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Determination of Tri-n-butyltin and Di-n-butyltin Compounds in Yellowtails

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Various tri-n-butyltin and di-n-butyltin compounds have been widely used as stabilizers of poly(vinyl chloride), fungicides and marine antifoulants, and they are known to be aquatic environmental contaminants. In Japan bis(tri-n-butyltin)oxide (TBTO) has been used popularly as an antifoulant in marine farms.
It is well known that tri-n-butyltin compounds degrade in aqueous environment to di-n-butyltin by photolysis or biolysis (Maguire et al. 1983, 1985). Dealkylation reduces the toxicity of alkyltin compounds to aquatic organisms (Vighi et al. 1985). However, the toxicities of di-n-butyltin compounds administered orally to rat are known to be almost equivalent to tri-n-butyltin compounds (Sharratt et al. 1980). It is necessary therefore to monitor di-n-butyltin compounds as well as tri-n-butyltin compounds in marine products.

We previously reported an analytical method for TBTO and other tri- and di-n-butyltin compounds in fish (Sasaki et al. 1988). The method consists of a two-step extraction, cleanup by automated gel permeation chromatography, alkylation with Grignard reagent and gas chromatography with flame photometric detection (FPD-GC). This paper outlines the development of a simpler analytical method for these compounds, using one-step extraction, alkylation, silica gel column chromatography, and FPD-GC. This procedure is also used to assay tri- and di-n-butyltin compounds in yellowtails reared in marine farms.

MATERIALS AND METHODS

All chemicals used were analytical reagent grade. Organic solvents were distilled in glass before use. Distilled water was used at all times.

Tri-n-butyltin chloride (Bu_3SnCl) and di-n-butyltin dichloride (Bu_2SnCl_2) were purchased from Sankyo Organic Chemicals Co., Ltd. and Wako Pure Chemical Industries, Ltd., respectively.
Ethylmagnesium bromide (3M in diethyl ether) was purchased from

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Tokyo Chemical Industry Co., Ltd. Silica gel (Kieselgel 60, Art. 7734, Merck) was activated at 450°C for 3 h and 10% (w/w) water was added. Yellowtails, which were called Wakashi, Inada, Hamachi, and Buri in Japanese in order of the growth stage, were purchased from a retail market. Hamachi and Buri were thought to be probably farm reared while the Wakashi and Inada were of natural origin.

The liver, white muscle, and red muscle were removed from carcasses, and each sample was homogenized with 2 volumes of methanol. The homogenate which is equivalent to 5 g of tissue was blended with 10 g of NaCl, 50 mL of 3N HCl, and 100 mL of diethyl ether-hexane (3:2). After centrifugation, the organic phase was removed, and the remaining aqueous layer was re-extracted with 100 mL of the same solvent mixture. The combined organic extract was mixed with 0.1 g of $NaHCO_3$ and 0.5 mL of water, and dried over anhydrous Na_2SO_4. The extract was concentrated to ca. 2 mL and 2-4 mL of C_2H_5MgBr and 2 mL of diethyl ether were added. A excess amount of C_2H_5MgBr was added to the extract which bubbled vigorously upon addition of the reagent. After standing for 30 min, 10 mL of distilled water, ca. 0.2 g of Na_2SO_3, and 1-2 mL of conc. HCl were added to the reaction mixture. The organic layer was separated, and the aqueous layer was extracted twice with 5 mL of hexane. The combined extract was dried over anhydrous Na_2SO_4 and evaporated to near dryness. The concentrate was applied to a silica gel (10 g, i.d.10 mm) column. The ethyl derivatives were eluted with 35 mL of hexane. The eluate was evaporated just to dryness, and redissolved in 2 mL of hexane for FPD-GC.

The standard solution, prepared in the same way but without silica gel column chromatography, was stored with a small portion of crystallized Na_2SO_3.

A gas chromatograph, model 9A (Shimadzu Co.), equipped with a flame photometric detector was operated in the tin mode (filter for 610 nm). The sample was injected into a fused-silica capillary column (CBP 10, Shimadzu Co., 0.53 mm x 12 m). Column temperature: programmed from 130°C (hold 4 min) at the rate of 40°C/min to 210°C. He carrier gas: 20 mL/min, H_2: 150 mL/min and air: 100 mL/min.

RESULTS AND DISCUSSION

Figure 1 shows FPD gas-chromatograms of tri-n-butyl ethyltin (Bu_3EtSn) and di-n-butyl diethyltin (Bu_2Et_2Sn). Ethyl derivatives were used instead of the methyl derivatives used in our previous work (Sasaki et al. 1988). Methyl derivatives are so volatile that they can be lost when the solvent is removed carelessly under reduced pressure.

Ethyl derivatives showed slight tailing on the chromatograms. The tailing is ascribed to the structure of detector (Maguire et al. 1981). The detection limits for both Bu_3EtSn and Bu_2Et_2Sn were 0.05 ng.

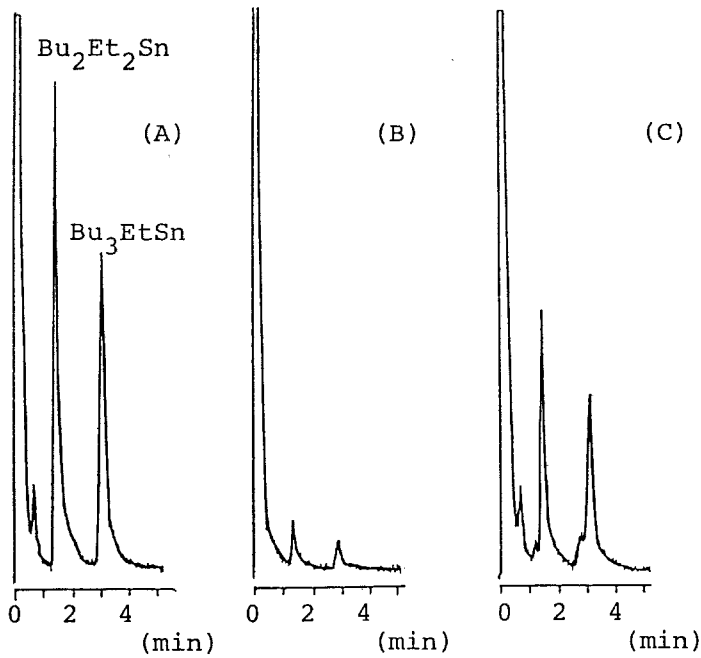

Figure 1. Gas chromatograms of Bu_2Et_2Sn and Bu_3EtSn (A) each 3 ng; (B) each 0.3 ng; (C) each 3 ng treated without Na_2SO_3

Bu_3SnCl and Bu_2SnCl_2 were quantitatively converted to the corresponding ethyl derivatives within 30 min at room temperature when the reaction mixture contained over 60% diethyl ether. Addition of Na_2SO_3 to the reaction mixture prevented the decomposition of the ethyl derivatives by free halogen which was liberated during the treatment (Figure 1).

It is well known that Bu_3SnCl and Bu_2SnCl_2 are polar and easily adsorbed to column packings such as silica gel or Florisil; therefore a silica-gel column-chromatographic cleanup procedure was used after formation of the ethyl derivatives.

Recoveries of Bu_3SnCl and Bu_2SnCl_2, which were added to fish at levels of 0.8 ppm and extracted after standing overnight, are given in Table 1. Average recoveries of the two compounds added to pond smelt ranged from 92 to 103% and were favorable regardless of solvent used for sample preparation.

On the other hand, Bu_3SnCl that was added to the tuna homogenized with water or 0.5 N HCl, was poorly recovered. The recovery of Bu_3SnCl that was added to the methanolic homogenate of tuna was 83%. This might be attributed to the binding of Bu_3SnCl to protein and the difference of protein contents between tuna and pond smelt samples.
Recoveries of Bu_3SnCl added to animal feed or casein were 65 and 31%, respectively. Recovery from the former could be improved to

Table 1. Recovery of Bu_3SnCl and Bu_2SnCl_2 from added fish samples

Sample	Homogenized with	Recovery (%)*	
		Bu_2SnCl_2	Bu_3SnCl
pond smelt	0.5N HCl	103 ± 5	92 ± 7
	CH_3OH	94 ± 4	96 ± 1
tuna	water	92 ± 2	29 ± 5
	0.5N HCl	77	22
	CH_3OH	91 ± 6	83 ± 2

Fortification level: 0.8 μg/g
*: average of duplicate or triplicate analysis

87% with addition of methanol to the solvent mixture used for extraction. Methanol therefore was used routinely for homogenization of the fish samples.

Table 2 shows the analytical results of tri- and di-n-butyltin compounds in yellowtails. Almost all samples had levels above the detection limit (0.005 μg/g). We previously reported that the residue values of Bu_3SnCl and Bu_2SnCl_2 in yellowtails with FPD-GC showed good agreement with the residues from GC-MS analysis. So in this work FPD-GC analysis was used.

Bu_2SnCl_2 was present at the highest levels in liver (from 0.025 to 3.7 μg/g) followed by red muscle and then white muscle. Bu_3SnCl on the other hand was present in liver and red muscle at the similar levels with the lowest levels in white muscle.
Wakashi and Inada contained more Bu_3SnCl than Bu_2SnCl_2 in both the liver and white muscle but the liver of Hamachi contained Bu_2SnCl_2 rather than Bu_3SnCl. Little Bu_2SnCl_2 was found in the white muscle of Hamachi.

The difference in the Bu_2SnCl_2/Bu_3SnCl ratio between Wakashi or Inada and Hamachi might be attributed to increased metabolic ability of fish as growth occurs.
As Bu_2SnCl_2 is more polar than Bu_3SnCl, the Bu_2SnCl_2 detected in fish might not come from absorption of Bu_2SnCl_2 in aquatic environment but from metabolism of Bu_3SnCl in fish liver.

The correlation coefficients between the concentration of Bu_3SnCl and that of Bu_2SnCl_2 in each tissue ranged from 0.864 to 0.991 (Table 3), i.e., concentrations of Bu_2SnCl_2 in liver and muscle are correlated with those of Bu_3SnCl in each tissue and Bu_2SnCl_2 and Bu_3SnCl in muscle are correlated with those in liver.

Table 2. Analysis for Bu_2SnCl_2 and Bu_3SnCl in yellowtails

Stage of growth	No.	Concentration (ppb)					
		Bu_2SnCl_2			Bu_3SnCl		
		White*	Red*	Liver	White*	Red*	Liver
Wakashi**	1	14	-	76	33	-	127
	2	5	-	67	26	-	93
	3	5	-	46	22	-	97
	4	7	-	100	34	-	190
	5	14	-	-	80	-	-
	6	8	-	109	55	-	122
Inada**	1	<5	4	25	9	18	30
	2	5	10	47	19	59	64
	3	8	20	75	52	122	127
	4	<5	6	35	11	21	26
Hamachi**	1	53	225	3740	1570	2390	1730
	2	<5	44	433	102	188	162
	3	<5	24	315	63	109	96
	4	<5	27	195	66	107	78
Buri**	1	5	17	-	62	96	-
	2	20	76	-	308	560	-
	3	8	32	-	130	200	-

-: not analyzed
*: White:white muscle; Red:red muscle
**: Yellowtails are called Wakashi, Inada, Hamachi, and Buri in Japanese in order of the growth stage.

Table 3. Correlation coefficient of Bu_2SnCl_2 and Bu_3SnCl in muscle and liver

	Bu_2SnCl_2 in			Bu_3SnCl in		
	white muscle	red muscle	liver	white muscle	red muscle	liver
Bu_2SnCl_2 in						
white muscle	1.000					
red muscle	0.984	1.000				
liver	0.926	0.976	1.000			
Bu_3SnCl in						
white muscle	0.963	-	-	1.000		
red muscle	-	0.987	-	0.991	1.000	
liver	-	-	0.864	0.928	0.991	1.000

In summary, butyltin compounds, especially dibutyltin, were at the highest levels in liver, and butyltin compounds were detected in both natural and marine farmed fish. It is therefore necessary to monitor tri- and di-n-butyltin compounds in both natural and reared fish. The proposed analytical method is suitable for the monitoring of of tri- and di-n-butyltin compounds in fish.

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