Journal of Chromatography, 258 (1983) 189–197
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,538

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF DIALKYLTIN HOMOLOGUES USING FLUORESCENCE DETECTION

TAI-HONG YU* and YASUAKI ARAKAWA
Department of Hygiene and Preventive Medicine, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bukyo-ku, Tokyo 113 (Japan)
(Received November 15th, 1982)

SUMMARY

A simple, sensitive and reproducible method has been developed for the simultaneous determination of dialkyltin compounds and their possible metabolites. Dialkyltin compounds were separated by high-performance liquid chromatography on a cyanopropyl-bonded column with *n*-hexane–ethyl acetate (95:5) containing 5% acetic acid and detected by a fluorescence detector with an excitation wavelength at *ca.* 420 nm and an emission wavelength at *ca.* 500 nm after post-column derivatization with Morin reagent. Detection limits ranged from 0.1 to 1 ng depending upon the dialkyltin species. Recoveries of dialkyltins added to various tissues at the 5-nmole level ranged from 91 to 99%.

INTRODUCTION

Dialkyltin compounds have been widely used as heat and light stabilizers for certain plastics. Dioctyltin derivatives are permitted by the U.S. Food and Drug Administration as additives in food-grade polyvinyl chloride (PVC) for wrapping and for containers. Dibutyltin derivatives are utilized for PVC medical devices as being more effective stabilizers. Dimethyltin derivatives have also been found to be suitable for the stabilization of PVC plastics.

Recently, deep concern has been expressed on the safety of these organotin stabilizers migrating from PVC products into food or finally into the body[1,2]. Consequently, the development of better analytical methods has been needed for the determination of the various organotin compounds present in environmental and biological samples.

A variety of analytical techniques and procedures have been applied to the determination of organotin species in aqueous environments. These include spectrophotometry[3,4], fluorometry[4], thin-layer chromatography[5], gas chromatography[6,7], gas chromatography–mass spectrometry[8], gas chromatography–flame photometry[9,10], atomic absorption spectrometry[11,12] and high-performance liquid chromatography (HPLC)–atomic absorption spectrometry[13,14]. Of the available methods,

0021-9673/83/0000–0000/$03.00

gas chromatography and HPLC appear to be the most versatile. Some of the methods, however, are too complicated while others suffer from unsatisfactory sensitivity, precision, reproducibility, specificity or lower recoveries in derivatization and separation of the alkyltins. Moreover, non of them gives optimum analytical conditions for the simultaneous determination of alkyltin homologues which have the same number of alkyl chain. In previous reports, we described gas chromatographic methods for the simultaneous determination of tetraalkyl-[15] and trialkyltins[16] in various kinds of biological material. Unfortunately, the gas chromatographic determination of dialkyltin homologues was not easy because of their adsorption and decomposition during chromatography.

For the purpose of applying HPLC to the determination of dialkyltins, therefore, we have developed a detection technique that makes use of spectrofluorometry. Basic studies on fluorometry revealed that Morin (2',3,4',5,7-pentahydroxyflavone) could be used to determine trace amounts of organotins, especially dialkyltins[17]. The work described here is based on this procedure, and is achieved by post-column fluorescence derivatization with Morin.

EXPERIMENTAL

Reagents

Dioctyltin dichloride (Oc_2SnCl_2) was purchased from Aldrich (Milwaukee, WI, U.S.A.). Dibutyltin dichloride (Bu_2SnCl_2) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Dipropyltin dichloride (Pr_2SnCl_2), diethyltin dichloride (Et_2SnCl_2) and dimethyltin dichloride (Me_2SnCl_2) were purchased from K & K Labs. (Plainview, NY, U.S.A.). The purity of the dialkyltin compounds was greater than 98%. When necessary, compounds were purified by distillation or by silica gel column

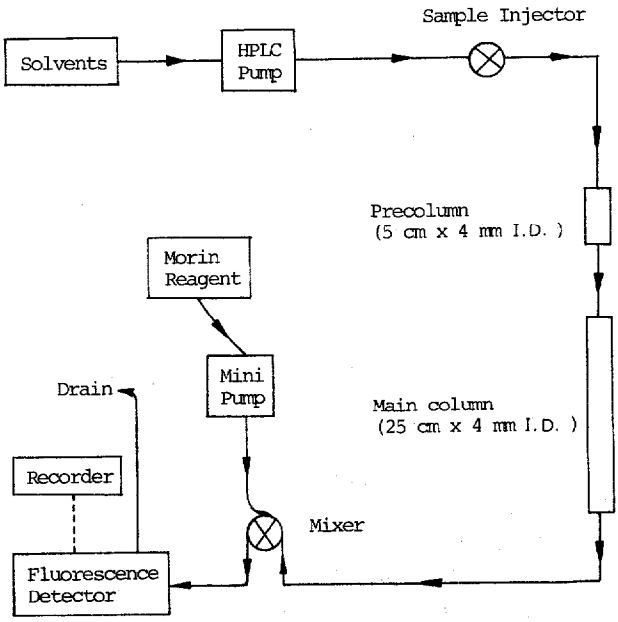

Fig. 1. The HPLC–fluorescence detector system.

chromatography on silica gel before use. Morin (2',3,4',5,7-pentahydroxyflavone) was obtained from Wako (Osaka, Japan). Other chemicals were purchased from commercial sources in reagent-grade purity. The pre-mixed mobile phase of *n*-hexane–ethyl acetate–acetic acid was passed through a minipore filter under reduced pressure in order to degass and remove obstructive particles.

HPLC

An outline of the HPLC–fluorescence detector system is shown in Fig. 1. The high-performance liquid chromatograph consisted of a constant-flow pump (Model KHD-16, Kyowa Seimitsu), a minimpump (Milton Roy), a sample injector (Model 7125, Rheodyne), a mixer (Kyowa Seimitsu) and a fluorescence detector (Shimadzu Model RF-500). The stainless-steel column (25 cm × 4.0 mm I.D.) was packed with Unisil QCN (cyanopropyl-bonded phase, Gasukuro Kogyo). The precolumn (5 cm × 4 mm I.D.) was also packed with the same fillers. The mobile phase was made up of *n*-hexane–ethyl acetate (95:5) containing 5% acetic acid. The flow-rate was 1.2 ml/min. The injected sample was modified with Morin post-column solution (0.005% Morin in ethanol; flow-rate, 0.5 ml/min) in the mixer after passing through the main column, and was then monitored by the fluorescence detector with an excitation wavelength at 420 nm and an emission wavelength at 500 nm. Other HPLC conditions are given in the figures.

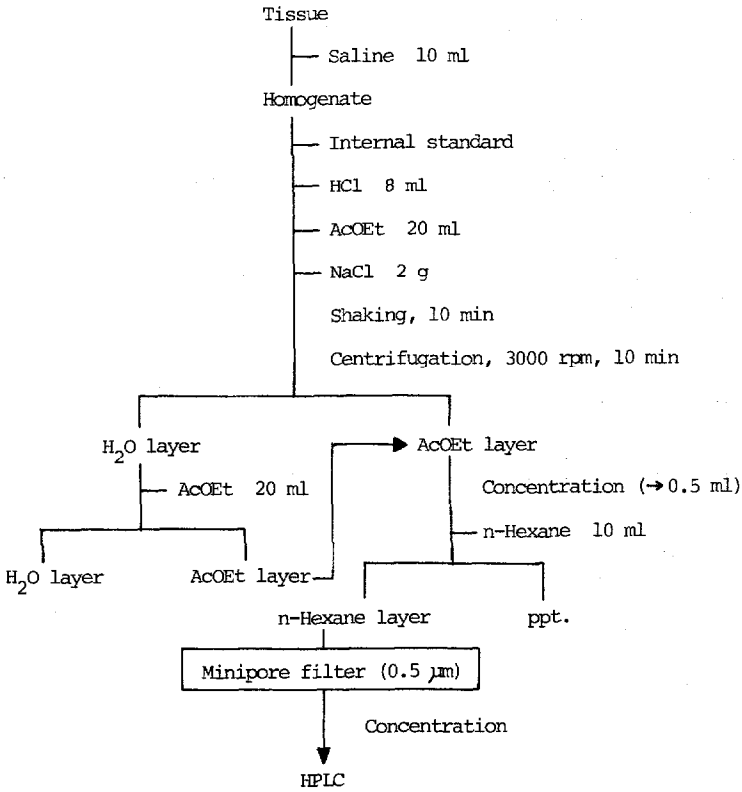

Fig. 2. Preparation of dialkyltin compounds from tissues. AcOEt = Ethyl acetate.

Preparation of dialkyltin compounds from tissues

As shown in Fig. 2, a sample of tissue weighing between 1.0 and 5.0 g (wet weight) was homogenized in 10 ml of normal saline solution. Hydrochloric acid (8 ml) was carefully added to the homogenate, followed by the addition of a suitable amount of internal standard (dialkyltin other than the object of analysis). The contents of the tube were mixed thoroughly and allowed to stand for 5 min. Ethyl acetate (20 ml) and sodium chloride (2 g) were then added, and the contents were extracted by shaking for 10 min. After centrifugation for 10 min at 1000 *g*, the ethyl acetate layer was transferred to a 100-ml pear-shaped flask. This extraction procedure was repeated twice. The combined ethyl acetate layers were then concentrated under reduced pressure at normal water temperatuire (*ca.* 20°C) to a volume of 0.5–1.0 ml. *n*-Hexane (10 ml) was added to the concentrated solution and the precipitate produced was removed by centrifugation. The *n*-hexane layer of the supernatant was filtered by a minipore filter (pore size, 0.5 μm) and concentrated to a suitable volume for HPLC.

Oral treatment studies

A randomized group of five to eight male weanling rats (Wistar-derived, weighing 40–50 g, obtained from Nippon Bio-supp. Center, Tokyo, Japan) was used. The dialkyltin compounds were first dissolved in 100% ethanol and then thoroughly mixed with a powdery stock diet at levels of 100 ppm by a spray method. Animals fed the test diet were killed 1, 2 or 3 weeks after treatment, and the liver, kidney, brain, spleen and thymus were prepared for HPLC analysis of the dialkyltins.

RESULTS AND DISCUSSION

Post-column fluorescence derivatization with Morin

The basic studies on the fluorometry of organotins revealed that Morin could be used to determine trace amounts of dialkyltins[17]. The formation of the fluorescent Morin–dialkyltin complex progressed very rapidly and quantitatively at room temperature. Moreover, the complex was stable for a long time in organic solvents such as *n*-hexane and ethyl acetate. The maximum excitation and emission wavelengths

TABLE I

MAXIMUM EXCITATION AND EMISSION WAVELENGTHS OF DIALKYLTIN–MORIN COMPLEXES

Sample: 3 ml of 10 μ*M* solution of dialkyltin in *n*-hexane–ethyl acetate (90:10) containing 5% of acetic acid with 3 ml of 0.005% solution of Morin in ethanol.

Compound	*Approximate wavelength (nm)*	
	Excitation	*Emission*
Me_2SnCl_2	420	499
Et_2SnCl_2	420	498
Pr_2SnCl_2	420	496
Bu_2SnCl_2	420	497
Oc_2SnCl_2	420	495

of the fluorescent complexes are shown in Table I. Although quercetin and 3-hydroxyflavone are similar to Morin in structure, they are unsuitable because of their sensitivity and instability. From these findings, Morin was selected as the most suitable fluorometric reagent for the simultaneous determination of dialkyltin homologues by a combination of HPLC and spectrofluorometry. The fluorescent complex, however, could not be passed through any stationary phases in spite of varying the mobile phases because of their adsorption and decomposition. Therefore, it was decided to conduct the derivatization of dialkyltins with Morin by the post column system.

Selection of analytical conditions

HPLC. Using the HPLC–fluorescence detector system shown in Fig. 1, the resolution of dialkyltins was examined on various stationary and mobile phases. It was possible to elute dialkyltins through an adsorbent column: in particlar, a complete separation of dialkyltins was achieved on a cyanopropyl-bonded phase column (Unisil QCN) with *n*-hexane–ethyl acetate (95:5) containing 5% acetic acid. This column gave satisfactory peak shapes and sensitivity. A reversed-phase column such as C_{18} could be also used by using aqueous bufferized acetonitrile as the mobile phase. However, the separation of the dialkyltins was rather poor and their retention times were affected by their polarity. The dialkyltins were separated according to their molecular weights on a cyanopropyl-bonded phase column. The addition of 5%

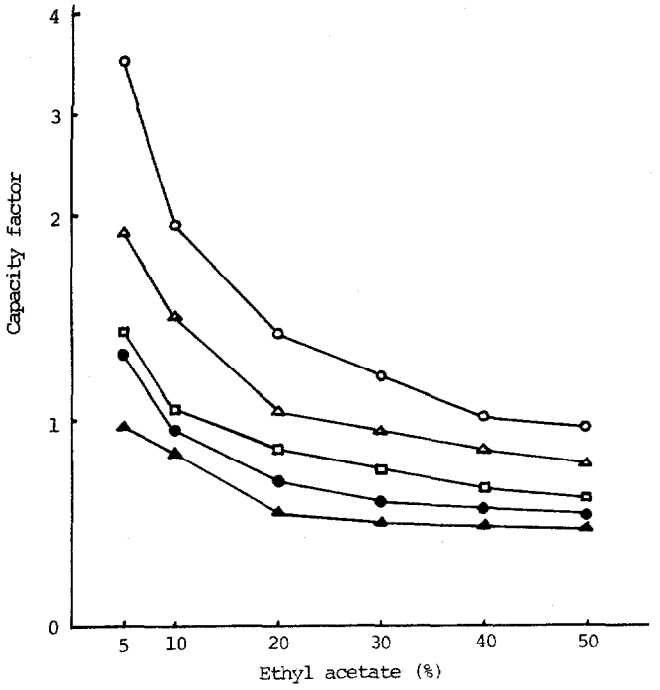

Fig. 3. Effect of the composition ratio of ethyl acetate to *n*-hexane in the mobile phase for the separation of dialkyltin compounds. Column: Unisil QCN, 25 cm × 4 mm I.D. Mobile phase: *n*-hexane–ethyl acetate mixtures containing 5% acetic acid. Other conditions are described in Fig. 4. ○, Me_2SnCl_2; △, Et_2SnCl_2; □, Pr_2SnCl_2; ●, Bu_2SnCl_2; ▲, Oc_2SnCl_2.

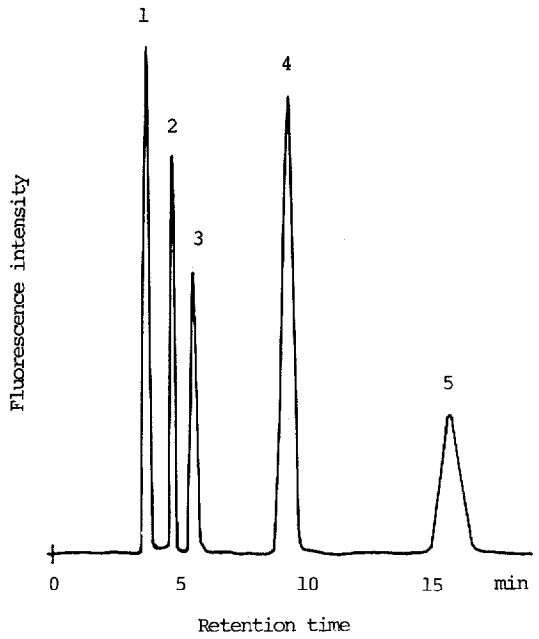

Fig. 4. Chromatogram of dialkyltin compounds. Column: Unisil QCN, 25 cm × 4 mm I.D. Mobile phase: *n*-hexane–ethyl acetate (95:5) containing 5% acetic acid. Flow-rate: 1.2 ml/min. Post-column reagent: 0.005% Morin in ethanol. Post-column flow-rate: 0.5 ml/min. Fluorescence detector: excitation wavelength at 420 nm, emission wavelength at 500 nm. Peaks: 1 = Oc_2SnCl_2; 2 = Bu_2SnCl_2; 3 = Pr_2SnCl_2; 4 = Et_2SnCl_2; 5 = Me_2SnCl_2.

acetic acid to the mobile phase was necessary to prevent any adsorption of dialkyltins on the support surface; also, the ratio of ethyl acetate in the mobile phase needed to be lower (Fig. 3).

Internal standard. To minimize errors due to mechanical losses, an internal standard was added to the crude sample before preparation. A dialkyltin other than the object of analysis was used as an internal standard.

Calibration graphs. Standard mixtures containing various amounts (0.01–1.0 μg/ml) of dialkyltin dichlorides and an approximately equal amount of internal standard (a dialkyltin other than the object of analysis) in *n*-hexane–ethyl acetate (9:1) was prepared. Under the HPLC conditions specified in the legend to Fig. 4, calibration graphs were established for the peak heights of dimethyltin, diethyltin, dipropyltin, dibutyltin and dioctyltin. The linearity of the calibration graphs indicated good working ranges for the compounds tested. Detection limits ranged from 0.1 to 1 ng depending upon the species of dialkyltin.

Addition studies

The application of this method to the analysis of dialkyltins in mammals was studied by conducting recovery tests on animal tissues. Approximately equal amounts (*ca.* 5 nmole of each) of dialkyltin dichlorides were added to various rat tissues and the recoveries were determined (Table II and Fig. 5). The average recoveries ranged from 91 to 99%. No difference in recoveries was seen among the different organs.

TABLE II

RECOVERY OF DIALKYLTIN COMPOUNDS ADDED TO RAT TISSUES *IN VITRO*

Five dialkyltins (5–15 nmole of each) were added to various tissues (1–5 g) and subjected to the HPLC method using 2.0 μg of Et_2SnCl_2 as internal standard. HPLC conditions are described in the legend to Fig. 4. Each result is the average of five determinations (mean ± standard error).

Compound	*Added (μg)*	*Organ*	*Average*	
			Found (μg)	*Recovery (%)*
Oc_2SnCl_2	2.08	Liver	2.03 ± 0.01	97.7 ± 0.6
		Kidney	2.03 ± 0.03	97.5 ± 1.0
		Spleen	2.03 ± 0.02	97.7 ± 0.6
		Brain	2.01 ± 0.04	96.5 ± 1.3
		Thymus	2.03 ± 0.01	97.5 ± 0.4
Bu_2SnCl_2	3.04	Liver	3.00 ± 0.01	98.9 ± 0.6
		Kidney	2.99 ± 0.02	98.4 ± 0.9
		Spleen	3.00 ± 0.01	98.7 ± 0.5
		Brain	2.98 ± 0.01	98.0 ± 0.5
		Thymus	3.01 ± 0.01	99.1 ± 0.2
Pr_2SnCl_2	2.76	Liver	2.63 ± 0.02	95.3 ± 0.9
		Kidney	2.64 ± 0.02	95.6 ± 0.9
		Spleen	2.61 ± 0.02	94.8 ± 1.0
		Brain	2.65 ± 0.02	95.6 ± 1.0
		Thymus	2.69 ± 0.01	97.5 ± 0.5
Et_2SnCl_2	2.48	Liver	2.28 ± 0.01	91.8 ± 0.4
		Kidney	2.30 ± 0.02	92.6 ± 0.9
		Spleen	2.30 ± 0.01	92.7 ± 0.7
		Brain	2.29 ± 0.01	92.3 ± 0.3
		Thymus	2.32 ± 0.01	93.4 ± 0.4
Me_2SnCl_2	3.30	Liver	3.01 ± 0.02	91.3 ± 0.8
		Kidney	3.02 ± 0.02	91.5 ± 0.6
		Spleen	3.02 ± 0.02	91.7 ± 0.7
		Brain	3.02 ± 0.01	91.6 ± 0.4
		Thymus	3.03 ± 0.01	92.1 ± 0.5

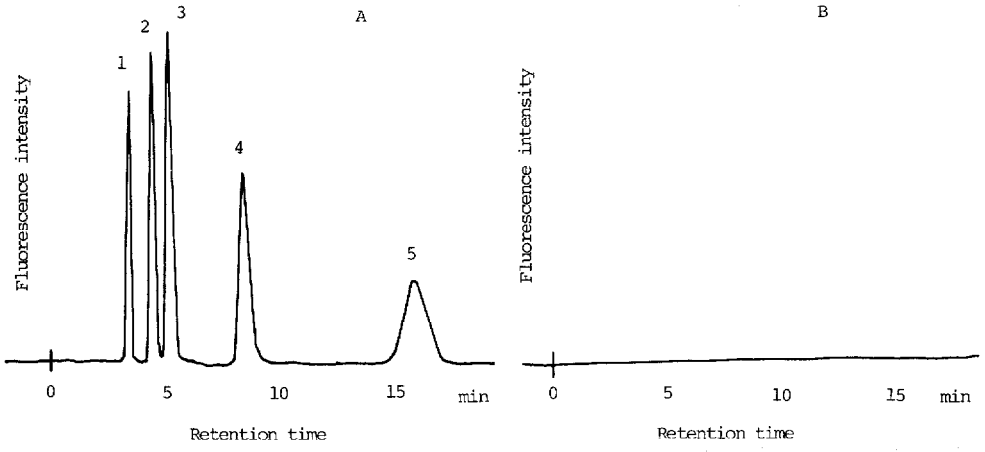

Fig. 5. Chromatograms of the extracts of rat liver treated with dialkyltin compounds (A) and untreated (B). Details as in Fig. 4.

TABLE III

DISTRIBUTION OF DIALKYLTIN COMPOUNDS IN RAT ORGANS AFTER ORAL ADMINISTRATION

Tissue samples (1–5 g) from rats given dibutyltin dichloride at dietary levels of 100 ppm were subjected to the HPLC method. HPLC conditions are described in the legend to Fig. 4. Dibutyltin is expressed as ng per g of tissue (wet weight). Results are means ± standard errors (5–8 animals per group).

Feeding period (weeks)	*Organ*				
	Liver	*Kidney*	*Spleen*	*Brain*	*Thymus*
1	16.4 ± 0.1	20.0 ± 0.1	6.3 ± 0.1	2.7 ± 0.1	4.1 ± 0.1
2	26.8 ± 0.1	41.6 ± 0.1	5.3 ± 0.1	4.1 ± 0.1	4.0 ± 0.1
3	13.4 ± 0.1	17.9 ± 0.1	3.9 ± 0.1	3.2 ± 0.1	4.2 ± 0.1

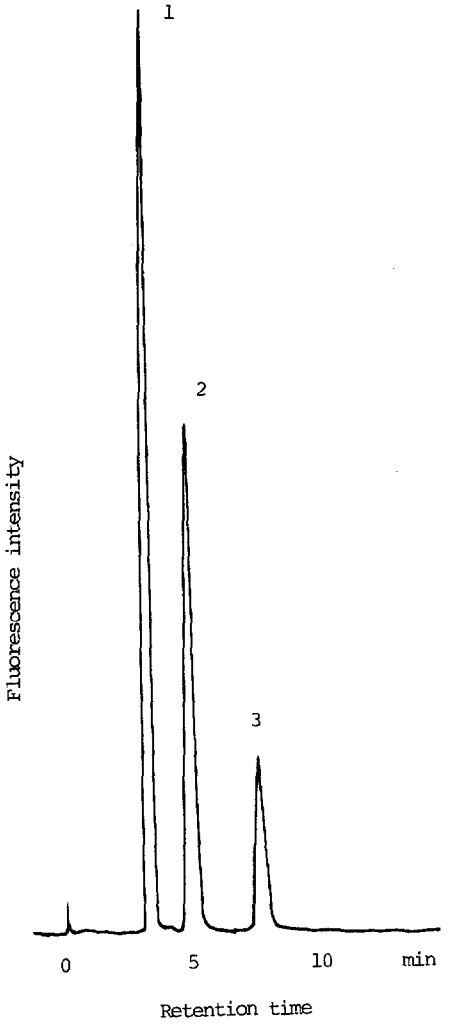

Fig. 6. Chromatogram of dibutyltin metabolites in rat kidney 3 weeks after oral administration. Column: Unisil QCN, 25 cm × 4 mm I.D. Mobile phase: *n*-hexane–ethyl acetate (80:20) containing 5% acetic acid. Post-column reagent: 0.005% Morin in ethanol. Other conditions are described in Fig. 4. Peaks: 1 = original dibutyltin; 2 = metabolite I; 3 = metabolite II.

Moreover, no peak interfering with the determination was seen in the control extracts, as shown in Fig. 5B.

Application to in vivo studies

The HPLC method was applied to dibutyltin metabolites in rats after oral administration. Table III shows the weekly distribution of dibutyltin compounds in organs after oral treatment. Dibutyltin was distributed to every organ; however, the levels in the liver and kidney were much higher than in other organs and reached a maximum after 2 weeks. On the other hand, the level in the thymus did not change and also was lower than expected throughout the 3 weeks observation period when thymus atrophy occurred to a significant extent. In addition, on and after 2 weeks some metabolites of dibutyltin, probably hydroxybutyltin, were found in the liver and kidney (peaks 2 and 3 in Fig. 6). The metabolites collected were confirmed as containing tin by atomic absorption spectrometry; their structural analyses are now in progress.

By these experiments, it has been shown that the method can be used for the simultaneous determination of dialkyltin homologues or their possible metabolites in biomaterials containing more than 1 ng/g of tissue (wet weight). The application of this method to studies on the metabolism of dibutyltin compounds in mammals will be published elsewhere.

REFERENCES

1 W. T. Piver, *Environ. Health Perspect.* 4 (1973) 61.
2 M. Nimni, *J. Pharm. Sci.*, 53 (1964) 1262.
3 R. T. Skeel and C. E. Bricker, *Anal. Chem.*, 33 (1961) 428.
4 W. N. Aldridge and B. W. Street, *Analyst (London)*, 106 (1981) 60.
5 J. E. Casida and E. C. Kimmel, *Acta Chem. Scand.*, 25 (1971) 1497.
6 C. J. Soderquist and D. G. Crosby, *Anal. Chem,.*, 50 (1978) 1435.
7 R. S. Braman and M. A. Tompkins, *Anal. Chem.*, 51 (1979) 12.
8 H. A. Meinema, T. B. Wiersma, G. V. Haan and E. C. Gevers, *Environ. Sci. Technol.*, 12 (1978) 288.
9 S. Kapila and C. R. Vogt, *J. Chromatogr. Sci.*, 18 (1980) 144.
10 W. A. Aue and C. G. Flinn, *J. Chromatogr.*, 142 (1977) 145.
11 V. F. Hodge, S. L. Seidel and E. D. Goldberg, *Anal. Chem.*, 51 (1979) 1256.
12 H. L. Trachman, A. J. Tyberg and P. D. Branigan, *Anal. Chem.*, 49 (1977) 1090.
13 K. L. Jewett and F. E. Brinckman, *J. Chromatogr. Sci.*, 19 (1981) 583.
14 F. E. Brinckman, W. R. Blair, K. L. Jewett and W. P. Iverson, *J. Chromatogr. Sci.*, 15 (1977) 493.
15 Y. Arakawa, O. Wada, T. H. Yu and H. Iwai, *J. Chromatogr.*, 207 (1981) 237.
16 Y. Arakawa, O. Wada, T. H. Yu and H. Iwai, *J. Chromatogr.*, 216 (1981) 209.
17 Y. Arakawa, O. Wada and M. Manabe, *Anal. Chem.*, submitted for publication.