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RAPID METHOD FOR THE DETERMINATION OF TETRAALKYLTIN COMPOUNDS IN VARIOUS KINDS OF BIOLOGICAL MATERIAL BY GAS CHROMATOGRAPHY

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SUMMARY

A rapid gas chromatographic method is described for the simultaneous determination of tetraalkyltin compounds in biological materials. Tetraalkyltins were rapidly purified by direct passage through a silica gel column after extraction from the homogenized tissues with *n*-hexane. Gas chromatographic analysis was alternatively carried out with PEG 20M at temperatures from 50 to 150°C. A hydrogen flame-ionization detector was more sensitive and selective towards tetraakyltins than an electron-capture detector. Detection limits reached $1 \cdot 10^{-8}$ g for tetraalkyltins. Recoveries of tetraalkyltins added to various tissues at the 85-nmole level ranged from 97 to 104%. *In vivo* studies indicated that for a sample containing more than 0.1 μg of tetraalkyltins per gram of tissue, the proposed method is accurate enough for quantitative analysis.

INTRODUCTION

Organotin compounds have been widely used as polymer stabilizers, fungicides, insecticides, organic catalysts, oil additives, etc. In recent years, there has been concern over the potential health danger from these compounds. Toxicological reports[1–4] have confirmed that tetraalkyltins produce the same effect in animals as trialkyltins, which are the most toxic organotin compounds towards the central nervous system. Therefore, it is important that sensitive and accurate methods are

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established for the determination of official tolerance limits of tetraalkyltin and other organotin compounds. In addition, such methods should also be simple and rapid and capable of determining organotin compounds in various kinds of biological material.

Numerous methods have been published for the determination of organotin compounds. However, most of the methods described for the determination of tetraalkyltins are incidental to those of tri-, di- or monoalkyltins. These include the determination of elements in organotin compounds such as tin[5–20], carbon and hydrogen[21,22], halogens, nitrogen and sulphur, and the determination of organotin compounds themselves[23–44]. The determinations of tin in organotin compounds are based on their conversion into tin(II) and tin(IV) oxides by oxidation with various oxidizing agents, and have been conducted by titrimetric[5,6], complexometric[6–11], spectrometric[12,13], gravimetric[9,14], volumetric[5,15,16], photometric[5,17], X-ray fluorescence[18], X-ray spectrophotometric[19] and polarographic[20] methods after destruction of the organotin compounds. For the determination of organotin compounds themselves, paper chromatographic[23,24], thin-layer chromatographic[25,26], ultraviolet and infrared spectrophotometric[27], nuclear magnetic resonance spectrometric[27,28] and gas chromatographic[29–44] methods have been developed.

Some of these methods, however, are too complicated and others suffer from unsatisfactory sensitivity, precision, reproducibility and specificity and are therefore unsuitable for application to the determination of tetraalkyltins in biological materials. Of the available methods, gas chromatography appears to be the most versatile and applicable to the determination of tetraalkyltins in mammals.

In this work, we examined the gas chromatographic separation of tetraalkyltins and the purification of tetraalkyltins from biological materials, and established a rapid procedure for the simultaneous determination of tetraalkyltins in various kinds of biological materials by gas chromatography.

EXPERIMENTAL

Reagents

Tetraethyltin (Et_4Sn), tetrapropyltin (Pr_4Sn), tetrabutyltin (Bu_4Sn) and tetraethyllead (Et_4Pb) were obtained from Aldrich (Milwaukee, WI, U.S.A.). The purity of these compounds was not less than 98%. When not of acceptable purity, the compounds were purified by distillation or by silica gel column chromatography (see Fig. 1). Other reagents included special-grade materials and organic solvents, such as silica gel (No. IIA, 100–200 mesh, obtained from Nakarai Chemical, Tokyo, Japan) and *n*-hexane (provided by Wako, Tokyo, Japan).

Gas chromatography

The instrument was a Shimadzu Model GC-6AM gas chromatograph equipped with a hydrogen flame-ionization detector (HFID). A glass tube (200 cm × 3 mm I.D.) was packed with 10% PEG 20M on Shimalite W (80–100 mesh) support. Other gas chromatographic conditions are given in the figures.

Preparation of tetraalkyltin compounds from tissues

The procedure for the preparation of samples for analysis of tetraalkyltin com-

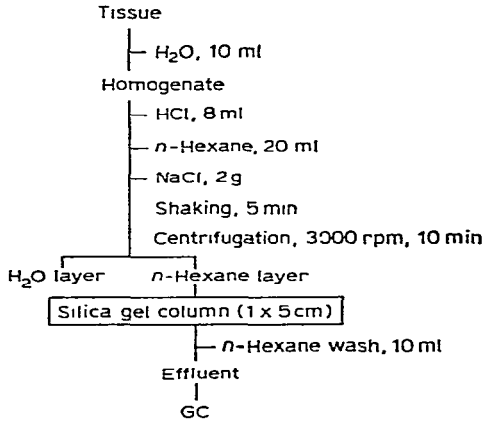

Fig. 1. Preparation of tetraalkyltin compounds from tissues.

pounds in tissues is shown in Fig. 1. A sample of tissue weighing between 1.0 and 5.0 g (wet weight) was homogenized in 10 ml of water. Concentrated hydrochloric acid (8 ml) was carefully added to the homogenate and the contents of the tube was mixed thoroughly and allowed to stand for 5 min. *n*-Hexane (20 ml), sodium chloride (2 g) and a suitable amount of Et_4Pb as internal standard were added and the tetraalkyltins were extracted by shaking for 5 min. After centrifuging for 10 min at 3000 rpm (1000 *g*), the upper *n*-hexane layer was transferred to another flask. The extraction procedure was repeated twice. The *n*-hexane layers were combined and passed directly through a silica gel column (1 × 5 cm, conditioned by washing with *n*-hexane), then washed with 10 ml of *n*-hexane. The effluent was collected in a 50-ml pear-shaped flask and concentrated to an appropriate volume under reduced pressure at about 20°C. A volume of 1–2 μl of the concentrated solution was injected directly into the gas chromatograph under suitable conditions.

Animals

Randomized groups of five to eight mature male rabbits (Japanese White rabbit, 3.5–4.0 months old, 2.0–2.3 kg body weight, obtained from Nippon Bio-supp. Centre, Tokyo, Japan) were used.

Administration of tetraalkyltins

For intravenous administration, tetraalkyltin homologues were first dissolved in 100% ethanol, then were carefully mixed with saline solution in a syringe (1 part of ethanol to 2–3 parts of saline solution). The final concentration of ethanol in the preparation was 20–30%. The preparation was slowly injected into the ear vein of rabbits, the dose levels used being 2.0 mg/kg body weight for tetraethyltin, 2.5 mg/kg for tetrapropyltin and 3.0 mg/kg for tetrabutyltin (equivalent to 8.5 μmole/kg of each tetraalkyltin). The rabbits were killed at 30 or 180 min after administration and liver, kidney, brain and whole-blood samples were prepared for gas chromatographic analysis of tetraalkyltins.

RESULTS AND DISCUSSION

Selection of analytical conditions

Gas chromatographic conditions. Using the HFID and an electron-capture detector (ECD), the resolution of tetraalkyltins was examined on various stationary liquid phases and retention times, separating state, peak sharpness and sensitivities were established. The HFID was more sensitive and selective towards tetraalkyltins than the ECD. It was possible to elute tetraalkyltins through polar stationary phases such as polyethylene glycol. In particular, the complete separation of tetraalkyltins was achieved on a 10% PEG 20M column within 25 min at temperatures from 50 to 150°C (Fig. 2). This column also gave satisfactory peak shapes and sensitivity. Low-polarity and non-polar stationary phases such as QF-1, SE-52, SE-30, OV-1, OV-17 and squalane could not be used because of adsorption and decomposition of the tetraalkyltins. The retention time of tetraalkyltins was affected by the polarity of the stationary phase. With polar stationary phases, tetraalkyltins were separated according to their molecular weights and boiling points. The solid support (Shimalite W, 80–100 mesh) used was treated by baking it at 300°C for 5 h, washing it with acid and alkali, drying it at 50°C and silylating it with dimethyldichlorosilane. The air, hydrogen and nitrogen flow-rates were 70, 90 and 60 ml/min, respectively.

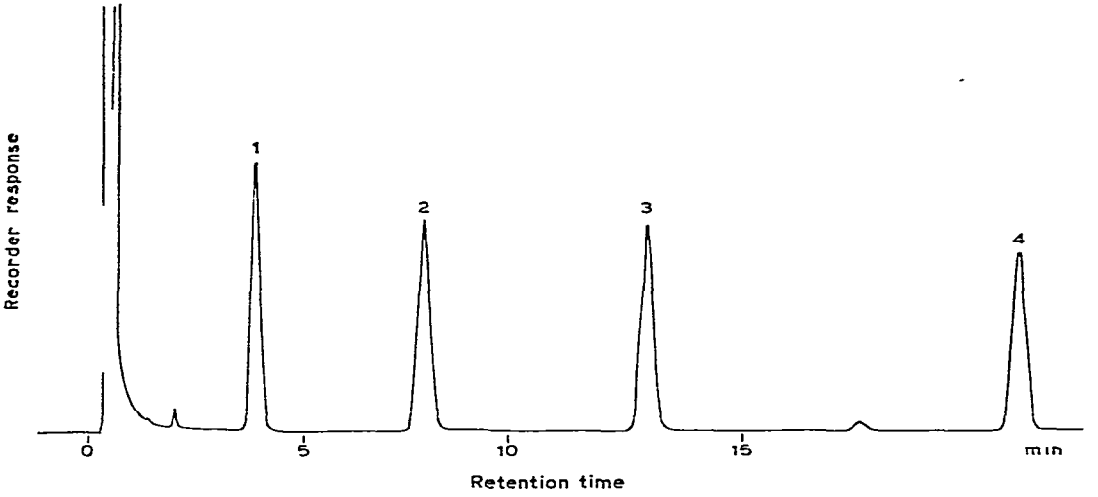

Fig. 2. Gas chromatogram of tetraalkyltin compounds. Column: 10% PEG 20M on Shimalite W (80–100 mesh), 2.0 m × 3 mm I.D. Temperatures: column, programmed from 50 to 150°C at 4°C/min; HFID, 180°C. Flow-rates: air, 70 ml/min; H_2, 90 ml/min; N_2, 60 ml/min. Sensitivity: 100. Range: 0.8 V. Peaks: 1 = Et_4Sn; 2 = Et_4Pb (internal standard); 3 = Pr_4Sn; 4 = Bu_4Sn.

Internal standard. The internal standard should have a retention time at about the mid-point of the chromatogram or in this instance an an elution temperature of about 90–100°C. To minimize errors due to mechanical losses, the internal standard was added to the crude sample before the preparation steps were begun. Tetraethyllead seemed to possess the necessary characteristics and hence was selected as the internal standard.

Calibration graphs. Standard solutions containing approximately equal concentrations (about 10 μg/ml) of tetraethyllead and varying concentrations (about 5–20 μg/ml) of the standard tetraalkyltin compounds in *n*-hexane were prepared. Under the gas chromatographic conditions specified in the legend to Fig. 2, calibration graphs were established for peak heights of tetraethyltin, tetrapropyltin and tetrabutyltin. Linear calibration graphs indicated good working ranges for the compounds tested. Detection limits reached $1 \cdot 10^{-8}$ g for tetraalkyltin compounds.

Sample preparation. Extraction of tetraalkyltins from tissues with various solvents was examined. Tetraalkyltins with a high solubility in organic solvents could be easily extracted by using low-polarity and non-polar solvents such as benzene, toluene, *n*-hexane and ethyl acetate. *n*-Hexane was selected as the most suitable solvent for the extraction of tetraalkyltins because it was also suitable for the next step, silica gel column chromatography. The recovery of double extractions with *n*-hexane was about 98%.

For the purification of tetraalkyltins from *n*-hexane-soluble substances in biological materials, column chromatography using silica gel was examined. Tetraalkyltins were not adsorbed on *n*-hexane-conditioned silica gel and could be easily separated from other slightly polar substances. Mono-, di- and trialkyltin compounds were adsorbed.

Analysis of tetraalkyltin mixtures

Standard solutions containing various amounts of tetraalkyltins in *n*-hexane were analysed according to the proposed procedure. The overall recovery of tetraalkyltins was 98–102% (Table I).

TABLE I

ANALYSIS OF STANDARD SAMPLE

Five standard solutions of tetraalkyltins were subjected to the gas chromatographic method using 10 μg of tetraethyllead as internal standard. Gas chromatographic conditions are described in the legend of Fig. 2.

Compound	*Added (μg)*	*Found (μg)*					*Average*	
		1	*2*	*3*	*4*	*5*	*μg*	*Recovery (%)*
Tetraethyltin	10	10.2	9.8	11.3	9.5	10.0	10.2	102
Tetrapropyltin	15	14.8	15.1	15.0	15.4	15.2	15.1	101
Tetrabutyltin	20	19.8	20.0	19.8	19.5	18.9	19.6	98

Addition studies

The application of the method to the analysis of tetraalkyltins in mammals was studied by conducting recovery tests on animal tissues. Equal amounts (85 nmole) of tetraalkyltins were added to various rabbit tissues and the recoveries were determined (Table II). The average recovery was 97–104%. There was no difference in the recoveries from different organs.

Application to in vivo studies

Rabbits given tetraalkyltins (8.5 μmole/kg of each) intravenously were killed 30 or 180 min after administration, and the concentrations of tetraalkyltins in the

TABLE II

RECOVERY OF TETRAALKYLTIN COMPOUNDS ADDED TO RABBIT TISSUES *IN VITRO*

Three tetraalkyltins (85 nmole of each) were added to various tissues (5 g) and subjected to the gas chromatographic method using 20 μg of tetraethyllead as internal standard. Gas chromatographic conditions are described in the legend of Fig. 2. Each result is the average of five determinations (mean ± standard error).

Compound	*Added (μg)*	*Organ*	*Average*	
			Found (μg)	*Recovery (%)*
Et_4Sn	20	Blood	20.8 ± 0.2	104.0 ± 0.9
		Liver	20.1 ± 0.3	100.4 ± 1.5
		Kidney	20.4 ± 0.3	101.8 ± 1.5
		Brain	20.6 ± 0.3	103.0 ± 1.4
Pr_4Sn	25	Blood	25.6 ± 0.2	102.2 ± 0.9
		Liver	25.6 ± 0.3	102.6 ± 1.1
		Kidney	25.5 ± 0.3	102.2 ± 1.3
		Brain	25.5 ± 0.2	101.9 ± 0.8
Bu_4Sn	30	Blood	29.0 ± 0.3	96.8 ± 1.1
		Liver	30.0 ± 0.4	100.1 ± 1.2
		Kidney	29.8 ± 0.4	99.3 ± 1.4
		Brain	29.9 ± 0.2	99.8 ± 0.7

liver, kidney, brain and whole blood were determined. The results are shown in Table III and Fig. 3. This experiment indicated that for a sample containing more than 0.1 μg of tetraalkyltins per gram of tissue (wet weight), the method is accurate enough for quantitative analysis.

TABLE III

DISTRIBUTION OF TETRAALKYLTIN COMPOUNDS IN RABBIT ORGANS AFTER INTRAVENOUS ADMINISTRATION

Tissue samples (1–5 g) from rabbits given three tetraalkyltins (8.5 μmole/kg of each) were subjected to the gas chromatographic method using 10 μg of tetraethyllead as internal standard. Gas chromatographic conditions are described in the legend of Fig. 2. Tetraalkyltins are expressed as μg/g of tissue (wet weight). Results are means ± standard errors (5–8 animals per group).

Organ	*Compound*	*Time after administration (min)*	
		30	*180*
Blood	Et_4Sn	12.3 ± 0.25	7.3 ± 0.10
	Pr_4Sn	13.2 ± 0.23	2.4 ± 0.04
	Bu_4Sn	12.5 ± 0.25	2.3 ± 0.04
Liver	Et_4Sn	5.5 ± 0.07	2.0 ± 0.02
	Pr_4Sn	1.5 ± 0.02	7.5 ± 0.17
	Bu_4Sn	1.2 ± 0.02	7.8 ± 0.15
Kidney	Et_4Sn	3.7 ± 0.05	6.4 ± 0.12
	Pr_4Sn	3.9 ± 0.05	1.6 ± 0.03
	Bu_4Sn	2.7 ± 0.03	1.0 ± 0.03
Brain	Et_4Sn	1.2 ± 0.02	0.5 ± 0.01
	Pr_4Sn	0.5 ± 0.01	0.6 ± 0.01
	Bu_4Sn	0.5 ± 0.01	0.6 ± 0.02

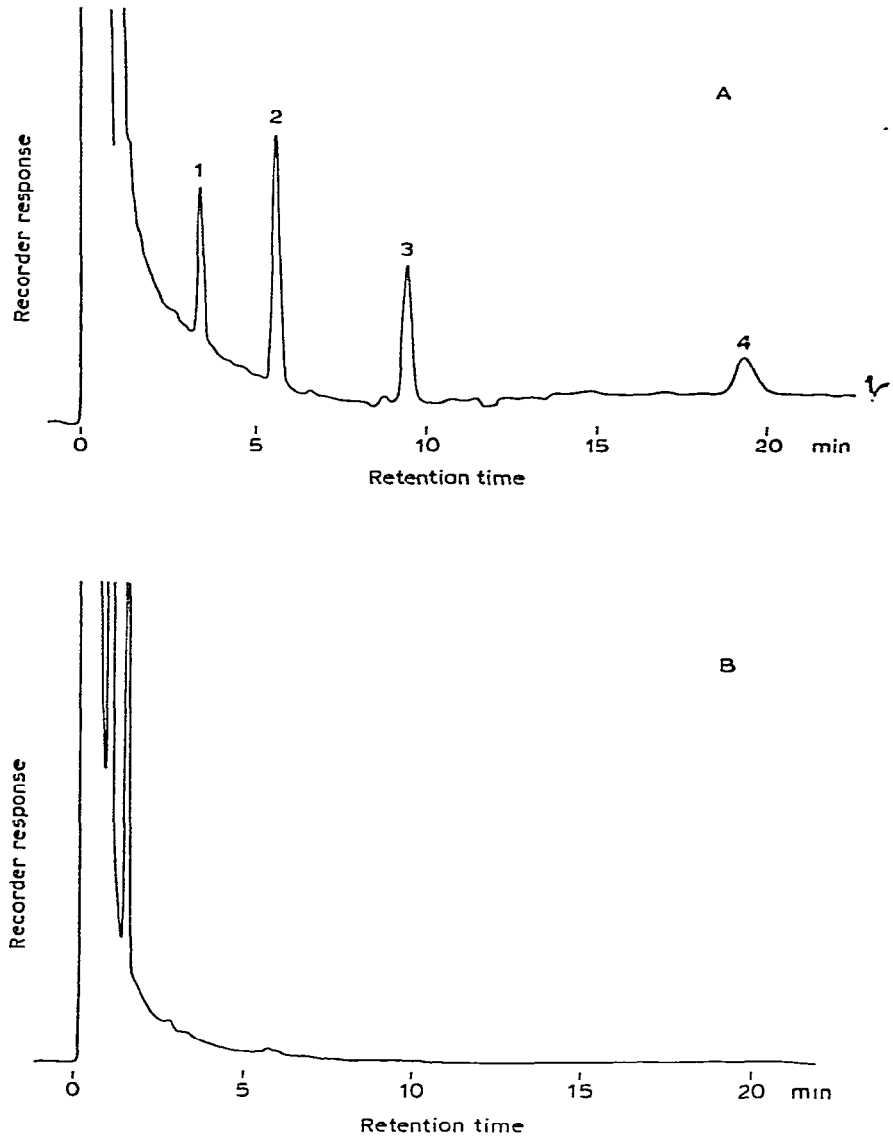

Fig. 3. Gas chromatograms of the liver extracts of rabbit (A) treated with tetraalkyltin compounds and (b) untreated. Column: 10% PEG 20M on Shimalite W (80–100 mesh), 2.0 m × 3 mm I.D. Temperatures: column, programmed from 70 to 130°C at 4°C/min; HFID, 180°C. Carrier gas: N_2 at 90 ml/min. Sensitivity: 100. Range: 0.8 V. Peaks: 1 = Et_4Sn; 2 = Et_4Pb (internal standard;) 3 = Pr_4Sn; 4 = Bu_4Sn.

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