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Speciation of butyltins in fish and sediment by means of gas chromatography with flame photometric detection

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A modified method for the determination of tributyl-, dibutyl-, and monobutyl-tin in fish and sediment samples is proposed. The samples are digested with hydrochloric acid and the butyltin compounds are extracted into a tropolone solution in pentane and pentylated by a Grignard reaction. The products are cleaned up by washing with a sodium hydroxide solution, dried over sodium sulphate, concentrated by evaporation and analysed by gas chromatography with flame photometric detection, using an interference filter at 610 nm. Problems peculiar to the fish and sediment samples are overcome by this improved clean-up procedure. The limit of detection for tributyltin in fish is 0.04 $\mu g\ g^{-1}$ and the reproducibility at 0.06 $\mu g\ g^{-1}$, expressed as the relative standard deviation, is 6.8%. Contaminated sediment samples were found to contain the mixed methylbutyltin compounds Me_2BuSn^+ and $MeBu_2Sn^+$.

Keywords: Organotin determination, gas chromatography, fish samples, sediment samples, speciation

INTRODUCTION

Organotin compounds have a wide range of industrial applications, including their use as stabilizers for poly(vinyl chloride) polymers, as catalysts, and as biocidal agents (antifouling paints and plant protection). For this last application trialkyl or triaryl derivatives, mainly butyl, cyclohexyl or phenyl, are the most commonly used. Tributyltin (TBT) compounds are very effective against common marine fouling organisms, but they are also highly toxic to various non-target aquatic organisms at very low levels. Concentrations of about 1 $\mu g\ cm^{-3}$ on the surface of the antifouling paint are sufficient to prevent fouling organisms (algae or barnacles) attaching themselves.[1] Very much lower concentrations, about 1 $\mu g\ dm^{-3}$ or even less, are lethal or affect the growth rate and reproduction of commercial shellfish.[2] In fact, one of the qualities of the tributyltin in such environmental uses is based on its degradation to dibutyl- and monobutyl-tin, which are comparatively innocuous, and eventually on its degradation to non-toxic tin(IV). There is also the possibility of other degradation pathways for tributyltin including redistribution[3, 4] and methylation[5, 6] reactions yielding products whose toxicity is still unknown.

Since some years ago, increasing concern has arisen about the impact of TBT and its degradation products in the marine environment. This concern has resulted in regulations restricting their use in EEC countries. Nevertheless, there is still a necessity for highly sensitive and selective analytical methods, whether for checking the levels of the toxic organotins or for obtaining information on their fate in the environment.

Several methods have been proposed for determining TBT, including graphite furnace–atomic absorption spectrometry (GF AA)[7, 8] or measurement of fluorescence in micellar solution.[9] There are also a variety of analytical methods for speciating organotin compounds. Some of them involve a non-chromatographic separation and use a series of selective solvent extractions[10, 11] or a generation of the hydrides, which are then collected in a trap cooled in liquid nitrogen, after which they are eluted following the sequence of their boiling points and finally detected by AA.[12–15] Chromatographic methods have been more widely used. High-performance thin-layer chromtography (HPTLC) has been proposed for phenyltin compounds.[16] High-performance liquid chromatography (HPLC) coupled with flame-AA or flameless-AA as detection system is a very good approach that has been used by several

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authors.[17-21] Whereas HPLC methods are able to distinguish between organotin compounds without requiring derivatization, gas chromatography (GC) methods are also widely used, but due to the low volatility and thermal lability of the majority of organotin compounds, conversion to volatile derivatives is desirable for GC analysis. A widely utilized technique is the formation of tetra-alkyltin compounds via a Grignard reaction. Several procedures have been described involving the formation of n-hexyl,[22] ethyl,[23] pentyl[24] and methyl[25] derivatives prior to gas chromatography. The formation of the hydrides of the organotins as volatile derivatives has also been reported by several authors.[26-28]

A variety of detectors for the anaslysis of organotins by GC in different samples has been used: flame photometry for fish,[27] harbour waters,[28] natural waters,[24-26] human urine[26,29] and sediments;[23,25] flame ionization for biological materials[30] and waters;[33] electron capture for waters,[32] biological materials and sediments;[31] mass spectrometry (MS) for natural waters and sediments.[25] AA has also been used as a detection system for GC analysis of organotins in waters.[33,34] Table 1 summarizes different methods for organotin speciation.

TBT-treated netting has been used on salmon farms, necessitating the determination of TBT and its degradation products, dibutyl- and monobutyl-tin, in order to assess the levels of the toxic TBT and their fate in farmed-salmon environments and in the environment generally. With this aim, in this paper a GC–flame photometric (GC FPD) system is proposed to speciate butyltin compounds in salmon samples, the method also being reliable for other environmental samples, such as sediments, with low levels of organotins. Problems peculiar to the fish and sediment samples due to a high content of fatty compounds, that are not encountered in water samples, have been overcome in this work by an improved clean-up procedure.

EXPERIMENTAL

Reagents and standards

All reagents were of at least analytical reagent grade. the organotin standards (Aldrich, BDH) were used without further purification. Standard pentyl derivatives were obtained by reaction with 1 mol dm^{-3} pentylmagnesium bromide solution in diethyl ether that was prepared weekly. Dimethyl chloride was used as internal standard, and standard stock solutions (*ca* 279 μg cm^{-3}) were prepared in absolute ethanol. The purity and stability of the standards were checked by GC FID and GC FPD under the same operating conditions as the procedure.

All glassware was washed with aqua regia, double-distilled water, ethanol and diethyl ether, in this order.

Apparatus

A Shimadzu GC8AFP gas chromatograph equipped with a flame photometric detector with an EMI 9601B photomultiplier (range 300–800 nm, maximum at 380 nm), a 610 nm interference filter (Infrared Engineering) and a glass capillary column (12 m × 0.53 mm i.d. coated with a 3.0 μm film of BP-1) was used. The injector and detector were held at 250 °C and the column was temperature programmed from 50 to 250 °C at a heating rate of 10 °C min^{-1}. Nitrogen was used as the carrier gas at a flow rate of 8.5 cm^3 min^{-1} through the column and 37 cm^3 min^{-1} through the splitter. The detector was operated with a hydrogen-rich flame, the hydrogen and air flow-rates being 50 and 85 cm^3 min^{-1}, respectively. The detector output was collected on a Pye–Unicam PU4810 computing integrator. Aliquots of 5 μl were injected and peak heights were recorded. The amount of each organotin present was determined from suitable calibration graphs using Me_2Pe_2Sn ($Me = CH_3$; $Pe = C_5H_{11}$) as the internal standard.

Procedure

Between 5 and 25 g of wet fish tissue or sediment was weighed accurately into a 250 cm^3 separating funnel fitted with a PTFE tap, 40 cm^3 of concentrated hydrochloric acid was added, the funnel shaken for two hours and left to stand for a further two hours. Then 10 cm^3 of hydrobromic acid (48 %) was added and after 15 min the mixture was extracted with 100 cm^3 of a 0.05% (w/v) solution of tropolone in pentane by shaking vigorously for 2 min. After one hour, the organic layer was separated and dried over anhydrous magnesium sulphate. The dried organic layer was placed in a 250 cm^3 round-bottomed flask together with a suitable amount of the internal standard (about

Table 1 Methods of speciation of organotin compounds

Compound	Sample	Separation method	Quantitation method	Reference
Phenyltins	Water	Solvent extraction	Photometry	10
Cyclohexyltins	Water	Solvent extraction	Photometry with toluene-3,4-dithiol	11
Methyltins, butyltins (11–25 ng g^{-1})	Oysters	HG, separation by boiling points	AA	12
Methyltins, butyltins (0.4–1.5 ng)	Seawater	HG, separation by boiling points	AA	13
Tributyltin, dibutyltin (2 ng dm^{-3})	Seawater	HG, separation by boiling points	AA	14
Methyltins (50 pg)	Seawater	HG, separation by boiling points	GF AA	15
Phenyltins (1 ng)		HPTLC	Fluorescence with morin	16
Tin(II), tin(IV), tributyltin (0.47 μg dm^{-3})	Harbour water	HPLC	FAA	17
Methyltins, butyltins, phenyltins (0.03 μg dm^{-3})	Water	HPLC	AA	21
Propyltins, butyltins (5–30 ng)	Water	HPLC	GF AA	18
Methyltins, butyltins, phenyltins (1 ng)		HPLC	ICP AE ICP MS	38
Methyltins, ethyltins, butyltins, phenyltins (2 ng)	Water	GC	FID	33
Butyltins (20 ng dm^{-3})	Water	GC	MS	22
butyltins, phenyltins (1–5 ng g^{-1})	Sediments and biological samples	GC	ECD	31
Butyltins (100 pg)	Fish	GC	FPD	27
Methyltins (0.1 ng)	Water	GC	AA	34

Abbreviations: AA, atomic absorption spectrometry; GF AA, graphite furnace–atomic absorption spectrometry; FAA, flame atomic absorption spectrometry; ICP AE, inductively coupled plasma emission spectrometry; ICP MS, inductively coupled plasma mass spectrometry; GC, gas chromatography; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin-layer chromatography; FID, flame ionization detector; MS, mass spectrometry; ECD, electron capture detector; FPD, flame photometric detector; HG, hydride generation. Cited amounts refer to limit of detection.

3 μg of Me_2SnCl_2). The magnesium sulphate was washed with 5 cm^3 of diethyl ether and this was added to the organic phase. To the dried organic extract 20 cm^3 of 1 mol dm^{-3} pentylmagnesium bromide in diethyl ether was added and the mixture refluxed, with constant stirring, for one hour. The excess of Grignard reagent was destroyed by slowly adding 25 cm^3 of 0.5 mol dm^{-3} sulphuric acid. The organic phase was separated and the aqueous phase extracted with two 10 cm^3 portions of diethyl ether. At this point 100 cm^3 of 3 % (w/v) sodium hydroxide solution was added to the organic phase, which was shaken for 5 min and allowed to stand overnight. The organic phase was filtered into a 250 cm^3 round-bottomed flask and concentrated on a rotary evaporator under reduced pressure at room temperature to a volume of about 2 cm^3. This liquid was transferred to a micro-evaporator (Fig. 1), rinsing the walls of the flask with 5 cm^3 of diethyl ether, and concentrated to 1 cm^3 under a stream of nitrogen. Aliquots of 5 μl were used for the GC analysis.

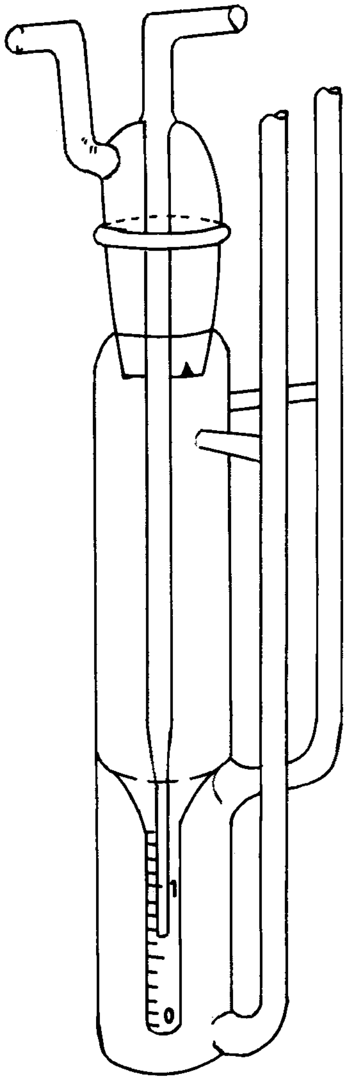

Figure 1 Micro-evaporator.

RESULTS AND DISCUSSION

Extraction of the butyltins from the salmon tissue

To extract the butyltins from the tissue it has to be previously digested. The sample is finely divided to facilitate the action of the hydrochloric acid. Contact with the acid for four hours was adequate; but overnight treatment was convenient in some instances.

Solvents such as benzene, chloroform, methylene chloride,[35] ethyl acetate,[31] hexane[22] and pentane[25] have been used to extract methyltin and butyltin species. However, because of the high polarity of the monobutyl- and dibutyl-tin it is necessary to use tropolone as complexing agent.[35] Solutions of tropolone in the previously mentioned solvents has been reported to extract organotin compounds with good recoveries.[22, 24, 34, 35] Such solutions also extract inorganic tin. As benzene is toxic, and chloroform and methylene chloride react with the Grignard reagent, solutions of tropolone in pentane were used. Hexane was not considered because its properties are similar to those of pentane but it is less volatile.

Table 2 Recoveries after extraction of butyltin species from an acid digest of salmon[a]

Species	Recovery (%)[b]
Bu_3Sn^+	95.3 ± 12.7
Bu_2Sn^{2+}	100.4 ± 3.3
$BuSn^{3+}$	72.7 ± 8.4

[a] Extraction into a 100 cm^3 of a 0.05% (w/v) tropolone solution in pentane. [b] Average of five extractions.

Addition of hydrobromic acid (HBr) prior to the extraction has been reported[35] to assist the recovery of butyltin species, probably because it helps the desorption of these species from the glass of the vessel. Therefore, 10 cm^3 of 48% HBr was added to the acid digest some minutes before the extraction was carried out.

The internal standard, dimethyltin dichloride, is added to the extract just before the derivatization step, i.e. after the extraction step, because hydrobromic acid supresses the extraction of these species.[34]

Experiments were carried out with about 5 g of clean fish spiked with 4 μg of each organotin to check the recoveries. Different volumes of 0.05% tropolone solution in pentane were assayed. Volumes of less than 100 cm^3 gave low recoveries, probably because the organotins were adsorbed on the emulsion which was formed. By extracting once with 100 cm^3 of tropolone solution no emulsion was produced and good recoveries were obtained. Table 2 shows the recoveries.

Table 3 Yields of the derivatization reactions to obtain volatile pentyl derivatives of butyltins

Compound	Product	Yield (%)[a]
Bu_3Sn^+	Bu_3PeSn	99.6
Bu_2Sn^{2+}	Bu_2Pe_2Sn	101.5
$BuSn^{3+}$	$BuPe_3Sn$	92.4

[a] Calculated considering the tin metal content.

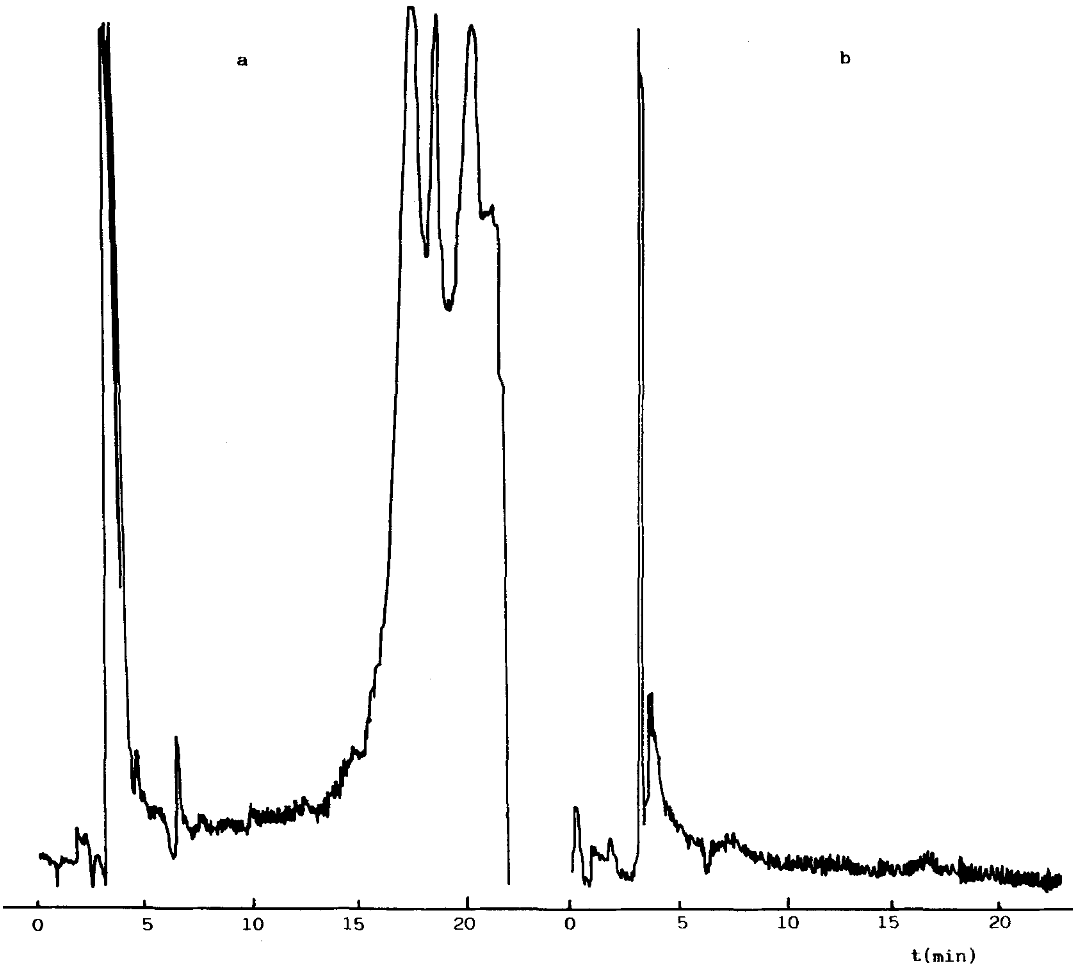

Figure 2 (a) Chromatogram of a fish digest not treated with 3 % NaOH solution. (b) Chromatogram of a fish digest after treatment with 3 % NaOH solution.

Derivatization

As has been stated before, the determination of trialkyl-, dialkyl- and monoalkyl-tin species by GC requires derivatization in order to obtain volatile compounds. We chose to make the n-pentyl derivatives, by means of a Grignard reaction, since some ethyl and propyl derivatives are volatile enough to be lost during routine concentration procedures.[24] Derivatization was carried out under refluxing conditions (at 40 °C) to ensure

Table 4 Linearity range of response of the flame photometric detector for butyltins

Compound	Linear range (ng)	RSD (%)[a]
Bu_3PeSn	70–1.4	4.7
Bu_2Pe_2Sn	80–1.3	6.8
$BuPe_3Sn$	65–1.5	9.4

[a] Evaluated for five injections of 5 ng of each compound.

Table 5 Concentration of butyltin species in salmon liver

	Compound (μg g^{-1})		
Sample	$BuSn^{3+}$	Bu_2Sn^{2+}	Bu_3Sn^{+}
1	0.54	2.01	0.38
2	0.24	1.00	0.28
3[a]	0.22	0.59	4.01
4[a]	0.04	0.05	0.13
5[a]	0.21	0.33	0.21
6	0.56	0.27	0.85
7	0.64	0.42	1.39

[a] Concentration experiments in tanks with: 1.0 (3); 0.1 (4); 1.0 (5) μg g^{-1} of TBT in the water.

the quantitative reaction of the dibutyl- and monobutyl-tins. Derivatization yields for Bu_3Sn^+, Bu_2Sn^{2+} and $BuSn^{3+}$ to give Bu_3SnPe, $BuSnPe_2$ and $BuSnPe_3$ are included in Table 3 ($Bu = C_4H_9$). The magnesium used to prepare the Grignard reagent can be contaminated with tin metal, which after the derivatization step yields tetrapentyltin.

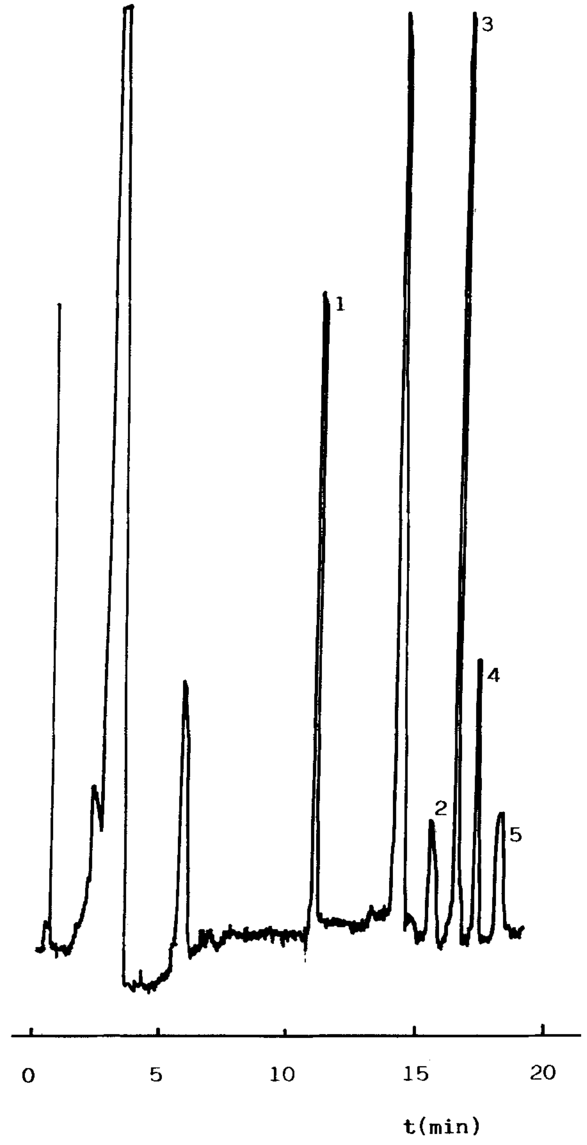

Figure 3 Chromatogram of a salmon liver sample. 1, Me_2Pe_2Sn (internal standard); 2, Bu_3PeSn; 3, Bu_2Pe_2Sn; 4, $BuPe_3Sn$; 5, Pe_4Sn.

Clean-up

Despite the high selectivity of the flame photometric detector (FPD) towards tin-containing compounds, direct injection of an extract from fish tissue led to a chromatogram which showed a very large background interference (Fig. 2). Since the main components of the extract are natural oils and fatty acids, their conversion into water-soluble substances by hydrolysis with sodium hydroxide was shown to be a suitable and easy method to eliminate those interferences. The use of $100\,cm^3$ of 3 % sodium hydroxide aqueous solution was found to be optimum for the clean-up. Recoveries ranging from 86 to 99 % were found from experiments carried out to check the influence of this step.

Concentration

After the extraction and derivatization steps the organotins are contained in a large volume ($\sim 120\,cm^3$) and at a low concentration, and so a concentration step is necessary. Concentration of the samples by rotary evaporation in a Wheaton flask was first attempted, in order to avoid the use of different flasks with consequent losses. However, this technique was very difficult to handle because of the continuous bumping of the solution through the system. A Kuderna–Danish concentrator was also tried but the process was very time-consuming, and was also rejected. Concentration to $1\,cm^3$ was carried out, first in a rotary evaporator and then in a cylindrical flask ending in a narrow graduated tube (Fig. 1) that has been described previously.[36]

Analysis by GC FPD

The chromatographic conditions have been optimized and the emission at 610 nm of the tin species is measured. The standard deviations are lower by using peak heights than by using peak

Table 6 Determination of butyltins in salmon flesh

	Compound ($\mu g\,g^{-1}$)		
Sample	$BuSn^{3+}$	Bu_2Sn^{2+}	Bu_3Sn^+
A	—[a]	0.05	—
B	—	0.07	—
C	—	—	0.18
D	—	—	0.06

[a] —, Not detected.

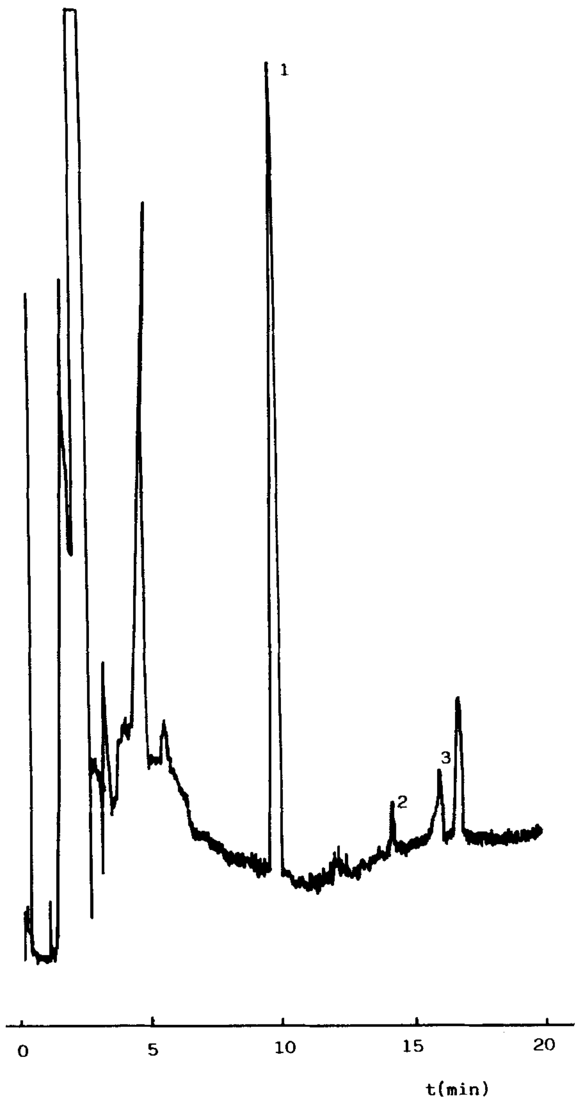

Figure 4 Chromatogram of a salmon flesh sample. 1, Me_2Pe_2Sn (internal standard); 2, Bu_2Pe_2Sn; 3, Pe_4Sn.

areas.[37] Dimethyldipentyltin was used as internal standard and calibration curves, using peak heights, were plotted. Table 4 shows the linearity range and the relative standard deviations (RSD) for 5 ng of each organotin (five injections). Absolute limit of detection is 1.5 ng of butyltin.

Determination of butyltins in salmon liver

The proposed method has been applied to the analysis of several salmon liver samples, obtained from a salmon farm and from experiments on the accumulation effects of tributyltin (carried out in the Marine Laboratory at Aberdeen). Table 5 shows the results, and the chromatogram of one of the samples can be seen in Fig. 3.

Determination of butyltins in salmon flesh

The same procedure was applied to the determination of butyltins in salmon flesh from a fish farm with TBT-treated netting. The results are presented in Table 6. Figure 4 shows the chromatogram of one of the samples.

Determination of organotins in sediment

A sample of sediment with a high percentage of hydrocarbons (36 %, w/w) was analysed by the proposed procedure and the results obtained were: $BuSn^{3+}$, 2.17; Bu_2Sn^{2+}, 3.72; Bu_3Sn^+, 0.64; Me_2BuSn^+, 12.92; $MeBu_2Sn^+$, 11.06 μg g^{-1}. The corresponding chromatogram is shown in Fig. 5.

CONCLUSIONS

The proposed method is shown to be suitable for the analysis of butyltins in fish either for checking the levels of the toxic species or to carry out studies on the degradation of small amounts of these compounds. Amounts of 0.4 ng, expressed as tin, of each butyltin compound can be determined. From the results of the analysed samples it can be concluded that degradation of tributyltin by debutylation to give dibutyl- and monobutyltin occurs, but the degradation is much more pronounced in the liver of the salmon than in the flesh. Taking into account the results of the analysis of salmon liver from concentration experiments (samples 3, 4, 5) it is possible to conclude that bioaccumulation occurs. It is remarkable to note the high levels of methylbutyltin compounds in the sample of sediment. They could be related to the high concentration of hydrocarbons present in the sample. This method (GC FPD) enables lower levels of TBT to be detected than other methods (e.g. GF AA[7]) the latter being based on a final measurement of the inorganic tin content. Similar results have been reported by Short.[39] We consider this could be due to contamination or poor selectivity during the extraction procedure in the GF AA method.

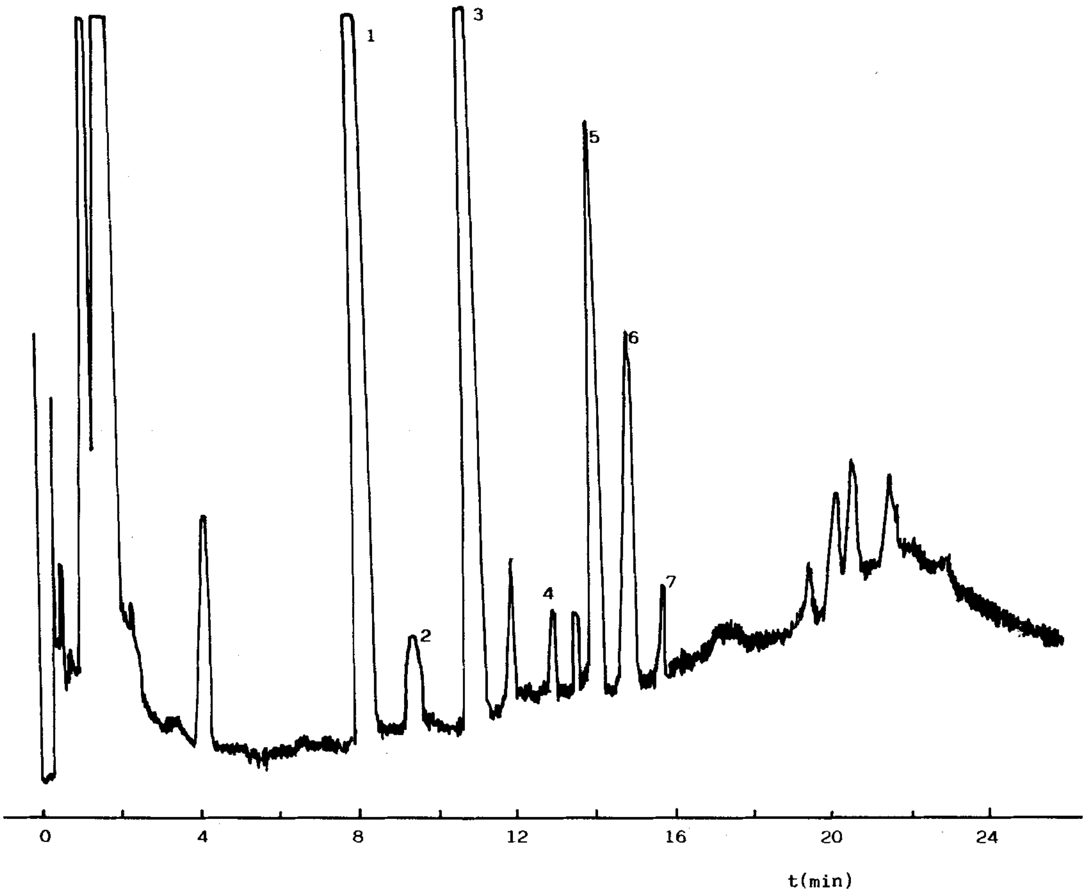

Figure 5 Chromatogram of sediment sample. 1, $Me_2BuPeSn$; 2, Me_2Pe_2Sn (internal standard); 3, $MeBu_2PeSn$; 4, Bu_3PeSn; 5, Bu_2Pe_2Sn; 6, $BuPe_3Sn$; 7, Pe_4Sn.

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