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Gas chromatographic determination of inorganic tin in rat urine after a single oral administration of stannous chloride and mono-, di-, and triphenyltin chloride

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ABSTRACT

A method is described for the determination of inorganic tin by gas chromatography with flame photometric detection. The inorganic tins, stannous and stannic, were extracted with hydrochloric acid and *n*-hexane–benzene in the presence of 0.05% tropolone, and both inorganic tins were pentylated to tetrapentyltin with a Grignard reagent prior to gas chromatography. The absolute limit of detection for tetrapentyltin was 3 pg as tin. The recovery of stannous chloride added to rat urine samples was 80.2 ± 2.4% (mean ± S.D., $n = 8$). The application of this method to the study of urinary excretion of inorganic tin and organotin compounds in rats following oral administration of tin compounds is presented. The urinary excretion of tin compounds was observed over a period of 96 h following administration of stannous chloride or phenyltin compounds. Most of the inorganic tin was excreted into urine within 24 h after administration of stannous chloride. In the experiments on organotin administration, the level of the excretion as total tin for monophenyltin reached a maximum *ca.* 0–24 h after administration, whereas the maxima for di- and triphenyltin were found after 24–48 h and 48–72 h, respectively. The predominant excretion product of these tin compounds found in urine was monophenyltin.

INTRODUCTION

Organotin compounds have been used mainly as poly(vinyl chloride) stabilizers, industrial catalysts, agricultural biocides, and marine antifoulants, etc. World consumption of organotin compounds in 1989 was $35 \cdot 10^6$ kg [1]. However, these compounds have wide ranging toxicological properties, and environmental pollution from some organotin derivatives has become a serious problem [1,2]. Although to date the use of these compounds in Japan is regulated, butyl- and phenyltin compounds are still found in environmental samples [3]. In recent years, we have developed a satisfactory method for measuring the concentrations of organotin compounds by capillary gas chromatography (GC) with flame photometric detection (FPD) [4], and we have applied this method to the study of the biological fate of orally administered organotin chemicals in rats [4,5]. Since it is difficult to determine inorganic tin by gas chromatography, the data obtained from these studies did not include data on inorganic tin. However, the simultaneous determination of both the inorganic tin and the

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organotin compounds is necessary to get a complete picture.

The present paper describes the gas chromatographic quantitation of inorganic tin. The method is applied to the analysis of inorganic tin and organotin compounds in rat urine samples.

EXPERIMENTAL

Materials

All chemicals were used without additional purification. Triphenyltin chloride (TPhT) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Diphenyltin dichloride (DPhT) and phenyltin trichloride (MPhT) were from Alfa Products (Danvers, MA, USA). Pentylmagnesium bromide (2.0 *M* in diethyl ether) and tetrapentyltin were from Aldrich (Milwaukee, WI, USA). Tropolone (2-hydroxy-2,4,6,-cycloheptatrienone) was from Sigma (St. Louis, MO, USA). Anhydrous sodium sulphate, *n*-hexane, and benzene of pesticide grade, hydrochloric acid of trace-metal analysis grade, and stannous chloride solution (1 mg Sn per ml of 6 *M* HCl) for atomic absorption spectrometry were from Wako (Osaka, Japan). Florisil PR was also from Wako, activated at 130°C for 6 h, after which 10% (w/w) distilled water was added. The 10% water-deactivated Florisil was rinsed with *n*-hexane before use. All other reagents were of the highest grade commercially obtainable.

Standard solutions

Each phenyltin compound was dissolved in tetrahydrofuran (THF) at concentrations between 16.2–16.9 mg per 10 ml as stock solutions and stored at 4°C. Working standard solutions for determination of tin compounds in urine were prepared by diluting the stock solutions and stannous chloride solution with distilled water. These solutions were added to control urine (urine of the rats before treatment with tin compounds). For recovery studies standard solutions of tetrapentyltin were prepared by dissolving 0.2–0.8 μg as tin in 5 ml of toluene.

Animal treatment

Male Wistar rats weighing 190–210 g (Doken, Ibaraki, Japan), were housed individually in a metabolic cage (Metabolica MC-ST, Sugiyamagen, Tokyo, Japan) for the collection of urine samples. The rats were acclimated to constant laboratory conditions (25 ± 2°C and 60 ± 10% relative humidity) for at least 7 days prior to use in the experiments. The animals having fasted for 20 to 24 h prior to the treatment, were given a single oral administration of 15.4 mg as tin per kg of body weight (29.3 mg of $SnCl_2 \cdot 2H_2O$ was dissolved in 10 ml of distilled water. Fifty mg of TPhT, 44.6 mg of DPhT, and 39.2 mg of MPhT were suspended in 10 ml of sesame oil. Each solution was administered as 10 ml per kg of body weight). Urine samples were collected before dosing and at 0–6, 6–24, 24–48, 48–72, and 72–96 h after the start of the experiment. All samples were stored at −30°C until analyzed.

Extraction procedures

Samples for GC were prepared according to our previously described procedure [5] with some minor modifications. Urinary tin compounds were extracted as their chloride. To a 5-ml urine sample in a glass-stoppered tube, 10 ml of saturated sodium chloride solution and 0.5 ml of concentrated hydrochloric acid were added. The mixture was extracted twice with 12 ml of *n*-hexane–benzene (3:2, v/v. containing 0.05% tropolone) by agitating for 20 min in a mechanical shaker. After shaking, 0.1 g of sodium dodecyl sulfate (SDS) was added to the tube. The mixture was centrifuged for 10 min at 1800 *g*. The upper phase was collected. The second extraction was done in the same way without further addition of SDS. Both organic phases were combined and dried with 0.5 g of anhydrous sodium sulphate. The organic extracts were transferred to a separatory funnel, and 1 ml of pentylmagnesium bromide was added. The reaction mixture was incubated for 20 min under occasional gentle stirring at room temperature and subsequently extracted with 25 ml of diluted hydrochloric acid (0.5 *M*) for 2 min. The organic layer was rinsed with 25 ml of distilled water and evaporated to near dryness at 40°C after drying with 0.5 g of anhydrous sodium sulphate. The residue was dissolved in 1 ml of *n*-hexane and applied to a Florisil column (2 g, 10 mm I.D.). The pentyl derivatives of the tin compounds

were eluted with 25 ml of *n*-hexane (ca. 1 ml/min). The effluent was evaporated and made up to 5 ml with toluene for GC. A 1-μl volume of the sample solution was injected into the GC. The standard solution used for GC–mass spectrometry (GC–MS), was pentylated in the same way as the organic extracts of the urine samples.

Apparatus and analytic conditions

A Hewlett-Packard (Avondale, PA, USA) Model 5890 A GC equipped with an FPD (at 610 nm), an HP Model 7673 automatic sampler, an HP Model 3396 integrator, and a 12 m × 0.2 mm I.D. HP Ultra-1 capillary column (100% dimethyl polysiloxane gum, 0.33-μm film), were employed for measuring the tin compounds. GC analysis was carried out under the same condition as described previously [5].

Pentylation of the inorganic tin compounds was confirmed on an HP Model 5890 A GC connected with a 5970 B mass selective detector (quadrupole mass filter). The GC–MS was operated using HP 59970 C MS chemistation computer software. The capillary column, an HP Ultra-1 (25 m × 0.2 mm I.D., 0.33-μm film), was directly interfaced to the mass spectrometer ion source. Electron impact (EI) mass spectra were recorded at an ionization potential of 70 eV. The injector and detector temperatures were maintained at 260°C and 280°C, respectively. The column temperature was initially 60°C, held for 2 min, increased to 160°C at 40°C/min, and finally from 160°C to 260°C at 20°C/min. Helium was the carrier gas with a column-head pressure of 207 kPa (an average linear velocity of 36 cm/s).

For comparison of the GC method with the atomic-absorption spectrometric (AAS) method, an Hitachi Z-8100 AAS equipped with an SSC-200 autosampler (Hitachi Koki, Tokyo, Japan) was used. The AAS was performed according to Chiba's method [7].

RESULTS AND DISCUSSION

Determination of inorganic tin

Flame photometric detection has been used in the GC determination of the organotin compounds. Takahashi *et al.* [8] have reported that the flame emission band spectra, originating from Sn-H molecules in a strongly reductive hydrogen flame have a strong band at 609.5 nm. In our previous studies [5,6], we applied GC–FPD with a 610 nm filter for determination of organotin compounds in biological materials after pentylation had been used to form the volatile derivatives, and detected a tetrapentyltin derived from inorganic tin. In addition, since tropolone can act as a tin complexing reagent [9], the extraction procedure was performed with *n*-hexane–benzene containing this compound.

Fig. 1 shows the gas chromatograms of a tetrapentyltin standard (A) and the pentylated tin compound extracted from control rat urine spiked with stannous chloride (B). The peak of the pentyltin derivative (Fig. 1B) was found at a retention time of 7.3 min, which corresponds to that of tetrapentyltin. The chromatogram had a stable baseline, and showed high sensitivity.

Confirmation by GC–MS

Further confirmation was performed by GC–MS. Fig. 2 shows EI mass spectra obtained from standard solutions containing tetrapentyltin (A) and pentylated stannous chloride (B). Both spectra showed almost the same pattern. No molecular-ion cluster is observed, but a major mass peak at *m/z* 333 for tetrapentyltin is detected caused by the loss of one pentyl group. In addition, stannic chloride was pentylated in the same way as stannous chloride. This pentyltin derivative was also identified as a tetrapentyltin by the above mentioned GC–MS technique. These results show that both inorganic tins, stannic and stannous, were detected

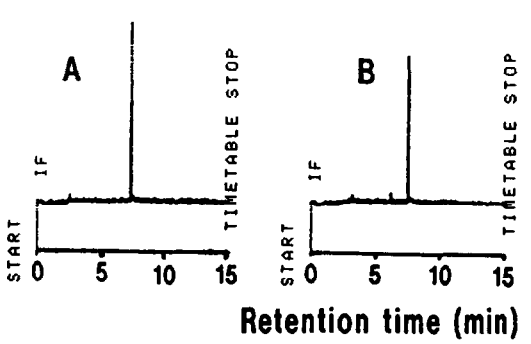

Fig. 1. Gas chromatograms of standard solutions of tetrapentyltin (0.2 μg as tin in 5 ml of toluene) (A) and pentylated tin compound extracted from control rat urine spiked with stannous chloride (the amount of stannous being 0.2 μg as tin per 5 ml of urine) (B).

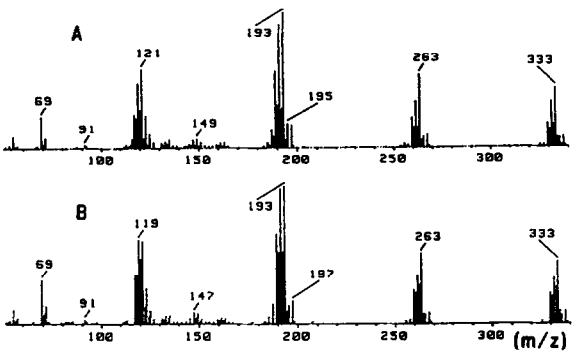

Fig. 2. EI mass spectrum of the pentyltin derivative. A: tetrapentyltin standard. B: pentylated derivatives of stannous chloride.

as tetrapentyltin by the GC method described here.

Calibration and detection limit

The calibration curve was obtained by the correlation of the known amounts of pentylated stannous chloride injected into the GC and the peak heights as tin. The peak height of the detector response with FPD is linear with a working concentration of stannous chloride in the range 0.2–0.8 μg as tin per 5 ml of urine.

The absolute limit of detection for tetrapentyltin, determined on the basis of a signal-to-noise ratio of 3, was 3 pg as tin. For the determination of inorganic tin in urine samples, the limit of detection was 3 ng as tin per ml of urine.

Precision and recovery

The precision of the method was estimated as the coefficient of variation (C.V.), which was quantified using five aliquots of the same control urine samples spiked with stannous chloride (0.4 μg as tin). The above calibration curve was used to determine the precision. The amount of tin was established at 0.402 ± 0.009 μg (mean $\pm$ S.D.) and the C.V. value was 2.2%.

Recovery experiments were carried out by adding stannous chloride at 0.2, 0.4, 0.6, and 0.8 μg as tin to 5 ml of the control rat urine samples or distilled water. The absolute recovery from the urinary solutions for inorganic tin was calculated as $80.2 \pm 2.4\%$ (mean $\pm$ S.D., $n = 8$) as measured from the experiments with direct injection of commercial tetrapentyltin. There was no difference in the recoveries from urinary solutions and aqueous solutions.

Comparison with AAS

To confirm the reliability of the GC method described here, fifteen urine samples from rats exposed to stannous chloride were collected as described in the Experimental section, and assayed by this method and the AAS method [7]. The results obtained with the two methods are shown in Fig. 3. The concentrations of inorganic tin obtained by GC and AAS on the same urine samples correlated well. The correlation coefficient is 0.988 ($n = 15$) and the line of least regression is $y = 1.088x + 0.185$.

Animal study

The GC method described in this paper was also applied to a study of the urinary excretion of tin compounds in rats. The urinary excretion of tin compounds was observed periodically for 96 h after a single oral administration of stannous chloride or phenyltin compounds (MPhT, DPhT, and TPhT). Fig. 4B shows a typical chromatogram of the tin compounds obtained from control rat urine spiked with stannous chloride and MPhT, DPhT, and TPhT. Fig. 4C shows a chromatogram of the tin compounds extracted from a urine specimen collected from

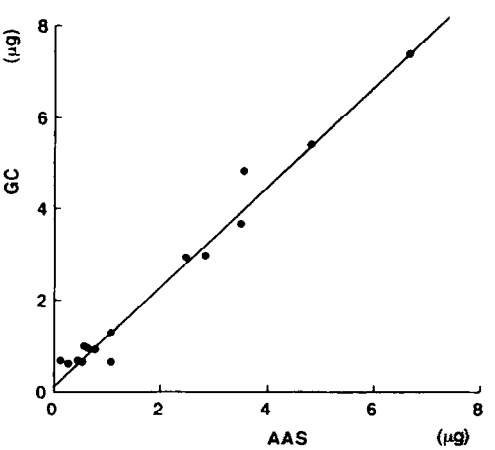

Fig. 3. Relation between the inorganic tin values obtained by the AAS method of Chiba [7] and the present method.

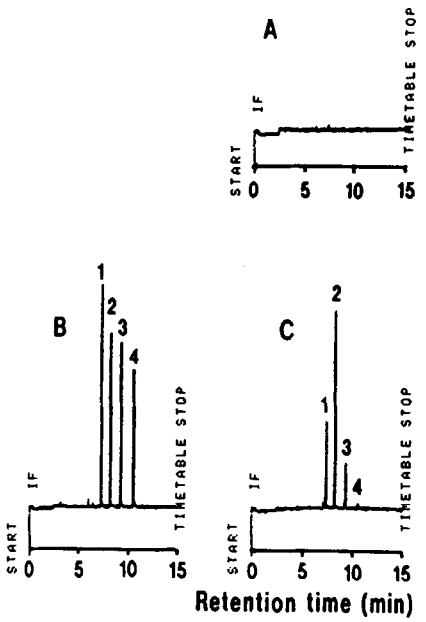

Fig. 4. Gas chromatograms of extracts from the rat urine samples. A: control sample of rat urine (no administration of tins). B: control sample of rat urine spiked with stannous chloride and mono-, di-, and triphenyltin chloride (the amount of each tin was 0.4 μg as tin per 5 ml of urine). C: rat urine sample collected from 24 to 48 h after a single oral administration of triphenyltin chloride. Peaks: 1 = tetrapentyltin, 2 = tripentylphenyltin, 3 = dipentyldiphenyltin, 4 = pentyltriphenyltin.

24 to 48 h after administration of TPhT. As shown in Fig. 4, peaks corresponding to triphenyltin and its metabolites, di- and monophenyltin and inorganic tin, were detected in the same sample of rat urine dosed with TPhT while no significant peaks were seen in the control sample (Fig. 4A). The chromatograms show that there are no serious interferences from the urine matrix and that the peaks are separated well.

Furthermore, the peak heights of both tetrapentyltin and the three phenyltin compounds exhibited a linear relationship with the concentrations of each tin compound and no significant amounts of the tin compounds were detected in control rat urine. These results show that it is possible to use the calibration curves to determine the amount of tin compounds in rat urine exposed to stannous or phenyltin compounds. Fig. 5 represents the time courses for the urinary excretion of tin compounds in rats after administration of stannous chloride (A), MPhT (B), DPhT (C), and TPhT (D).

Most of the inorganic tin was excreted into urine within 24 h after administration of stannous chloride. A relatively low level of inorganic tin excretion was observed in urine samples obtained after more than 24 h, showing a moderate decrease. This suggests that part of the administered inorganic tin is accumulated in the rat body (Fig. 5A).

In the experiments on organotin administration, the level of the excretion as total tin for the monophenyltin reached a maximum *ca.* 0–24 h following administration, whereas the maxima for di- and triphenyltin were found after 24–48 h and 48–72 h, respectively. Kimmel *et al.* studied the metabolism of triphenyltin acetate in rats with a 113 Sn-labelled compound and the determination of tin in urine [10]. For administration of TPhT, our results were similar to those of Kimmel *et al.* Additionally, we found that although all three different types of phenyltins were excreted, *ca.* 70% of the total tin was excreted as monophenyltin. Also in the case of dosing with MPhT and DPhT, the principal excretion product found in urine was monophenyltin. These findings show that the monophenyltin form has an efficient polarity for excretion into urine. The average amount of total tin excretion during the 96-h period following administration of each phenyltin compound was 14.9 μg for MPhT, 23.8 μg for DPhT, and 28.2 μg for TPhT. These results are probably due to the differences in the lipophilicity of the phenyltin compounds, triphenyltin being more lipophilic than mono- and diphenyltin.

The GC method reported in this paper will probably be widely applicable for studies on tin compounds.

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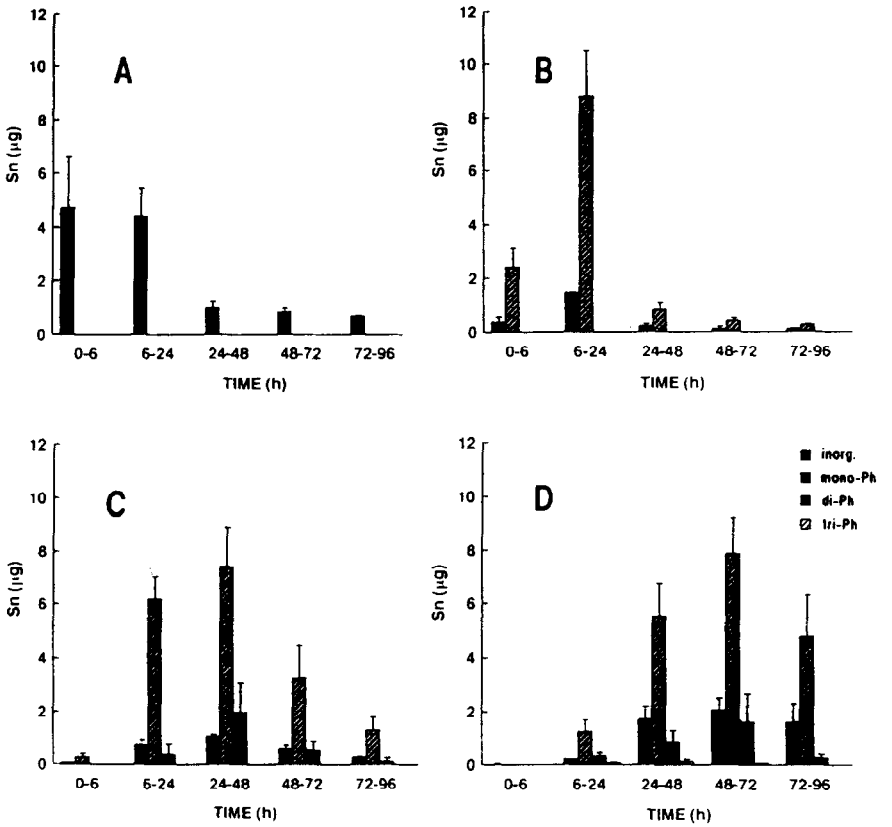

Fig. 5. Time courses of urinary excretion of tin compounds in rats after a single oral administration of stannous chloride (A), phenyltin trichloride (B), diphenyltin dichloride (C), and triphenyltin chloride (D). Values represent mean ± S.D. of three animals.

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