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An Evaluation of Reversed-Phase and Ion-Exchange Chromatography for Use with Inductively Coupled Plasma–Mass Spectrometry for the Determination of Organotin Compounds

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An evaluation of reversed-phase high-performance liquid chromatography (HPLC) employing mobile phases compatible with direct coupling to inductively coupled plasma–mass spectrometry (ICP–MS) is described for the selective and sensitive detection of organotin species. The findings of this study are compared with established methods, employing ion-exchange chromatography.

In order to achieve optimum performance, both the HPLC and ICP–MS were optimized for speciation work. The results from studies using various mobile phases for the separation of a range of tin compounds (inorganic tin, tributyltin, dibutyltin and monobutyltin) are discussed both in terms of resolution and compatibility with ICP–MS instrumentation. Tropolone, a commonly used complexing agent for organotin species, is also discussed with reference to the chromatographic separation of tin species.

Finally, the role of isotope dilution analysis in conjunction with HPLC–ICP–MS for organotin speciation is described with respect to the European Community Standards, Measurements and Testing (BCR) certified material programme.

Keywords: Organotin compounds; speciation; high-performance liquid chromatography; inductively coupled plasma–mass spectrometry

INTRODUCTION

The environmental impact of organotin species is now well established.[1-7] It is known that the organic species of tin are more toxic than the inorganic forms, following the general trend monobutyltin (MBT) < dibutyltin < (DBT) < tributyltin (TBT). It is also recognized that levels as low as 1 ng ml^{-1} in water of TBT may cause deformation in shellfish,[8] which in turn may affect the fertility, mariculture and population of shellfish in affected areas. The preconcentration of organotins in shellfish may also lead to toxic effects in man, the nature of which is related to the exact chemical form of the analyte.

One of the major applications of tributyltin has been its use as the active ingredient in antifouling paints, but other organotin compounds have also been used as bactericides, fungicides, insecticides, wood preservatives and poly(vinyl chloride) polymer stabilizers. Toxic levels of these compounds are present in aquatic and sedimentary environments in many areas. Consequently, analytical methods for the precise and accurate determination of mono-, di- and tri-substituted organotin compounds (speciation) are of clear importance.

The aim of this work is therefore to develop an accurate and precise analytical method for the determination of organotin species in environmental samples. To achieve this goal, one of the most promising approaches is to separate the species of interest by high-performance liquid chromatography (HPLC) and then introduce them into a highly sensitive element-specific detector such as the inductively coupled plasma–mass spectrometer (ICP–MS).[9-15] This route has been reviewed and selected as the most appropriate methodology for this study since it avoids derivatization procedures which may alter speciation and be adversely affected by matrix components. The results presented in this paper were obtained using both ion-exchange and reversed-phase chromatographic systems to separate inor-

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ganic tin, TBT, DBT and MBT. In the case of the reversed-phase system, two different procedures were used. In the first, a methanol, water and acetic acid–ammonium acetate mobile phase (in varying ratios) was used in both isocratic and gradient mode. In the second procedure, the complexation of the different organotins with tropolone was investigated.

An additional approach to enhance the accuracy of the analytical methodology is to utilize isotope dilution analysis (IDA), since it provides compensation for several sources of variability. Although this technique has been successfully used in lead speciation studies,[16] it has not yet been reported for use with organotin compounds. Thus in later stages of this study isotope dilution analysis will be used, following the synthesis of organotin compounds with altered but known isotopic composition. Environmental matrices will then be spiked with the synthesized organotin species to allow the accuracy of an analytical methodology to be fully evaluated as part of a European Community Measurements and Testing certification programme. Thus a fundamental objective of this present study is to identify chromatographic conditions compatible with this technique.

EXPERIMENTAL

Chemicals

The organotin standards, 96% tributyltin chloride (TBT), 96% dibutyltin chloride (DBT) and 95% monobutyltin chloride (MBT), were obtained from Aldrich Chemical Co. Ltd, Dorset, UK.

Stock solutions (1000 μg g^{-1}) of these were prepared in HPLC-grade methanol (Rathburn Chemicals Ltd, Peebleshire, Scotland, UK) and stored in darkness at 4 °C. The same methanol was used to prepare the mobile phases for HPLC studies.

Inorganic tin calibrant was prepared from an inorganic tin ICP/DCP standard solution in hydrochloric acid, 9990 μg ml^{-1}, obtained from Aldrich Chemical Co. Ltd.

The triammonium citrate, citric acid and ammonium acetate used as buffers in this work were purchased from Fisons Analytical Reagents, Loughborough, UK. The tropolone was obtained from Aldrich Chemical Co. Ltd. Acetic acid and nitric acid, AnalaR grade, were obtained from BDH, Poole, UK.

Milli-Q deionized water (Millipore, Bedford, MA, USA) was used throughout the study.

Instrumentation and operating conditions

The high-performance liquid chromatography system consisted of an inert HPLC gradient pump (Varian Ltd, Model 9010, Warrington, UK), with an injection valve fitted with a 200-μl sample loop (Anachem, Model Rheodyne No. 7010, Bedfordshire, UK).

Ion-exchange chromatography was performed using a Partisil 10-SCX column of 25 cm length and 4.6 mm inner diameter. The mobile phase used when isocratic elution was employed was methanol/water (70:30, v/v) with ammonium citrate–citric acid buffer at pH 5.8. In the case of gradient elution, two step gradients were applied, varying the mobile phase from methanol/water (70:30, v/v), pH 5.8, to methanol/water 85:15, v/v, pH 5.8, and finally to methanol/water (85:15, v/v), pH 3.4.

The reversed-phase chromatography system employed a 5-μm Hamilton PRP-1 column of 15 cm length and 4.1 mm i.d. Various compositions of mobile phases were used with this column, employing an ammonium acetate–acetic acid buffer at pH 6.5. The flow rate used for both the ion-exchange and reversed-phase systems was 1 ml min^{-1}.

The inductively coupled plasma–atomic emission spectrometer used was a Varian Liberty 200 (Varian Ltd, Warrington, UK). The flow rates of coolant, auxiliary and nebulizer gases were 15, 1.5 and 0.75 l min^{-1} of argon, respectively. The rf power was 1500 W.

The inductively coupled plasma–mass spectrometer employed was a VG Plasmaquad II (Fisons Instruments Elemental, Cheshire, UK). The ICP was operated at 1400 W. Coolant, auxiliary and nebulizer flow gases were at 15, 0.75 and 0.75 l min^{-1}, respectively. An oxygen bleed (2.4%) into the nebulizer gas was used to negate the effects of the organic solvent. The nebulizer employed was a V-groove type (Fisons Instruments Elemental, Cheshire, UK) coupled to a Scott-type double-pass spray chamber. An auxiliary chiller unit (Techne Ltd, Cambridge, UK) using 2-propanol was connected to the spray chamber to reduce the temperature to −15 °C. The sampler–skimmer distance was 1 mm. The mass spectrometer ion lenses were optimized every day in order to obtain the maximal signal for a solution of 100 ng ml^{-1} of ^{115}In in 2% HNO_3 or the mobile phase employed.

The HPLC–ICP–MS interface consisted of a short length of PTFE tubing (0.3 mm i.d.) to connect the end of the HPLC column to the nebulizer. No further refinements were required for this study as there was no need for desolvation or additional sheath gases.

RESULTS

Evaluation of separation methods utilizing reversed-phase chromatography

Separation of the different organotin species (inorganic tin, TBT, DBT and MBT) was first evaluated using the reversed-phase column. There are several methods reported in the literature to separate these compounds using this form of chromatography.[14, 15, 17–22] The first attempts were made using various polar mobile phases with different concentrations of methanol and buffer (acetic acid–ammonium acetate) at pH 6.5. The compositions of the mobile phase ranged from 98% methanol and 2% buffer, down to 90% methanol, 2% buffer and 8% water. Using the 98% methanol/2% buffer mobile phase system, the first species to elute was MBT, followed by TBT, with DBT eluting last. The inorganic tin was thought to be coeluting with the MBT, a small shoulder being observed on the peak profile. A typical chromatogram obtained using this composition of mobile phase is shown in Fig. 1(a).

Decreasing the methanol content of the mobile phase to 90% did not help the resolution of MBT and inorganic tin, both again coeluting as a single peak. The elution of the other species was retarded however, with a degradation in peak shape.

A range of intermediate mobile phases with a methanol/water ratio between 90 and 98% were also investigated. The best performance was obtained with 93–95% methanol. However, the differences between these and the chromatogram in Fig. 1(a) are small, the TBT and DBT being slightly better resolved although the retention times were longer (Fig. 1(b)). The use of gradient elution was also evaluated in order to separate the inorganic tin and MBT species. Various compositions of the mobile phase were used employing a range of flow rates and gradient steps. Examples of the chromatograms obtained are shown in Figs 2(a) and 2(b). As can be seen, little success was achieved in the separation of the inorganic tin and MBT species employing gradients.

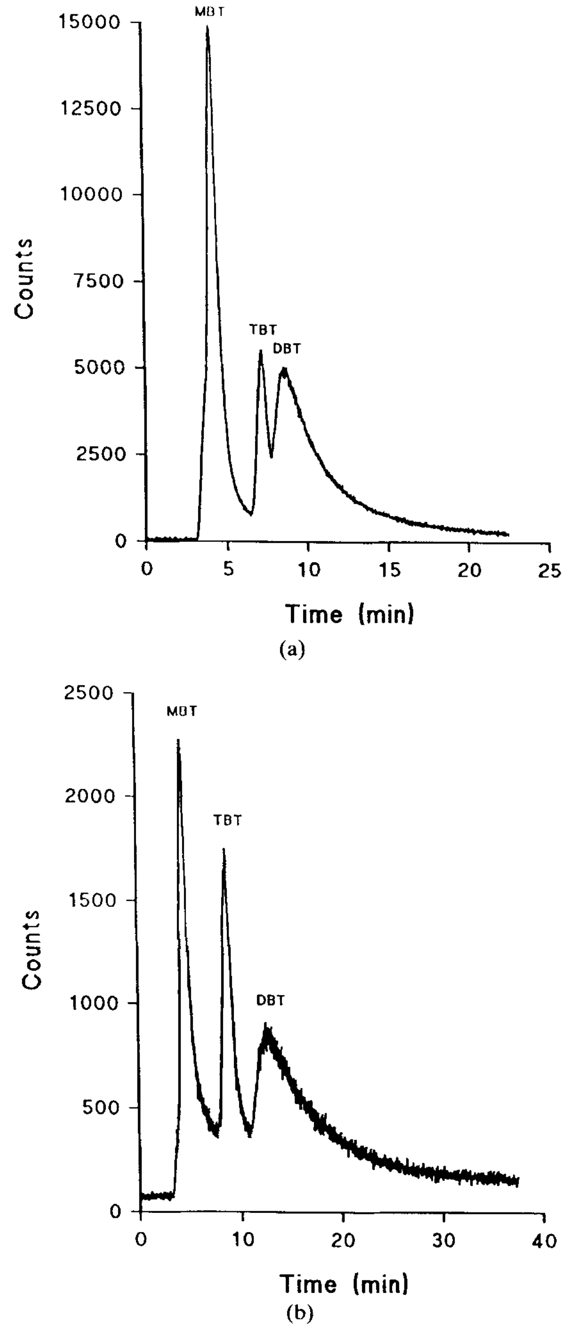


Figure 1 Reversed-phase chromatograms of inorganic tin, MBT, DBT and TBT. Mobile phase: (a) 98% methanol/water, acetate buffer, pH 6.5; (b) 95% methanol/water, acetate buffer, pH 6.5.

One of the most common problems in speciation studies on organotin compounds is the low extraction efficiency of the species from the original sample. Several workers have proposed the use of a complexing agent at some stage of the extraction procedure to improve the efficiency. One of the best known of these complexing agents for organotin species is tropolone, which has been used in several methods.[12, 14, 23, 24] In order to study the effect of tropolone on the chromatography, a range of organotin calibrants were complexed with different amounts of tropo-

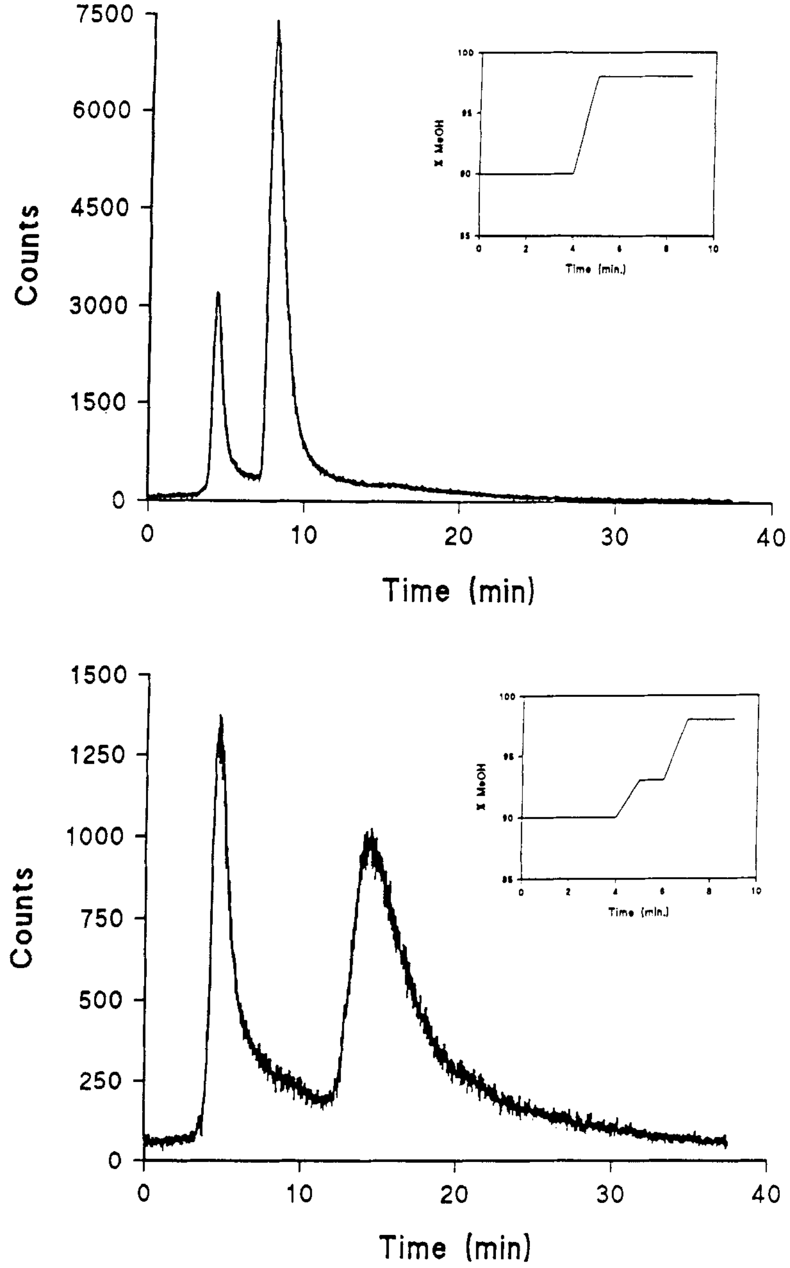


Figure 2 Reversed-phase chromatograms of inorganic tin, MBT, DBT and TBT. Mobile phase: methanol/water, acetate buffer, gradient elution, pH 6.5. The gradient elution profiles are shown in the insets.

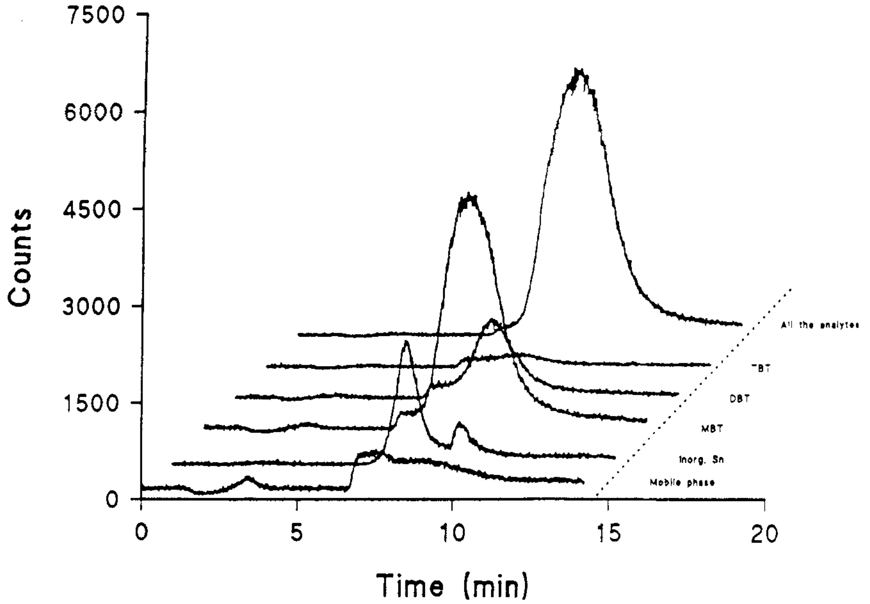


Figure 3 Reversed-phase chromatogram of inorganic tin, MBT, DBT and TBT complexed with 0.005% (m/v) of tropolone. Mobile phase: 95% methanol/water.

lone. Two experiments were carried out with these calibrants; the first involved a study of the effect of adding the complexing agent to the sample and the second included the tropolone as a component of the mobile phase. Two concentrations of tropolone in two different mobile phases were tried, i.e. 0.0050% and 0.0075% (m/v) tropolone in mobile phases of 85 and 95% methanol/water. In both cases a single peak was obtained with all species coeluting.

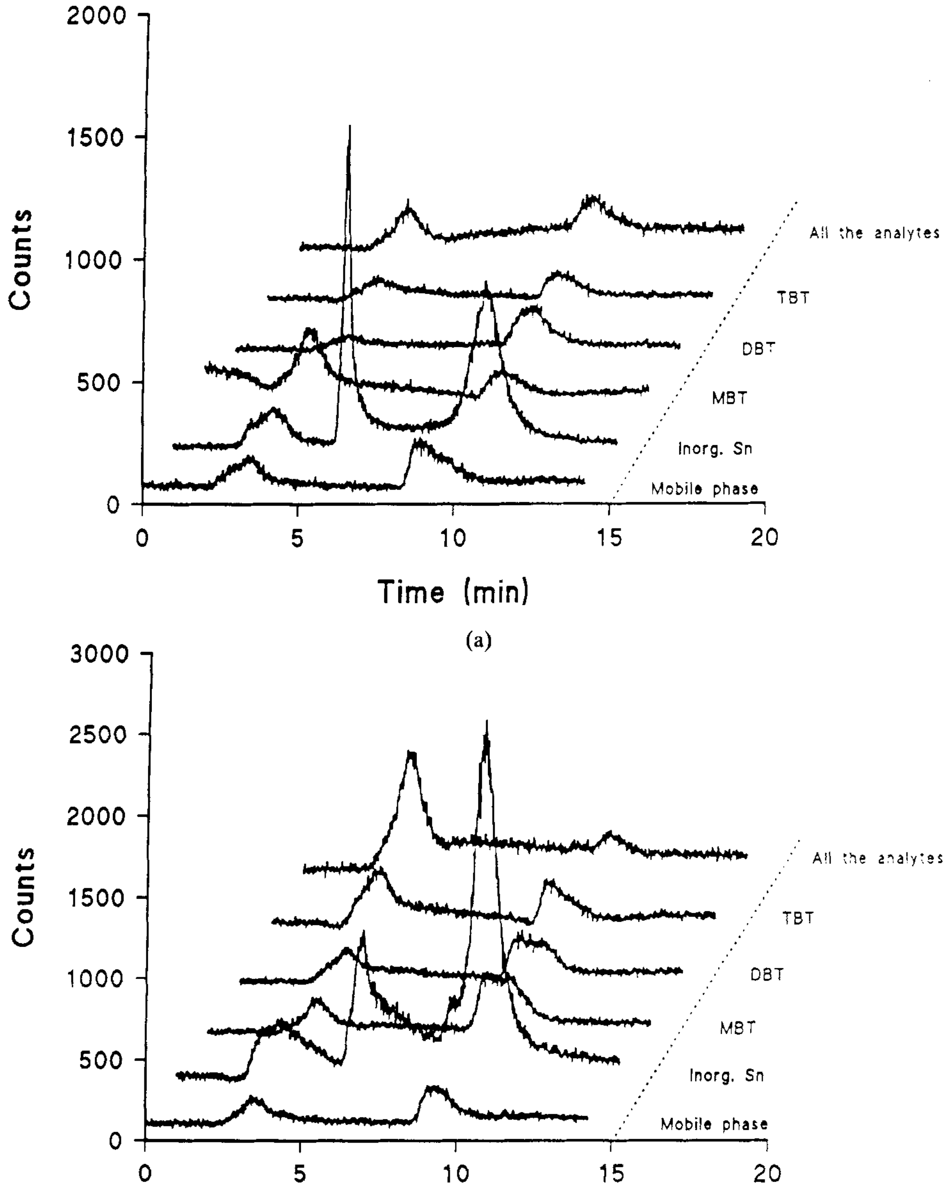
The same methanol/water mobile phases (without tropolone addition) were then used to investigate the effect of adding the tropolone (0.005 and 0.01%, m/v) to the calibrant rather than to the mobile phase. In the case of the 95% methanol with 0.005% of tropolone as complexing agent in the calibrant, again all the peaks coeluted, as shown in Fig. 3. The same was found for the same mobile phase and 0.01% of tropolone in the calibrant.

For the mobile phase of 85% methanol with 0.005 and 0.01% (m/v) of tropolone in the calibrants, the chromatograms were similar. The chromatogram obtained for the inorganic tin species showed three peaks, the first being the solvent peak. The other two may correspond to two different eluted species, one of them inorganic tin non-complexed, the second one a more polar complexed species, i.e. Sn(trop)$_n$ complex. This species was found to elute for both concentrations of tropolone. The chromatograms obtained are shown in Figs 4(a) and 4(b).

An alternative mobile phase system was also evaluated. The use of tetrahydrofuran, water, acetic acid and tropolone (54, 38, 8 and 0.2%, respectively) has been reported for the separation of several organotin species: triphenyltin (TPhT), diphenyltin (DPhT), TBT, DBT, MBT, trimethyltin (TMT), dimethyltin (DMT) and monomethyltin (MMT). Here a mobile phase of tetrahydrofuran, water, acetic acid and tropolone (54, 38, 8 and 0.2%, respectively) was employed with atomic absorption spectrometric detection.[17] Reversed-phase chromatography was again employed, using a TSK gel ODS-8TM column (250 mm × 4.6 mm). Kadokami *et al.*[17] also suggested that the separation could be achieved with 54% methanol in the mobile phase instead of tetrahydrofuran (THF), although no experimental results were given to support this. Since this option offers advantages when the chromatography is coupled to ICP–MS detection (inductively coupled plasmas only tolerate low amounts of THF without desolvation), and additionally offers the possibility of extending the method to the separation of inorganic tin and other species of interest, the substitution of methanol for THF was evaluated. Inorganic tin, MBT, DBT and TBT were injected both as single calibrants and as a mixed calibrant. The suggested flow rate in the original paper could not be used, due to a very high back-pressure on the column, so it was reduced until a normal back-pressure (approx. 14×10^6 Pa) was achieved. Unfortunately, the chromatograms obtained were again disappointing, with all the peaks coeluting at or near the solvent front. The percentage of methanol in the mobile phase was both increased in steps up to 90%, and decreased to 30%. In all cases the organotin species coeluted, and no improvement was obtained.

Ion-exchange chromatography

Following the disappointing results obtained utilizing reversed-phase chromatography as described above, the use of ion-exchange chromato-



(a)

(b)

Figure 4 Reversed-phase chromatograms of inorganic tin, MBT, DBT and TBT complexed with (a) 0.005% (m/v) and (b) 0.01% (m/v) of troplone. Mobile phase: 85% methanol/water.

graphy was evaluated.[9,25,26] This approach has already been used for water[1,27] and shellfish[2] and so the aim of this study was to evaluate further the chromatography for potential use in later isotope dilution studies on potential candidate BCR reference materials, such as sediments. The organotin species were extracted from an organotin-free sediment spiked with MBT, DBT and TBT (250 ng as Sn of each analyte), using acetic acid in the following manner: 25 ml of acetic acid was added to 2 g of sediment and the analytes extracted for 4 h; shaking the samples helped the extraction. The sediments were centrifuged at 2500 rpm for 15 min. The extraction with acetic acid and centrifugation were then repeated. The solid was washed twice with 5 ml of acetic acid. The extract was diluted to 50 ml with Milli-Q water. The organotins were back-extracted into toluene (3 × 10 ml). A few drops of sodium hydroxide (4 mol l^{-1}) were added. Finally, the extracts were rotary-evaporated to dryness. The sediments were dissolved in the mobile phase prior to the analysis. Extraction efficiencies of 103 ± 25% for TBT, 33 ± 3% for DBT and 12 ± 4% for MBT were obtained using this method.

As in previous studies,[1,2,27] a 25-cm Partisil 10-μm SCX column was used with a methanol/water (70:30, v/v) mobile phase employing ammonium citrate/citric acid buffer at ph 5.8. An example of a chromatogram obtained following this procedure is shown in Fig. 5(a)). To improve the resolution for DBT and MBT, gradient elution (methanol/water, 70:30, v/v, pH 5.8, to methanol/water, 85:15, v/v, pH 5.8, and finally to methanol/water, 85:15, v/v, pH 3.4) has been employed, as shown in Fig. 5(b).

CONCLUSIONS

The detailed study presented here of the use of reversed-phase chromatography for the separation of organotin species demonstrates the limitations of this approach when employing methanol/water mobile phases. The restriction on mobile phase composition is imposed in this study due to the need to develop a separation system compatible with the ICP–MS detection system to be used in later isotope dilution studies. Although good resolution was obtained for MBT, DBT and TBT using methanol/water (95:5, v/v) mobile phase, the inorganic tin remained unresolved from the MBT and thus remains a problem when

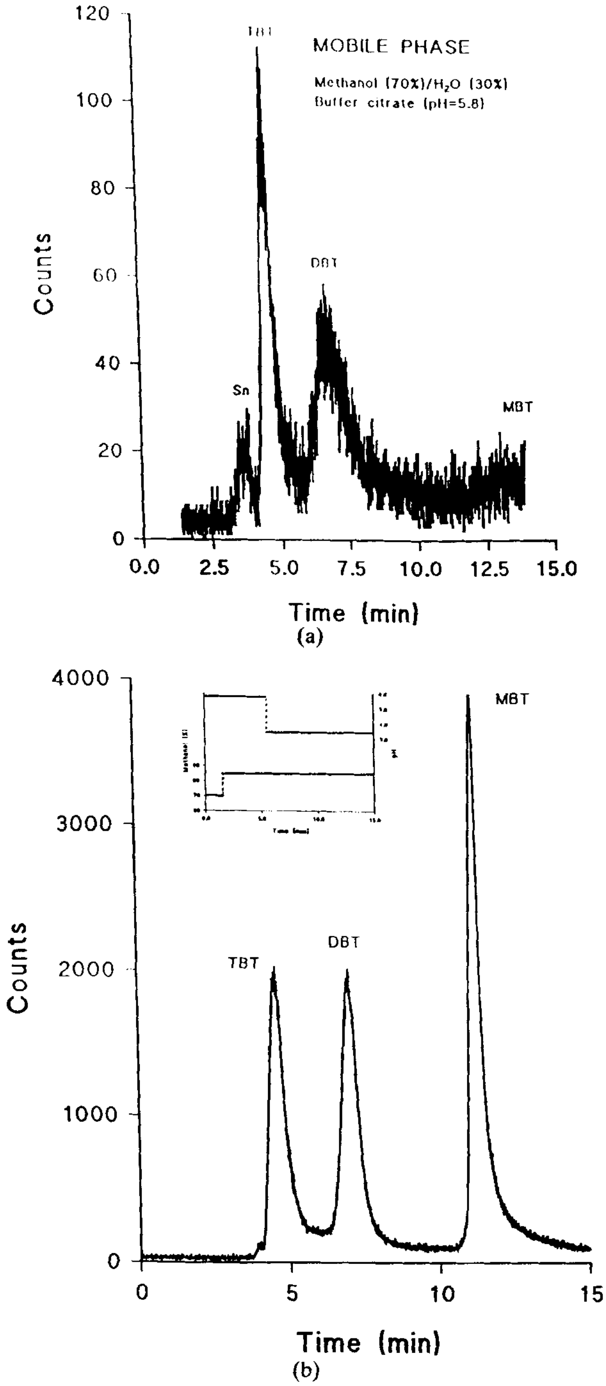


Figure 5 (a) Ion-exchange chromatogram using isocratic elution of inorganic tin, TBT, DBT and MBT. Mobile phase: 70% methanol/water, citrate buffer, pH 5.8. (b) Ion-exchange chromatogram using gradient elution of inorganic tin, TBT, DBT and MBT. Mobile phase: 70% methanol/water, citrate buffer, pH 5.8; 85% methanol/water, citrate buffer, pH 5.8; 85% methanol/water, citrate buffer, pH 3.4.

dealing with real samples. The use of tropolone was found to offer no advantage, and if present in the sample following extraction it may totally degrade the chromatography, possibly due to the formation of Sn(trop)$_n$ complexes. A similar approach based on a methanol/water/acetic acid/tropolone mobile phase as published in the literature was also unsuccessful, although a reversed-phase column from a different manufacturer was used for this study.

The use of ion-exchange chromatography still seems to offer the most potential for use in future work. This approach has already been used for natural waters[1,27] and biological samples[2] and has successfully been used to determine TBT in a sediment candidate reference material.

The next stage of this work with respect to the BCR programme is to utilize the advantages of isotope dilution analysis as a highly accurate and precise analytical method to determine organotin species in environmental samples. The chromatography identified as optimal above will be used with organotin compounds of altered but known isotopic composition. Environmental matrices will then be spiked with the synthesized organotin species and determined by isotope dilution analysis–high-performance liquid chromatography–inductively coupled plasma–mass spectrometry (IDA–HPLC–ICP–MS) to assist in the validation of future certified reference materials.

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