MS several parameters were optimized for the detection of the ^{120}Sn isotope in an organic solvent. The main problem in using organic solvents with ICP–MS is the increased volatility of the aspirated solutions. The sample loading to the plasma becomes intolerable and the plasma can not be maintained. Also, if too much solvent reaches the plasma and the MS interface, the running vacuum of the quadropole can not be controlled and kept below the optimum level. The chiller temperature had a marked effect on the running vacuum of the ICP–MS instrument. However, below $-15°C$ there was no further improvement in the vacuum or the observed ^{120}Sn signal. As recommended by other authors [11,12], oxygen addition was kept to within 2–3% of the total nebulization gas flow while utilizing a power level of 1250 W. Under these conditions, although the Pt sampler cone became covered by a thin layer of deposited carbon, no degradation in the analytical signal was observed, even after 6–7 h of continuous operation. Finally, the ion lenses were optimized on the ^{120}Sn signal and were fixed at the values given in Table 1 for the rest of this study.

3.2. HPLC columns

The reactivity of organo-tin compounds to silanol sites on HPLC packing materials is a topic which has received virtually no systematic study. Initial work for this study showed that the use of poly(styrene–divinylbenzene) co-polymer materials, together with mobile phases based on either acetonitrile or methanol, gave no measurable advantage in the chromatography of the compounds when compared to silica based columns. This perhaps suggests that the role of silanol groups in impairing the separation of organotin compounds may be less significant than previously thought. Nevertheless, in an attempt to improve and develop a greater understanding of the separation, a systematic study was undertaken on three silica-based materials.

It was decided to concentrate on C_{18}-based packing materials and reversed-phase ion-pair chromatog-

raphy. Ion-exchange chromatography was not considered for this study as silica-based cation exchangers are notoriously unstable and have a tendency for their ion-exchange capacity to decrease when eluted with aqueous mobile phases. Three materials, Spherisorb ODS2, Spherisorb ODSB and Kromasil-100 were chosen to be evaluated. These column packing materials were chosen for several reasons. The ODS2 column was included to provide a bench mark separation from which to compare the other packings and mobile phases. The Spherisorb ODSB column was tested as it is a base-stabilized material and therefore may be less likely to exhibit undesirable chromatographic effects such as peak-broadening and non-quantitative recoveries, which can be common with this type of chromatography. Kromasil-100 is an extremely pure, fully end-capped, silica-based material, so the number of adsorption sites should be minimized thus aiding the separation of these organo-tin compounds. Several ion-pairing reagents were used during the study such as 1-pentane sulphonic acid sodium monohydrate [11], 1-heptane sulphonic acid sodium monohydrate and triethylamine. The different reagents all affected the chromatography in a similar manner, although the triethylamine seemed to produce the best signal-to-noise ratio, as detected by the ICP–MS instrumentation. However, it is worth noting that triethylamine makes the system compatible with LC–MS sample introduction interfaces such as electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI). For these reasons, triethylamine was used in the mobile phase for the column packing study. It was found that ion-pairing reagents caused the tri-substituted compounds to elute slightly more rapidly and with better peak shapes, although there was negligible improvement in the chromatography of the mono- and di- substituted compounds. Subsequently, in practice it was found to be possible to omit the ion-pairing reagents altogether.

Typical chromatograms obtained from each column for a mixture of TBT and TPhT are shown in Fig. 1a–c. As can be seen the Kromasil-100 column clearly gives a better performance than either the ODS2 or the ODSB columns. In fact this has been the case in all other experiments regardless of mobile phase and analyte. Typical retention times using the

described system are given in Table 2. As can be seen, PhT and DPhT both would interfere with the determination of TPhT, if present in the same sample. BT and DBT under these chromatographic conditions are not eluted at all. It should be stated however, that the peak shapes for PhT and DPhT were not good and that no quantification of these compounds could be made using this system.

3.3. Effect of multifunctional carboxylic acids

To help with the elution and resolution of mono- and di- substituted organo-tin compounds, the influence of various carboxylic acids was investigated. Inclusion of carboxylic acids in eluents used to separate organo-tin compounds has chiefly been justified by the effects such acids would have in suppressing silanol activity by reducing the eluent pH.

The following alternatives to acetic acid were investigated; oxalic, citric, tartaric and malonic acids were all tested for their effect on organo-tin compounds. It was found that they all affected the chromatography in the same manner except malonic acid which had no appreciable effect on retention times. Typically, all the mono- and di- substituted compounds were non-retained and eluted with the solvent front. Retention times obtained after the addition of oxalic acid are given in Table 3 with a typical chromatogram for the tri-substituted compounds shown in Fig. 2. Thus, the addition of oxalic acid to the mobile phase allows for the interference free determination of the two tri-substituted compounds of interest. Further experiments showed that even when using the ODS2 column (in the presence of ion-pairing reagents) oxalic acid did not alter, to any significant extent, the retention times of the tri-substituted compounds. It was subsequently found that it was possible to achieve the separation of TBT from TPhT using a mobile phase of 80% (v/v) methanol, 20% (v/v) water and 10 mg per 100 ml oxalic acid (retention times 130 s for TPhT and 190 s for TBT). This tends to suggest that silanol activity is probably not the reason for the poor chromatography of organo-tin compounds and that it may have more to do with eluent composition.
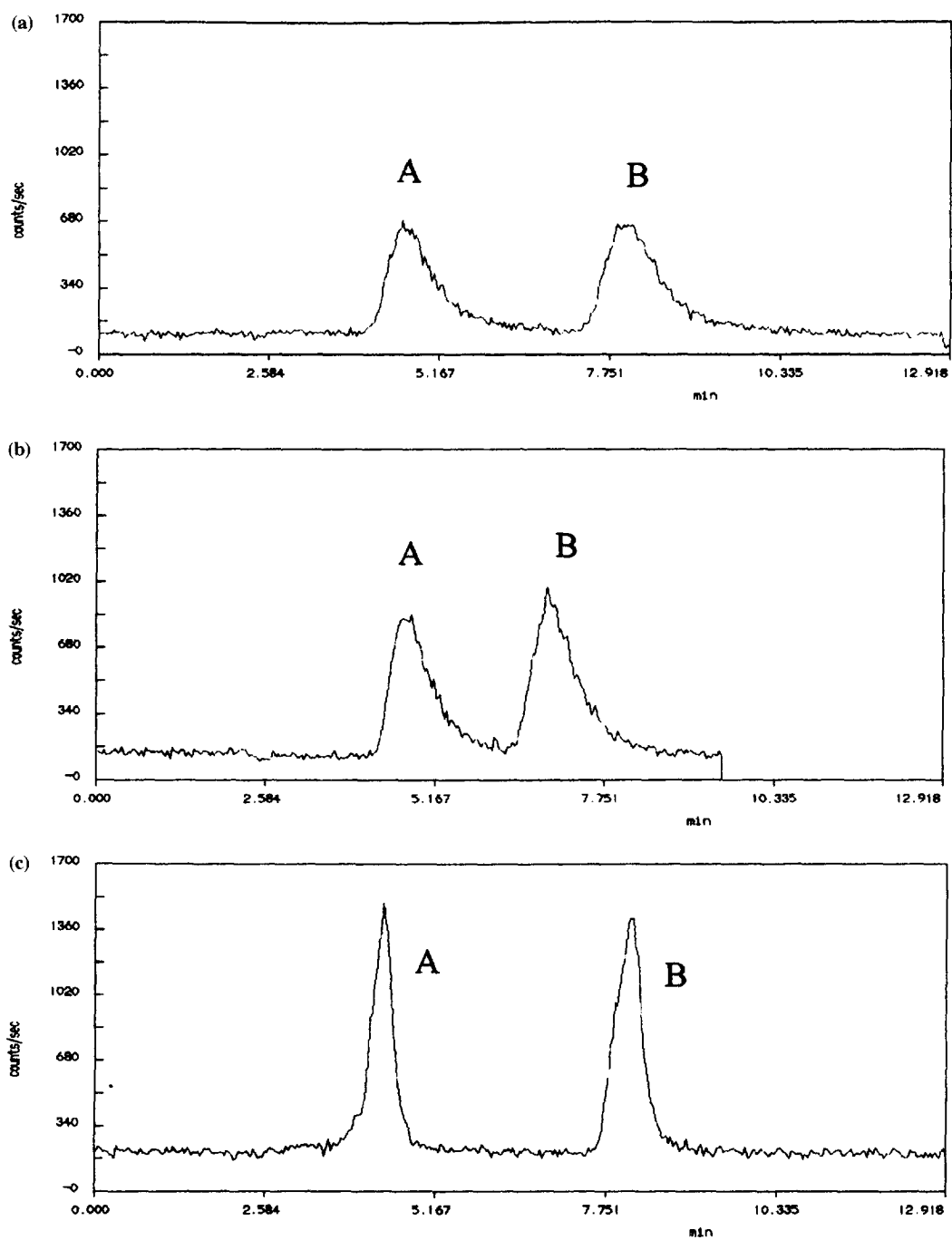
Fig. 1. Ion-pair chromatograms of triphenyl tin (A) and tributyl tin (B) separated on various silca-based column packing materials using a 84% (v/v) methanol, 0.3% (v/v) triethylamine and 2.5% (v/v) acetic acid mobile phase; 100-μl injection of a 4.76 ng ml^{-1} mixture of both compounds as their salts: (a) ODS2, (b) ODSB and (c) Kromasil-100.

Table 2

Retention times for organo-tin compounds separated using the Kromasil-100 column with a mobile phase of 84% (v/v) methanol, 0.3% (v/v) triethylamine and 2.5% (v/v) acetic acid

Compound	Retention time (s)
Phenyl tin	300
Diphenyl tin	200
Triphenyl tin	300
Triethyl tin	220
Butyl tin	Not eluted
Dibutyl tin	Not eluted
Tributyl tin	580

Table 3

Retention times for organo-tin compounds separated using the Kromasil-100 column with a mobile phase of 84% (v/v) methanol, 0.3% (v/v) triethylamine and 2.5% (v/v) acetic acid and 10 mg/100 ml oxalic acid

Compound	Retention time (s)
Phenyl tin	143
Diphenyl tin	175
Triphenyl tin	312
Triethyl tin	221
Butyl tin	143
Dibutyl tin	175
Tributyl tin	630

3.4. Formic acid

Because of the effect obtained while using the multifunctional carboxylic acids it was decided to investigate whether monocarboxylic acids such as formic acid would provide a further insight into the separation mechanisms. A typical chromatogram obtained using 80% (v/v) methanol and 1% (v/v) formic acid (and no ion-pairing reagent) can be seen in Fig. 3. When compared to Fig. 2, the formic acid has given rise to a more rapid elution of the tri-substituted compounds, while not affecting the mono- or di- substituted compounds. This faster elution may be because the pH of the eluent containing formic acid is much lower (pH 2–3) than those based around acetic acid.

3.5. Preconcentration and recovery data

The present system had a detection limit of 200 ng TBTCl 1^{-1}. As it stands this is not good enough for the analysis of real samples as it has been demonstrated that low ng 1^{-1} levels are toxic to certain marine organisms [14]. The preconcentration procedure was based on that of Garcia Alonso et al. [13], but optimised for both TBT and TPhT. The
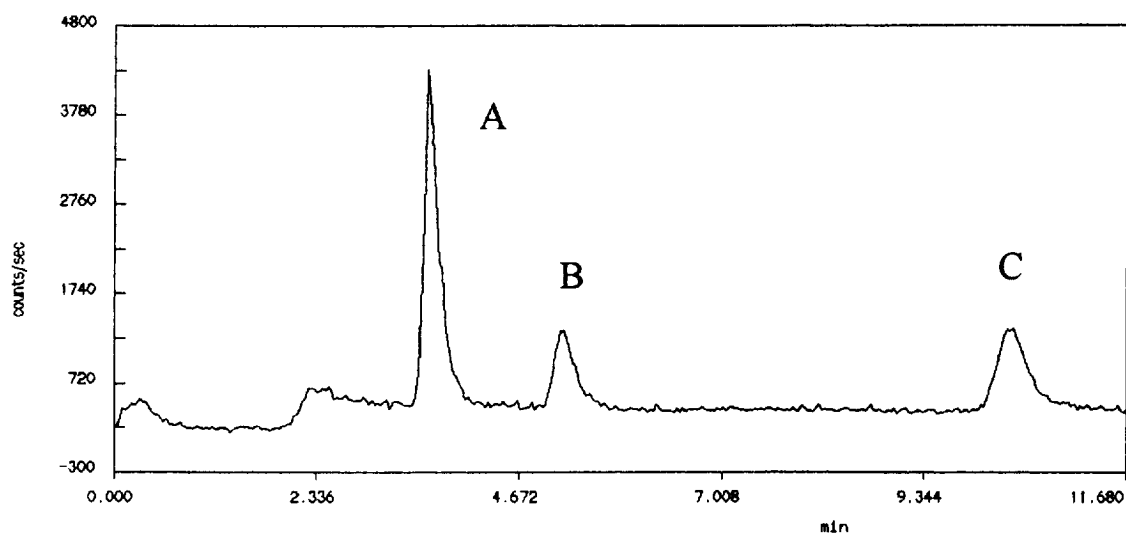

Fig. 2. Ion-pair chromatogram of triethyl tin (A), triphenyl tin (B) and tributyl tin (C) separated on the Kromasil-100 column using a mobile phase of 84% (v/v) methanol, 0.3% (v/v) triethylamine, 2.5% (v/v) acetic acid and 10 mg/100 ml oxalic acid; 100-μl injection of a mixture of 4.81 ng ml^{-1} triethyl tin and 4.76 ng ml^{-1} of both triphenyl and tributyl tin as their salts.
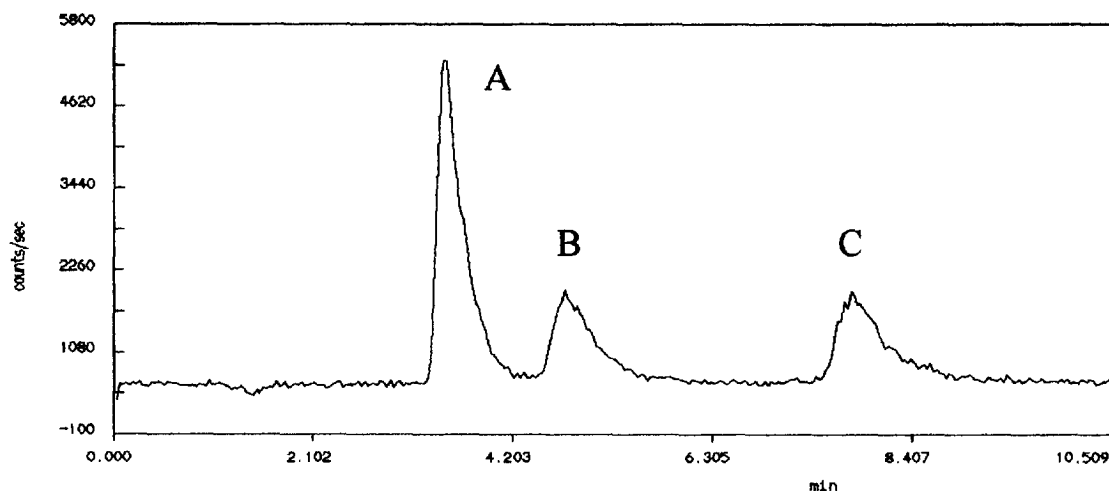
Fig. 3. Chromatogram of triethyl tin (A), triphenyl tin (B) and tributyl tin (C) separated on the Kromasil-100 column using a mobile phase of 80% (v/v) methanol and 1% (v/v) formic acid 100-μl injection of a mixture of 4.81 ng ml^{-1} triethyl tin and 4.76 ng ml^{-1} of both triphenyl and tributyl tin as their salts.

procedure was optimized for the different flow-rates for column loading and elution. The elution flow-rate of 0.5 ml min^{-1} was found to be the most critical factor. Recovery data for both TBT and TPhT spiked waters, as well as data for HCl extracts of incinerator ash are given in Table 4. As can be seen, for the water samples near 100% recoveries for both compounds can be obtained. This lead to a detection limit of typically 2 ng l^{-1} for both TBT and TPhT.

4. Conclusions

A HPLC–ICP–MS method for the preconcentration and determination of TBT and TPhT in water samples has been developed. The reported system guarantees the separation of the tri-substituted tins from the mono- and di- substituted forms. A proprietary silica-based packing material Kromasil-100 has been demonstrated to provide the best chromatograms. Indications have been found that ion-paring agents are not essential to achieve acceptable chromatographic separations of tri-substituted organo-tin compounds in the presence of multi-functional carboxylic acids. Although not conclusively proved here the role of silanol activity in the separation of these compounds may have been over estimated in the past and that more work is required to assertion the exact role of ion-pairing reagents in this type of separation.

Acknowledgments

Table 4
Preconcentration recovery data for spiked water and hydrochloric acid samples for TBT and TPhT using the Sep-Pak C_{18} preconcentration procedure

	Recovery %	
	TPhT $n=3$	TBT $n=3$
Non-acidic water	87–100	103
HCl extracts	0	87–106

Samples were spiked at the 50 pg ml^{-1} level for both compounds as their salts.

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