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Speciation of inorganic and organotin compounds in biological samples by liquid chromatography with inductively coupled plasma mass spectrometric detection☆

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ABSTRACT

This paper describes the effect of inorganic tin chloride on the separation of trimethyl-, tributyl- and triphenyltinchlorides by reversed-phase ion-pair high-performance liquid chromatography with detection by inductively coupled plasma mass spectrometry. The detection limits are 1.6 pg, 1.5 pg and 2.3 pg as tin for trimethyltin, tributyltin and triphenyltin, respectively. The relative standard deviation for ten injections of 20 ng of the tin compounds was less than 5%. Inorganic tin was held strongly on the columns used, to a greater extent on the silica column compared to the polymer column. Extraction and determination of tributyltin and triphenyltin as chlorides in fish tissue (certified reference material) and tuna fish (grocery store) were performed. The recovery study from fish tissue showed an efficiency of over 90% for both tributyltin and triphenyltin and over 60% recovery for spiked tuna.

INTRODUCTION

Speciation of an element is the determination of the individual physico-chemical forms of that element which together make up its total concentration in a sample. Variation in the elemental species can dramatically change their bioavailability or toxicity. Therefore speciation information at subnanogram levels is essential.

Organotin compounds have numerous applications [1–4], for example, mono- and diorganotins are used to stabilize poly(vinyl chloride) (PVC) polymers. Triorganotins are used as biocides, catalysts, wood preservatives, fire retardants and reducing agents, as well as in the pharmaceutical, ceramic and glass industries. Tin compounds have also been used in the production of cans for food storage. These organotin compounds are of high environmental and toxicological concern, since they are released into the environment [5,6]. A large fraction of the total organotin compounds used as biocides and algicides in antifouling paints have directly entered into the aquatic environment. For example, coastal water

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pollution is one of the most serious environmental problems in Japan. Tributyltin and triphenyltin have been used as antifouling paints for fish nets and ship hulls. Pollution by triphenyltin is a more serious problem than pollution by tributyltin, since it accumulates in the lipophilic tissues in the fish. Also, the speciation of tin in natural waters is important because of the large amount of tin mining and the increased use of toxic organotin compounds such as tri(*n*-butyl)tin oxide and tri(*n*-butyl)tin acetate in antifouling paints.

Several approaches to organotin speciation have been suggested [7,8]. One of the more promising approaches is to couple the separatory power of chromatography with the selectivity and sensitivity of atomic spectrometry. Gas and liquid chromatography are suitable techniques for the analysis of complex samples. Although gas chromatography (GC) has been coupled to atomic spectrometry for organotin speciation [7], it is limited to volatile and thermally stable organometallic compounds. The use of high-performance liquid chromatography (HPLC) considerably expands the type of chemical and physical species that may be studied. The separation of ions and non-volatile organotin species, in addition to volatile species is possible using several LC configurations [8].

Atomic spectrometry offers the possibility of selectively detecting a wide range of metals and non-metals. The use of detectors responsive only to selected elements in a multi-component mixture reduces the constraints placed on the chromatographic step, since only those components in the mixture which contain the element of interest will be detected. Among the various atomic spectrometric techniques, plasma detectors have evolved into a dominant source for trace metal speciation. Inductively coupled plasma mass spectrometry (ICP-MS) is a sensitive technique and has been investigated as a detector for HPLC [9–18]. HPLC–ICP-MS offers the sensitivity necessary for trace metal speciation in real samples [16], and the on-line detection capability required to enable optimization of the separation and routine operation. ICP-MS can detect chromatographic eluents at subnanogram to picogram levels under the optimal conditions.

Previously, in this laboratory, Suyani *et al.* [18] have investigated the separation of three organotin compounds: trimethyltin chloride, tributyltin chloride and triphenyltin acetate by ion-pair reversed-phase HPLC on a Spherisorb ODS-2 C_{18} column. They observed relatively high background, about 3300 counts per second (cps), after several injections of organotin mixture. This suggested an accumulation of tin compounds on the column probably because of the equilibration between ion-pairing agent and the column packing material.

This paper describes the use of a PRP-1 column for the organotin separation. The more stable polymer-based column accepts a wide range of pH values giving greater flexibility in developing chromatographic conditions. The composition of the PRP-1 particle is more homogeneous from the surface to the center, thus exposure of active sites, which are chemically different, is reduced.

In this study, three alkyltin compounds are separated using reversed-phase ion-pair chromatography on PRP-1 column. Also presented, is the effect of inorganic tin on the organotin separation and the effect on background after repeated injections of organic and inorganic tin. The analytical figures of merit obtained by using the PRP-1 column are compared to those obtained using a Spherisorb ODS-2 column [18]. The accuracy of the method was determined by speciating organotin compounds that were extracted from fish tissue (certified reference material) and tuna fish.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Model 2350 pump, a Model 2360 gradient programmer (Isco, Lincoln, NE, USA) and a Model 7010 injector with 200-μl sample loop (Rheodyne). A PlasmaQuad (PQ I, VG Elemental, Winsford, Cheshire, UK) ICP-MS was used. A forward power of 1500 W was used with an argon coolant flow of 16 l/min, auxiliary flow of 1 l/min and nebulizer gas flow of 0.66 l/min of argon mixed with about 1% of oxygen to prevent carbon

accumulation on the sampler. A double-pass Scott-type spray chamber was used, jacketed, and cooled to −20°C. Cooling is necessary to remove organic solvent vapor which causes quenching of the plasma. The tin major isotope at m/z 120 (32.37% abundance) was monitored.

Reagents

Organotin compounds, trimethyltin chloride (TMT), tributyltin chloride (TBT) and triphenyltin chloride (TPT) were obtained from Alfa products (Ward Hill, MA, USA) and used without further purification. An inorganic tin chloride standard solution was obtained from Aldrich (St. Louis, MO, USA). Stock solutions (1000 μg/g as tin) were prepared in HPLC-grade methanol and working solutions were prepared daily using methanol for dilutions. HPLC-grade methanol (Fisher Scientific), deionized distilled water (Barnstead, 18 MΩ), reagent-grade glacial acetic acid (Fisher Scientific) and certified A.C.S ammonium acetate (Fisher Scientific) were used to prepare the mobile phase.

Biological samples

Fish tissue (certified reference material No. 11) was obtained from National Institute for Environmental Studies, Japan Environmental Agency. The tuna fish was from canned tuna obtained at a local grocery store. Tuna was dried in the oven and homogenized before the extraction procedure.

Chromatographic separations and conditions

The two columns that were employed for ion-pairing reversed-phase chromatography were: (1) Spherisorb ODS-2 (C_{18}) (Phase Separations), 250 mm × 4.6 mm I.D with 5 μm particle size (Alltech, Deerfield, IL, USA). (2) PRP-1 column (Anspec, Ann Arbor, MI, USA) 150 mm × 4.1 mm I.D with 5 μm particle size. The ion-pairing agent used was sodium pentane sulfonate (PIC-B5, Eastman Kodak Company, Rochester, NY, USA). For the Spherisorb ODS-2 C_{18} column, a mobile phase of methanol–water–acetic acid (80:19:1) was used at a pH of 3. All the mobile phases, for both columns, contained 0.004 *M* sodium pentane sulfonate. Mobile phases with the PRP-1 column were adjusted to the pH by using 1% acetate which was composed of ammonium acetate and acetic acid in a ratio that would give the desired pH. For example, mobile phase containing 94% methanol and 5% water had 0.046 *M* acetic acid and 0.012 *M* of ammonium acetate to yield a pH of 6. The mobile phase flow-rate for all the studies was 1 ml/min.

Extraction of tributyltin and triphenyltin

Tributyltin and triphenyltin were extracted from the samples following the procedure described by Tsuda *et al.* [19]. About 2.5 g of sample was placed in a separatory funnel and extracted with 12.5 ml of ethyl acetate for 30 min after adding 25 ml of deionised water (Barnstead, 18 MΩ), 3.75 g of sodium chloride and 2.5 ml of 6 *M* HCl. The mixture was then centrifuged for 5 min. The organic layer was evaporated nearly to dryness at low pressure and rotation at 40°C. The residue was then dissolved in 5 ml of methanol. A 200 μl volume of sample was injected into the liquid chromatograph for analysis.

RESULTS AND DISCUSSION

Initial studies were performed to find the optimal chromatographic conditions for the PRP-1 column. The effect of changing the pH and the methanol concentration of the mobile phase on the organotin (TMT-Cl + TBT-Cl + TPT-Cl) separation, with 500 ng g^{-1} inorganic tin also present was studied. At 94% methanol concentration of the mobile phase,the pH was varied from 3 to 6. As the pH increased, from 3 to 5, there was an increase in resolution between the organotin compounds but there was no base-line resolution between inorganic tin and trimethyltin. Whereas, at pH 6, a base-line resolution between TMT-Cl and the inorganic tin chloride, was observed. This is shown in Fig. 1. A shorter analysis time was observed with the PRP-1 column which yielded a complete separation within 6 min.

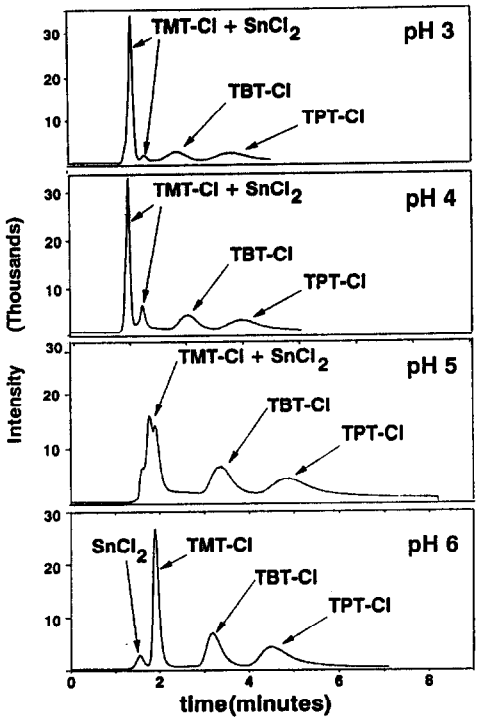

Fig. 1. The effect of pH on tin separation (500 ng g^{-1} inorganic tin chloride + 100 ng g^{-1} of organotin compounds) on PRP-1 column with mobile phase containing 94% methanol, 5% water, 1% acetate (*e.g.* for pH = 6, 0.046 *M* acetic acid and 0.012 *M* ammonium acetate) and 0.004 *M* sodium pentane sulfonate. Flow-rate = 1 ml/min.

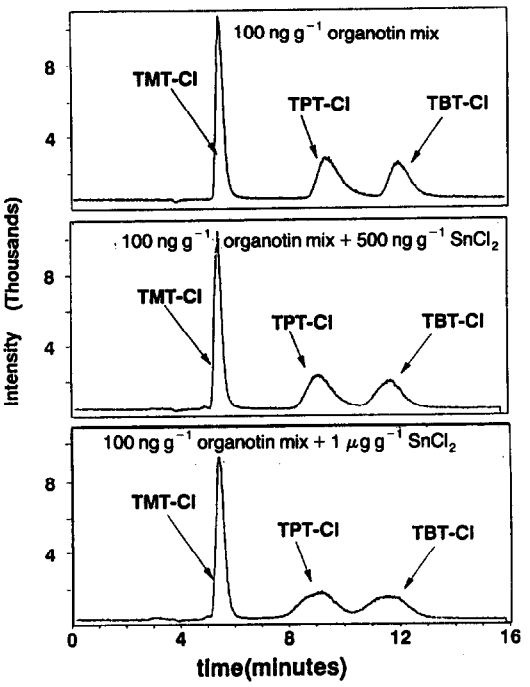

Fig. 2. The effect of inorganic tin on organotin separation with ODS-2 silica-based column, mobile phase containing 80% methanol, 19% water, 1% acetic acid (pH = 3) and 0.004 *M* sodium pentane sulfonate. Flow-rate = 1 ml/min.

Effect of inorganic tin on organotin separation

This study was done on both the silica-based column and the polymer-based column. Co-injection of the inorganic tin with organotin compounds for the silica-based column is shown in Fig. 2. Inorganic tin was retained on the column and did not affect the organotin separation, unless a concentration higher than 1 $\mu g\ g^{-1}$ of inorganic tin chloride was co-injected. Co-injection of inorganic tin chloride with organotins for the PRP-1 column is shown in Fig. 3. Inorganic tin eluted from the column, though not in a quantitative manner since some tin was retained even when using the PRP-1 column.

The above observations indicate that there was less retention of inorganic tin on the PRP-1 column than on the silica-based column. These observations also suggest that the silica-based column acted as an internal guard column, retaining the inorganic tin, and also acted as a chromatographic column by separating the organotins. An elevated background was observed with silica-based column after several injections of tin compounds which necessitated frequent reconditioning of the column by running the mobile phase for several hours.

Effect of organic and inorganic tins on background

The tin background intensities with the PRP-1 column and the silica-based column after ten 10 $\mu g\ g^{-1}$ injections of inorganic tin chloride and the organotin mixture (TMT-Cl + TBT-Cl + TPT-Cl) was studied. For the PRP-1 column, the background was monitored after the ten injections, including a 6-min period between each injection, which is the normal analysis time for the tin compounds. Similarly, for the silica-based column, there was a period of 13 min between each injection, which was the normal time for the tin compounds to elute. The base-line intensity of the mobile phase which was directly

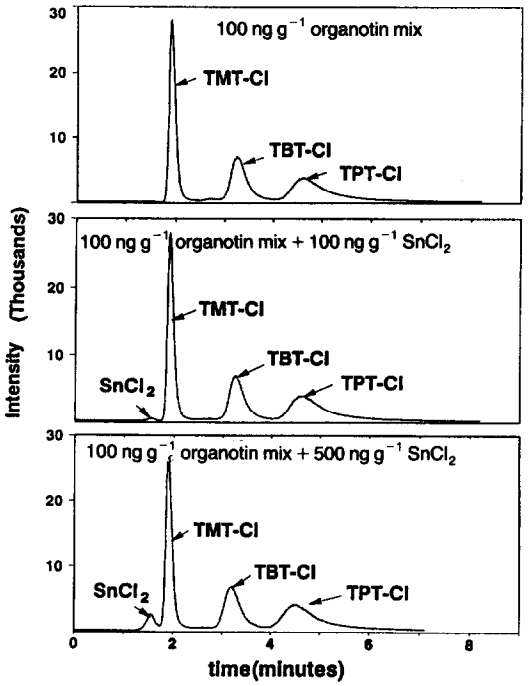

Fig. 3. The effect of inorganic tin on organotin separation with PRP-1 column, mobile phase containing 94% methanol, 5% water, 1% acetate (0.046 *M* acetic acid and 0.012 *M* ammonium acetate) and 0.004 *M* sodium pentane sulfonate. pH = 6. Flow-rate = 1 ml/min.

nebulized into the plasma without passing it through the column was observed to be approximately 50 cps. A tin bleed off the column was seen after repeated injections of both organic and inorganic tin. It was observed that organotins are retained less on the column than the inorganic tin chloride which is evident from their signal intensities. The intensity of the background was only in the hundreds of cps after the series of inorganic tin injections, whereas, the intensity was in thousands of cps (1500–3000 for both the columns) after the series of organotin injections of the mixture containing TMT-Cl, TBT-Cl and TPT-Cl. Inorganic tin was held strongly on both the columns, but to a greater extent on the silica-based column (300–400) compared to the PRP-1 column (600–700). It was also observed that organotins washed off with the mobile phase within 30–45 min whereas inorganic tin bleeds off the column with the mobile phase within 2 to 3 hours.

Analytical figures of merit

Detection limits. Detection limits for the organotin compounds with the PRP-1 column are presented in Table I. These are at the picogram level as compared to the nanogram level obtained by Suyani *et al.* [18] using a silica-based column and an earlier version of the plasma mass spectrometer.

Precision and linear dynamic range. Reproducibilities of peak areas were determined using ten replicate injections of 20 ng analyte as tin. The results are also listed in Table I, which are presented as relative standard deviations and are comparable with the work of Suyani *et al.* [18].

The linear dynamic range was 3 orders of magnitude for TMT-Cl and TBT-Cl, 2.5 orders of magnitude for TPT-Cl. These are again compared to the work of Suyani *et al.* [18] in Table II. Correlation coefficients obtained by using peak area measurements are also reported.

Detection of tributyltin and triphenyltin in biological samples

The chromatographic separation method described above was then applied to detect tributyltin and triphenyltin in fish tissue and tuna fish. The elution profile for organotins in fish tissue is shown in Fig. 4. The chromatogram indicates that there are no interfering peaks throughout the elution. The recovery study of

TABLE I

DETECTION LIMITS AND REPRODUCIBILITY OF PEAK AREA

	Detection limit[a]		Reproducibility of peak area[b]	
	PRP-1 (pg)	ODS-2[c] (ng)	PRP-1	ODS-2[c]
TMT-Cl	1.6	0.4	3.4	7.2
TBT-Cl	1.5	0.7	3.8	5.8
TPT-Cl	2.3	–	4.5	–
TPhT[d]	–	1.0	–	6.3

[a] Detection limit = $\frac{3 \times \text{standard deviation of background}}{\text{slope of calibration curve.}}$

[b] Relative standard deviation (%).

[c] Suyani *et al.* [18].

[d] Triphenyltin acetate.

TABLE II

SLOPE AND CORRELATION COEFFICIENTS FOR CALIBRATION PLOTS

	Slope[a]		LDR[b] (order of magnitude)		Correlation[c] coefficient	
	PRP-1	ODS-2[d]	PRP-1	ODS-2[d]	PRP-1	ODS-2[d]
TMT-Cl	0.9689	0.9872	3	2	0.9997	0.9999
TBT-Cl	0.9447	1.0251	3	2	0.9999	0.9999
TPT-Cl	1.0528	–	2.5	–	0.9999	–

[a] Obtained from log (integrated area) *vs.* log (concentration) plot.
[b] LDR = linear dynamic range.
[c] Obtained from integrated area *vs.* concentration plot.
[d] Suyani *et al.* [18].

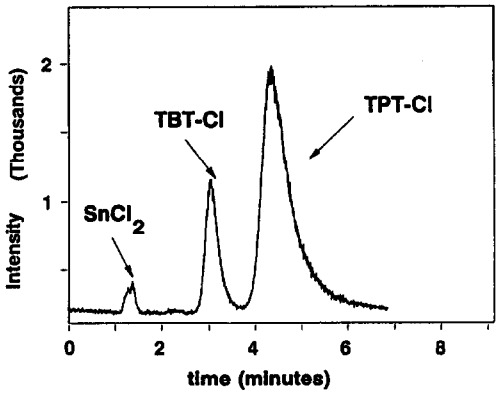

Fig. 4. Chromatogram of tin compounds from the fish tissue (certified reference material) extract. Mobile phase: 94% methanol, 5% water, 1% acetate (0.046 *M* acetic acid and 0.012 *M* ammonium acetate), 0.004 *M* sodium pentane sulfonate. pH = 6. Flow-rate = 1 ml/min.

these compounds shows good recovery of over 90% for both tributyltin and triphenyltin. The precision of the extraction method was evaluated by replicate analysis of the sample, which is shown in Table III.

This method was also used to analyze the amount of organotins in tuna fish. The elution profile for tins in tuna fish is shown in Fig. 5. An appreciable amount of inorganic tin was seen to be present in the tuna fish sample which might arise from the container, having a soldered seam, used to preserve the canned tuna. The amount of tributyltin and triphenyltin were estimated to be about 29.8 (± 1.2) ng g^{-1} and 21.5 (± 5.6) ng g^{-1}, respectively in 2.5 g of the dried tuna sample. Fig. 6. shows the chromatogram from the tuna fish extract which was spiked with 100 ng g^{-1} of TBT-Cl and TPT-Cl. The percent

TABLE III

RECOVERY OF TRIBUTYLTIN AND TRIPHENYLTIN FROM BIOLOGICAL SAMPLES (MEAN ± STANDARD DEVIATION, $n = 3$)

	Fish tissue (certified reference material)			Tuna fish (local grocery store)		
	Certified value ($\mu g\, g^{-1}$)	Amount detected ($\mu g\, g^{-1}$)	Recovery (%)	Amount spiked (ng g^{-1})	Amount detected (ng g^{-1})	Recovery (%)
TBT-Cl	1.3	1.2	94 ± 4.4	100	65.3	65.3 ± 1.7
TPT-Cl	6.3	6.2	99 ± 4.4	100	63.8	63.8 ± 4.9

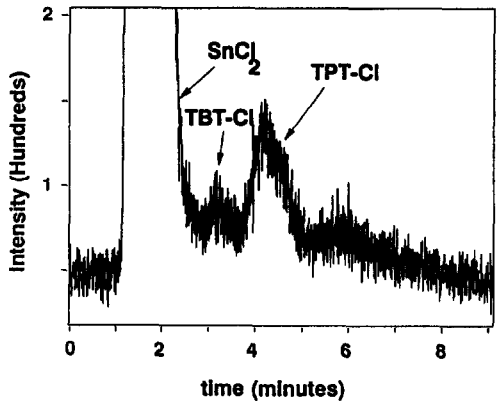

Fig. 5. Chromatogram (PRP-1 column) of tin compounds from the tuna fish (grocery store) extract. Mobile phase: 94% methanol, 5% water, 1% acetate (0.046 *M* acetic acid and 0.012 *M* ammonium acetate), 0.004 *M* sodium pentane sulfonate. pH = 6. Flow-rate = 1 ml/min.

recoveries for spikes of TBT-Cl and TPT-Cl compounds in tuna fish subjected to extraction conditions is also included in Table III.

CONCLUSIONS

Good separation between inorganic and organotin compounds was achieved at pH 6 using a PRP-1 column. Elution time for the analysis of tin compounds is reduced to about 6 min as compared to 13 min with the use of a silica-based column. Inorganic tin retains more on the silica-based column than on the polymer-based column. Thus, for a mixture of compounds having inorganic tin concentrations less than 1 $\mu g\ g^{-1}$, silica-based column acts as both a guard column by retaining inorganic tin, and as an analytical column by separating the organotin compounds. Whereas, the polymer-based column can be used to separate lower concentrations of inorganic tin from organotin compounds. Tin bleed off the column is observed for both columns after several injections of the tin compounds. Also, the organotins wash off the column faster than the inorganic tin. The detection limits were improved to the picogram level with the use of a PRP-1 column as compared to those of the silica-based column. This method of analysis was applied to detect tributyltin and triphenyltin compounds extracted from fish tissue (certified reference material) and tuna fish obtained from a local grocery store.

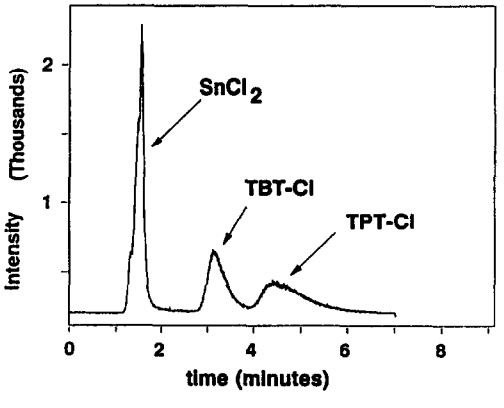

Fig. 6. Chromatogram (PRP-1 column) of tin compounds from tuna fish (grocery store) extract which was spiked with 100 ng g^{-1} of TBT-Cl and TPT-Cl. Mobile phase: 94% methanol, 5% water, 1% acetate (0.046 *M* acetic acid and 0.012 *M* ammonium acetate), 0.004 *M* sodium pentane sulfonate. pH = 6. Flow-rate = 1 ml/min.

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