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A Comprehensive Method for the Determination of Aquatic Butyltin Species at Ultratrace Levels Using Simultaneous Hydridization/Extraction With GC-FPD

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U.S. DEPARTMENT OF COMMERCE, Malcolm Baldrige, *Secretary*
NATIONAL BUREAU OF STANDARDS, Ernest Ambler, *Director*

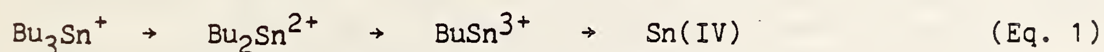
A COMPREHENSIVE METHOD FOR THE DETERMINATION OF AQUATIC BUTYLTIN AND BUTYLMETHYLTIN SPECIES AT ULTRA-TRACE LEVELS USING SIMULTANEOUS HYDRIDIZATION/EXTRACTION WITH GC-FPD

1.0 Introduction

The increasingly diverse and pervasive use of butyltin compounds in industrial, aquatic, and agricultural applications has led to concern regarding the impact of these alkyltin compounds on the environment and prompted recognition of the need for reliable environmental analytical monitoring methods (1-3). Aquatic uses of organotin compounds, in particular their incorporation as tributyltin biocides in controlled-release paints on ships, represent a phenomenal growth area in its infancy, yet pose the greatest immediate impact upon harbor and coastal aquatic biota (4). Tributyltin species are very effective against common marine fouling organisms such as barnacles. However, it is clear that tributyltin is also highly toxic to various non-target aquatic organisms at low concentrations. For example, tributyltin at low parts per billion (ppb) levels is acutely toxic to amphipod larvae (5); lobster larvae and zoeal shore crabs (6), sheepshead minnows (7), and mysid shrimp (8). At sub-ppb levels, tributyltin causes sublethal effects in zoeal mud crabs (9), mussel larvae (10) and copepods (11). Part of the presumed redeeming quality of tributyltin in such environmental uses rests in its degradation by Sn-C cleavage to comparatively innocuous di- and monobutyltin and inorganic tin residues. Mono- and dibutyltins are less

toxic than tributyltin to marine biota, consistent with the general trend for $R_nSn^{(4-n)+}$; which is increasing toxicity with increasing molecular size (12) from n=1 to n=3, and marked decrease in toxicity for n=4 (4). Inorganic tin is virtually non-toxic and may be an essential trace element in animals and man (13).

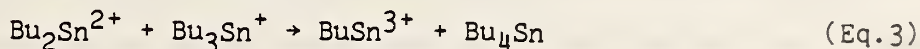
The degradation of tributyltin in the marine environment is widely assumed to follow a stepwise debutylation (4):



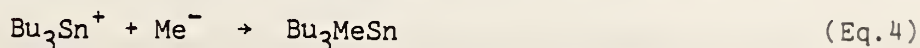
$$Bu_3Sn^+ \rightarrow Bu_2Sn^{2+} \rightarrow BuSn^{3+} \rightarrow Sn(IV) \qquad \text{(Eq. 1)}$$

The butyl groups may be oxidized to CO_2 by microbial activity (14). However, no consistent, quantitative picture of the persistence of the tributyltin species in its aquatic service environment has yet emerged. Complicating the issue of persistence is the possibility of other degradation pathways for tributyltin species including a number of possible redistribution reactions catalyzed by environmental molecules such as amines or sulfides (15) or other reactants (16). The possibility of environmental methylation of butyltins has been raised by a recent report of the presence of mixed butylmethyltin species in sediments (17), presumably arising by biological methylation of anthropogenic butyltins. This suggests additional pathways for tributyltin in the aquatic environment. The toxicity of many of the products of these methylation pathways is still unknown. A few of the possible Sn-C reactions include:

$$2Bu_3Sn^+ \rightarrow Bu_2Sn^{2+} + Bu_4Sn \qquad \text{(Eq.2)}$$



$$Bu_2Sn^{2+} + Bu_3Sn^+ \rightarrow BuSn^{3+} + Bu_4Sn \qquad \text{(Eq.3)}$$



$$Bu_3Sn^+ + Me^- \rightarrow Bu_3MeSn \qquad \text{(Eq.4)}$$

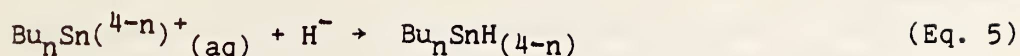
The source of methylcarbanion (Eq.4) presently is uncertain but may be due to redistribution with biogenic or anthropogenic methyltin species (18) or by intermediate oxidative methylation from algal metabolites (19,20).

In sum, it has been assumed that toxic anthropogenic organotin species in the environment lose their potency by degradation to less toxic organotin species and, finally, to innocuous inorganic tin(IV). Analytical methods reported heretofore, nevertheless, do not adequately test the validity of the presumed degradation pathway. For example, chromatographic procedures relying on charge separation fail to determine neutral species such as tetrabutyltin or dibutyldimethyltin. Moreover, laboratory derivitization using methyl Grignard reagents (21) obscure butylmethyltin species, if present.

Clearly, any analytical method for adequate environmental monitoring of tributyltin must be capable of determining at very low concentrations (ng/L levels) the entire set of both butyltin and mixed butylmethyltin species in order to assess the environmental fate of tributyltin. Because of the increase in the world use of tributyltin as the active agent in marine biocides, the need also exists for frequent routine monitoring of areas of high ship traffic, such as marinas and harbors, and of sensitive areas, such as oyster beds. The analytical method used for the large number of samples that such monitoring would generate must be relatively simple and fast, as well as highly sensitive.

Previous methods for the determination of butyltins have relied primarily upon derivatization to form hydrophobic, neutral organotin species. Some investigators used reduction of aquated alkyltin species

with sodium borohydride ($NaBH_4$) to the covalent hydrides



$$Bu_nSn^{(4-n)+}{}_{(aq)} + H^- \rightarrow Bu_nSnH_{(4-n)} \qquad \text{(Eq. 5)}$$

followed by inert gas purge of the aqueous sample. The evolved volatile alkyltin hydrides were trapped at liquid nitrogen temperatures, followed by the gradual warming of the cold trap to allow the separation of alkyltins by boiling point, and finally, tin was detected absorption spectroscopy (22, 23, 24). Others have used extraction of aqueous samples into organic solvents and alkylation of the extract with a Grignard reagent to produce neutral tetraalkyl compounds which can then be separated and identified using gas chromatography coupled with mass spectrometric (GC-MS) (21) or flame photometric detection (GC-FPD) (25). Liquid chromatography coupled with an atomic absorption detector has also been employed for butyltin speciation (26). The recent work of Mueller (27) employed a macroreticular resin for concentration and separation of tributyltin from water followed by methylation and detection by GC-FPD and GC-MS.

In the present work, we describe a relatively rapid, simple, sensitive method for the complete determination of butyl- and mixed butylmethyltin species in the water environment. The butyltin species are reduced to their respective volatile hydrides with aqueous $NaBH_4$ and simultaneously extracted into CH_2Cl_2 for appropriate pre-concentration and subsequent chromatographic analysis.

2.0 Experimental

2.1 Materials

All glassware and Teflon separatory funnels were cleaned prior to use by rinsing in methanol, washing with laboratory detergent and leaching with warm 10% nitric acid for at least 8 hr, followed by rinsing with copious amounts of deionized water (18 MΩcm). All other plasticware parts were similarly cleaned except that the acid leaching time was shortened to one hour. The $Bu_nSnCl_{(4-n)}$ compounds and Bu_3SnH used for preparation of standard solutions and spikes (95-98% purity, Alfa Products, Danvers, MA) were used as received without further purification. Di-n-propyltin dichloride (M & T Chemicals, Inc., Rahway, NJ) was used as an internal standard. Chromatographic grade dichloromethane was obtained from Burdick and Jackson Laboratories, Inc. (Muskegon, MI). In addition, a dilute (ppm), aqueous tributyltin research material was prepared chromatographically in our NBS laboratory (28). Organotin solutions were prepared at concentrations of approximately 1000 mg/L as tin in spectrograde methanol. Deionized water of 15-18 MΩcm resistivity obtained from a Milli-Q Reagent Grade water system (Millipore Corp, Bedford, MA) was used to dilute these stock solutions to the working range of approximately 550 ng/ml (0.5 ppm). Fresh solutions of 4% (w/v) sodium borohydride (Aldrich Chemical Co., Inc., Milwaukee, WI) were prepared daily in deionized water. No butyltin species were detected in the reagent blanks.

2.2 GC-FPD System

A Hewlett-Packard (Avondale, PA) (HP) Model 5730 gas chromatograph equipped with a HP flame photometric detector was used for this study. Chromatographic separations were carried out on a 2-mm i.d. X 6 ft. glass

column packed with 1.5% OV-101 (liquid methyl silicone) on Chromosorb G HP(100-200 mesh size) (Varian, Sunnyvale, CA). A hydrogen-rich flame was employed, supported by H_2 flowing at the measured rate of 110 mL/min, air at 70 mL/min, and N_2 (zero grade) carrier gas at 20 mL/min. The FPD was equipped with a 600-nm cut-on interference filter (band-pass 600-2000 nm) (Ditric Opitics, Inc., Hudson, MA) to monitor the SnH molecular emission (29, 30). The output signal from the FPD was recorded simultaneously on a strip chart recorder and an integrator-plotter (HP Model 3390A). For all runs reported herein, the column temperature was programmed at 23° C for 2 min. then heated to 170° C at 32° C/min. The detector temperature was maintained at 200° C and injection port at 150° C.

2.3 GC-MS System

The GC-MS system is described in ref. 29 with the following modifications for use with butyltins. The GC column was that described above for the FPD system. For tetrabutyltin analysis, samples were extracted into CH_2Cl_2 without hydridization. The temperature program for GC-MS tetrabutyltin was 50° C for 1 min. then to 170 °C at 30 °C/min.

The GC-MS system is interfaced with an on-line Computer Automation, Inc. computer, and software from Teknivet Inc. (St. Louis, MO). This system provided two modes for data acquisition: mass-spectrum mode and selected ion monitoring. Major representative peaks were selected from fragmentation patterns of the mass spectrum of tetrabutyltin (m/e = 119, 121, 177, 179, 233, 235) for selected ion monitoring. Total ion current mass spectra were obtained for tetrabutyltin and the mixed species.

2.4 Redistribution reactions

The mixed butylmethyltin species were prepared after the method of Calingaert *et al.*, (31) by refluxing at 80°C 5 mM of the appropriate

methyl- and butyltin chloride starting materials (Alfa Products) with 0.5 mM aluminum chloride catalyst (Fisher Scientific Co., Fairlawn, NJ) in 30 mL HPLC grade hexane (Fisher Scientific). The reactions were run for 5-6 hrs under N_2. Aliquots of the reaction mixtures were hydridized as CH_2Cl_2 dilutions in a two phase system with aqueous $NaBH_4$ and analyzed by GC-FPD to determine the retention times of the various mixed butylmethyltin products. The reaction products were identified as butylmethyl-, butyldimethyl, dibutyl- and dibutylmethyl-, dibutylmethyl- and tributyltin, and confirmed by GC-MS.

2.5 Analysis Procedure for Butyltins

For a typical analysis of saline water with a butyltin concentration in the sub-µg/L range (as tin), the following procedure was found to be optimal. To 100 mL of sample in a 125 mL glass separatory funnel equipped with a Teflon stopcock and Teflon-lined screw top (Wheaton Scientific, Millville, NJ), were added 2.8 mL dichloromethane and 2.0 mL of 4% (w/v) aqueous $NaBH_4$. In addition, a 10 µL spike of a 0.5 ppm aqueous solution of di-n-propyltin dichloride was added to certain samples as an internal standard. The funnel was capped and shaken by hand for 1 min, vented, and then shaken (240 strokes/min) on a wrist action shaker (Burrel Corp., Pittsburgh, PA) for 10 min. Following a 5 min settling period, the lower organic layer was removed. An additional 1.4 mL of dichloromethane was added and the extraction procedure repeated. The organic layers were combined (approx. 2 mL) in polypropylene centrifuge tubes and evaporated to 100-200 µL or less under a gentle stream of air. Appropriate reagent blanks were carried through the entire procedure. All quantitation was achieved using the method of standard additions to the sample matrix. For

samples of concentration greater than 500 ng Sn/L, no evaporation concentration step was required. While most of our work has been done using the 100 mL sample size, samples of up to 800-1000 mL have been analyzed using 1L Teflon screw-capped separatory funnels (Fisher Scientific) and proportionately larger volumes of all reagents. Extracts were concentrated to 50-100 µL in 15 mL glass centrifuge tubes (Wheaton).

2.6 Environmental Samples

Environmental samples were collected aboard the research vessel Ridgely Warfield or from docks or piers. Surface water samples were collected in 4 liter glass bottles at 1 m depth. Surface microlayer samples were collected by gently dipping a Teflon sheet (0.32 m^2) to the water surface and by rinsing of the adsorbed sample into a glass bottle with about 25 mL of deionized water. Sampling station designations are those of the Chesapeake Bay Institute. Samples from San Diego were provided by Dr. P. F. Seligman and were stored on dry ice until analysis.

3.0 Results and Discussion

3.1 Solvent Choice and Hydridization

Dichloromethane is often the solvent of choice for extracting of organic compounds from natural waters (32) and is effective in extracting of organotins from tissues (8). Preliminary experiments showed that the efficiency of dichloromethane in extracting Bu_nSnCl_{4-n} (n=1-4) from deionized water (1 µg Sn/L) ranged from 60% for tetrabutyltin to 95% for tributyltin cation after a single 10 min extraction (unpublished data). Hydride derivatization was required to insure sufficient volatility for

the GC analysis of butyltin species in the dichloromethane extracts Fig. 1, top). The highest sample recoveries were obtained with simultaneous extraction and hydridization as opposed to a conventional two-step extraction and derivatization sequence (Fig. 1). The extraction efficiency of the new procedure for tributyltin was determined by spiking at 1 ppb with tributyltin chloride a 100 mL Chesapeake Bay water sample of low intrinsic butyltin concentration and performing the simultaneous hydridization/extraction. A calibration curve of peak area vs. ng of tributyltin hydride was prepared with dilute dichloromethane solutions of commercially available neat tributyltin hydride (98% purity, Alfa Products, Inc.). The amount of tributyltin recovered in the extraction process was determined using this curve. Extraction efficiency from the environmental matrix was 112% (±10%) recovery of the spikes of tributyltin cation. The possibility that the analytical work up might induce either degradation or rearrangement reactions also has been of great concern. A severe limitation for analysts has been the lack of any tributyltin reference material for use in evaluating analytical schemes. A research material of aqueous tributyltin has been prepared chromatographically at NBS (28) and analyzed repeatedly at concentrations from the range of mg/L to < 50 ng/L with no evidence of any degradation or rearrangement products. Spikes of dibutyltin added to dilutions of the reference material indicate that at a concentration of 1 μg/L, detection of <3% conversion to the dibutyltin degradation product would be readily achieved (Fig. 2).

3.2 Detection Limits and Uncertainty

Calibration curves and the limits of detection at the 95% confidence interval (33) for the analysis procedures for four major butyltin species

are shown in Figure 3. The detection limits of the method were determined by analysis of spiked samples of 100 mL volume, with the dichloromethane extract concentrated to 0.05 mL to give a preconcentration factor of 5×10^4. The absolute detection limits for the GC-FPD instrument (as ng Sn) were also determined, by hydridization of dichloromethane solutions of known butyltin concentration (Fig. 3). Scale up to 800-1000 mL samples resulted in approximately 5-6 fold improvement in detection limits, with detection of 1-2 ng Sn/L as tributyltin and tetrabutyltin and less than 1 ng Sn/L as dibutyltin being possible for these larger samples. The resulting 5-6 fold lowering of detection limits with an 8-10 fold increase in sample volume could in part be explained by loss of the analyte to the separatory funnel walls, because of the large surface area.

Additionally, the extraction efficiency may be reduced for these large volumes because the large funnel could not be shaken as vigorously as the 125 mL funnel with the automatic mechanical shaker.

The detection limit for environmental samples is dependent upon the degree of extract concentration prior to analysis. Consequently, sample loss on evaporation was also investigated. In these experiments, 1 mL of the hydridized dicloromethane extract of Chesapeake Bay water spiked with 1 µg/L $Bu_nSn^{(4-n)+}$ (n = 1-4) was evaporated under a gentle stream of gas to 50 µL, then re-diluted to 1 mL with fresh dichloromethane. Comparison between dry air and N_2 and ambient temperature (22°C) and 0°C (ice-water bath) for use in evaporation were evaluated. Peak areas for the tin species before and after the concentration step were compared. Under all conditions, minimal losses (0-12%) on evaporation were seen for

di-, tri- and tetrabutyltin, approximately 50% losses were seen for monobutyltin. For convenience, air and ambient temperature were used on all subsequent analyses.

Reproducibility of the procedure was determined by carrying six 100 mL aliquots of the same sample through the entire analytical procedure. Instrumental reproducibility was estimated by replicate (n=7) injections of the same sample (Table I). Reproducibility of the analytical scheme appears to be instrument limited.

The sources of potential errors in the analysis of complex environmental samples are numerous. Errors in environmental analysis of aqueous butyltins at parts-per-trillion concentrations result from sample handling, storage and derivitization steps and can be attributed to the physical and chemical properties of the butyltin analytic within the matrix, as well as to instrumental limitations. For the analytical scheme described here, the overall analytical error is the sum of individual sources of error, i.e., sample losses to glass or Teflon, partition onto suspended matter, efficiency of hydridization, efficiency of extraction detector variability and syringe injection reproducibility. While this list of errors is extensive, it is by no means exhaustive. It is possible to minimize the effects of some of these potential errors by analyzing samples immediately after collection, by the use of an internal standard (dipropyltin dichloride) and quantitation using standard additions.

3.3 Application to Aquatic Environmental Samples

Because of the possibility of wide variations in pH and salinity of natural waters, the analysis procedure was carried out at several pH and salinity levels. Figure 4 shows that neither salinity nor pH

significantly affect simultaneous extraction and detection of butyltin species in Chesapeake Bay waters. Butyltins spiked into sample waters obtained from widely different locations in the Bay, representing salinities ranging from 0 (deionized water) to 22.4 0/00, were recovered with similiar efficiency. Adjustment of sample pH (mid-Bay site 858C, pH = 7.7) to pH 6.3 with 1.0M HCl or to 9.4 with 1.0M NaOH made little difference in detection of butyltin species. These pH values exceed the range expected for estuarine or marine samples. Evaluation of the extractions from different pH or salinity samples as replicates shows standard deviations of 3.2-12.3% for pH variation and 9.5-16.2% for salinity variation calculated from peak areas of the butyltin species. These values are comparable to the standard deviations for replicate analyses of the same sample reported above (Table I) and for this reason pH and salinity do not appear to contribute important errors in this analysis.

Several environmental samples have been analyzed, including bulk waters samples from Shelter Island (San Diego Harbor) and from Jones Falls (Baltimore Harbor) (Fig. 5) and microlayer samples from Jones Falls. Tributyltin concentrations of 68 ± 11 ng Sn per liter and dibutyltin concentrations of 108 ± 19 ng Sn per liter were found in the San Diego samples comparing closely with levels reported by Seligman *et al*. (8, 22). Tributyl- and dibutyltin species were also detected in surface waters from commercial marinas at Annapolis, MD (Table II). Of interest in the Jones Falls microlayer samples is a peak with retention time corresponding to that of tetrabutyltin (Fig. 6). In addition, a peak with the same retention time as tetrabutyltin is seen in washwater from samples collected during dry-dock cleaning of organotin paints ship hulls.

Identification of this peak as tetrabutyltin was confirmed by GC-MS (Fig. 7). The peaks on the mass spectrum at m/e 205 correspond to a large non-tin peak which is not completely resolved chromatographically from the tetrabutyltin peak (Fig. 8). This is the first report of tetrabutyltin in the environment, and suggests that the tributyltin transformation reactions shown in Eq. 2,3 may occur in harbor and marina waters. The hydrophobic nature of the surface microlayer and its elevated levels of microorganisms and metabolites is well documented (34). The surface microlayer may contain components capable of inducing redistribution reactions of butyltins, e.g. by a mechanism similar to the Lewis base induced redistribution mechanism for conversion of $(CH_3)_3SnOH$ to $(CH_3)_4Sn$ described by Guard and co-workers (35). The microlayer is a preferred sink for tetrabutyltin due to tetrabutyltin's low water solubility and low volatility.

The ability to detect ultratrace concentrations of environmentally methylated anthropogenic butyltin species is paramount for fully understanding the fate of tributyltin in the environment, and is achieved with this analytical scheme. We have observed several small peaks in environmental samples which suggest the presence of mixed methylbutyl species in estuarine and harbor waters, but due to very low concentrations (< 1 ng/L) of analyte and relatively poor sensitivity of the GC-MS system we have not as yet confirmed the identity of these peaks. This work is continuing.

The new technique was also used to detect biodegradation products of tributyltin spiked into natural waters. Here, (Fig. 9) a sample of Baltimore Harbor (Chesapeake Bay) water was amended with yeast extract (0.05% final concentration) to promote microbial activity and the NBS

research material tributyltin (100 µg/L, final concentration). After 12 weeks incubation at 20 °C, substantial degradation of tributyltin to dibutyl and monobutyltin species was noted. No mixed butylmethyltin species were detected. A sterile control showed very little tributyltin degradation. Further studies are now in progress to measure tributyltin degradation rates in natural waters. These experiments employ low levels of tributyltin (ca. 1 µg/L) and short incubation times (days).

4.0 Conclusions

This approach to the analysis a butyltins in aquatic samples at environmental action levels (ng/L) has been shown to be highly sensitive, simple and non-disruptive to the molecular speciation of the sample. Clearly, instant partitioning into the polar organic medium (CH_2Cl_2) immediately after hydridic derivatization of aquated $Bu_nSn^{(4-n)+}$ species greatly favors analytical sensitivities and precision. Currently, we are attempting to expand the scope and utility of the analytical method to sediments in order to evaluate more fully the fate of tributyltin in the estuarine environment.

5.0 Acknowledgements

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Center for environmental samples, and William R. Blair for assistance with the GC-MS.

Certain commercial products or equipment are mentioned in order to adequately describe experimental procedures. In no case does such identification imply endorsement by the National Bureau of Standards, nor does it imply that the material is necessarily the best available for the purpose. Contributions from the National Bureau of Standards are not subject to copyright.

This work will be included in the dissertation of C.L.M. to be submitted as a requirement for the Ph.D. degree from the University of Maryland.

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Table I. Reproducibility of Butyltins Analysis[1]

	n	BuSn	Bu_2Sn	Bu_3Sn	Bu_4Sn
Replicate injections of one sample extract[2]	7	12%	5%	12%	9%
Replicate extractions of same sample[2]	6	9	15	11	11

[1]Standard deviation

[2]Sample of Chesapeake Bay water (site 858) spiked with 1.0 µg/L Bu_nSnCl_{4-n} (n = 1-4)

Table II. Concentration of butyltin species (ng/L Sn) in natural waters[1]

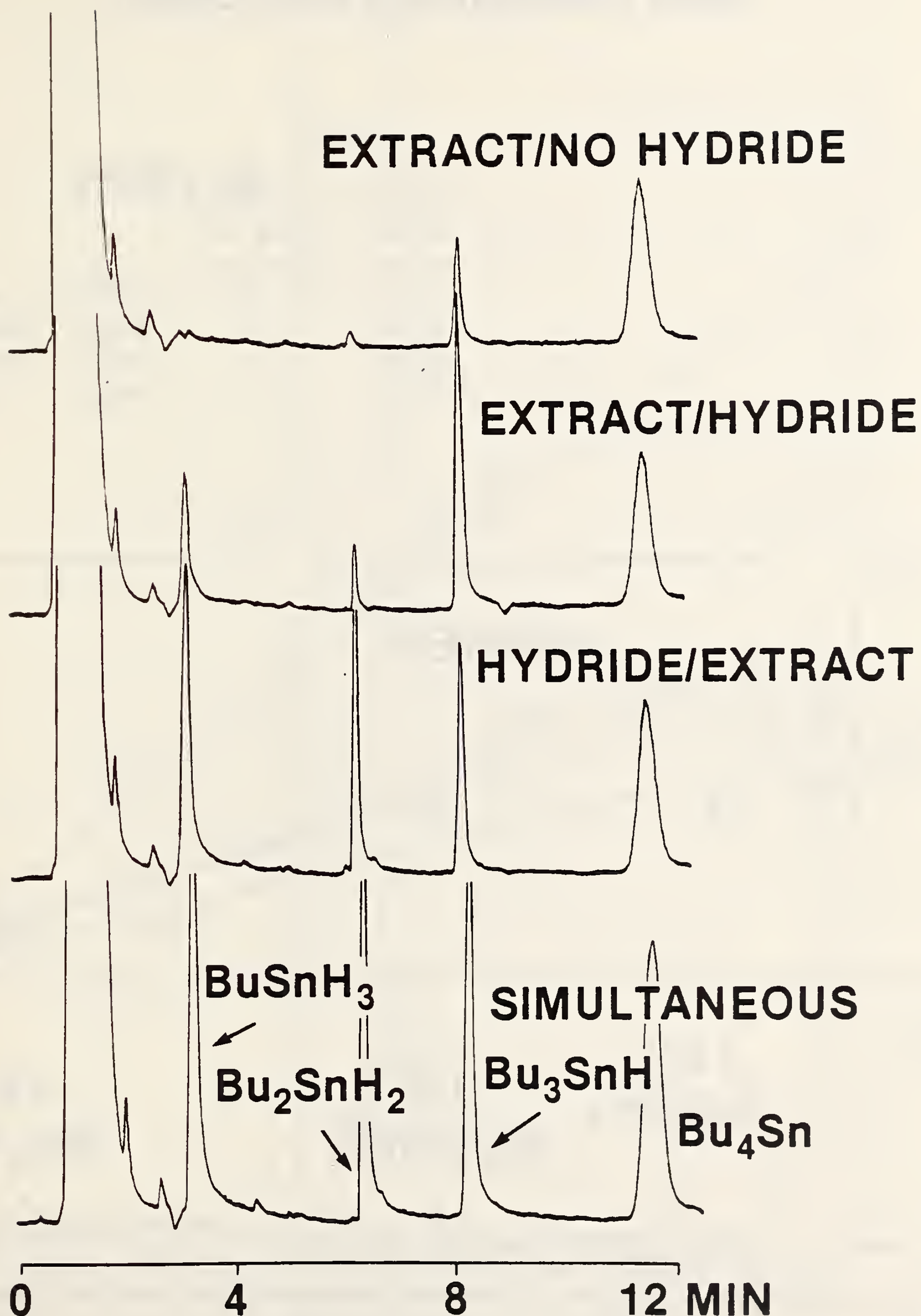
Site	$BuSn^{3+}$	Bu_2Sn^{2+}	Bu_3Sn^{+}	Bu_4Sn
Jones Falls	ND[3]	8	trace	ND
Jones Falls microlayer[2]	165	91	1872	395
Annapolis marina-1	ND	10	14	ND
Annapolis marina microlayer[2]	ND	150	trace	ND
Annapolis marina-2	ND	21	29	ND
Shelter Island	ND	109	68	ND
San Diego Bay[4]	ND	4	2	ND
Dry Dock Sample	ND	ND	900	4200

[1]Collected at 1 m depth unless otherwise noted, 100 mL sample extracted

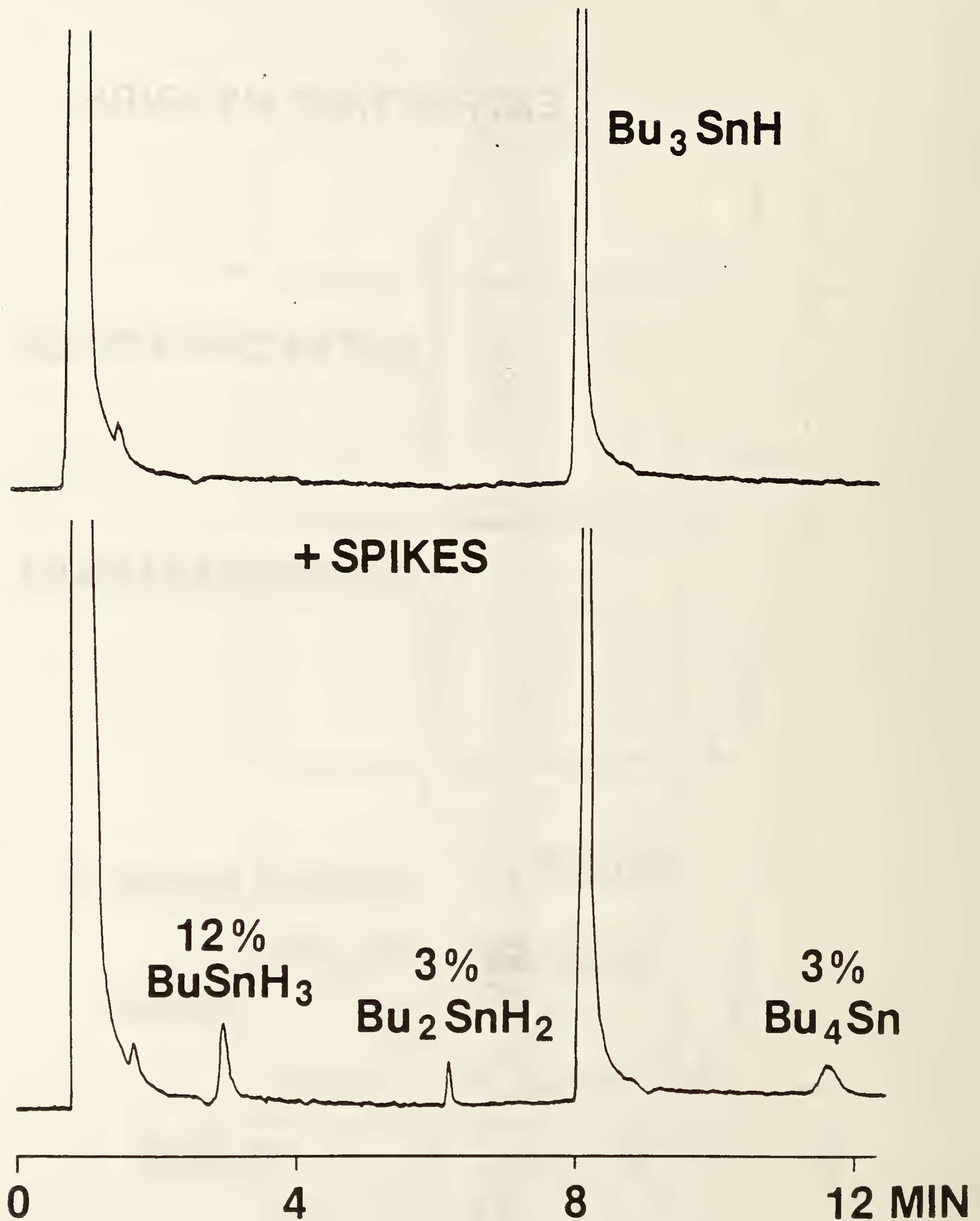
[2]Concentration in wash water (approximtely 50 mL) collected from Teflon sheet ($0.32m^2$)

[3]Not detected

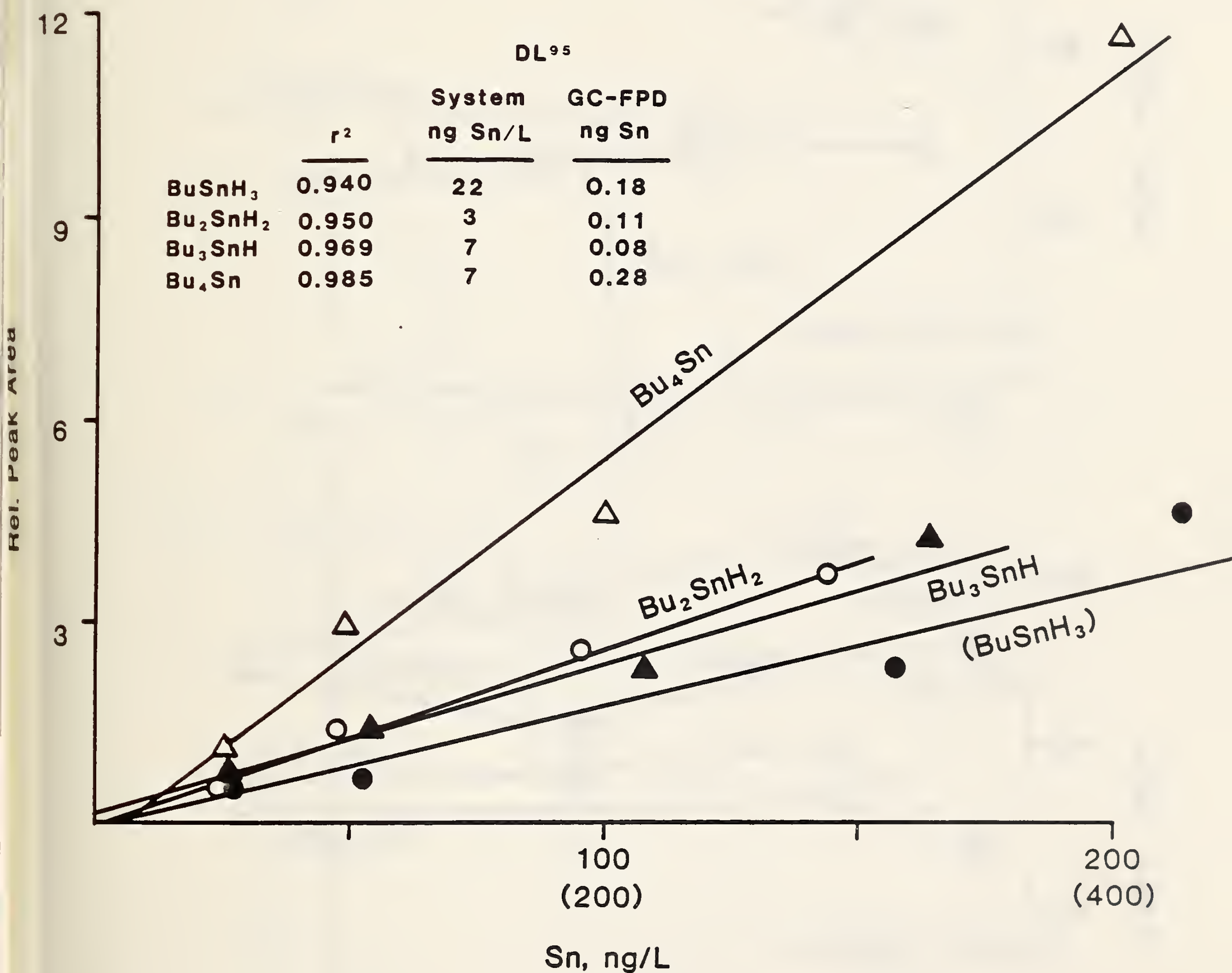
[4]Sample volume = 800 mL



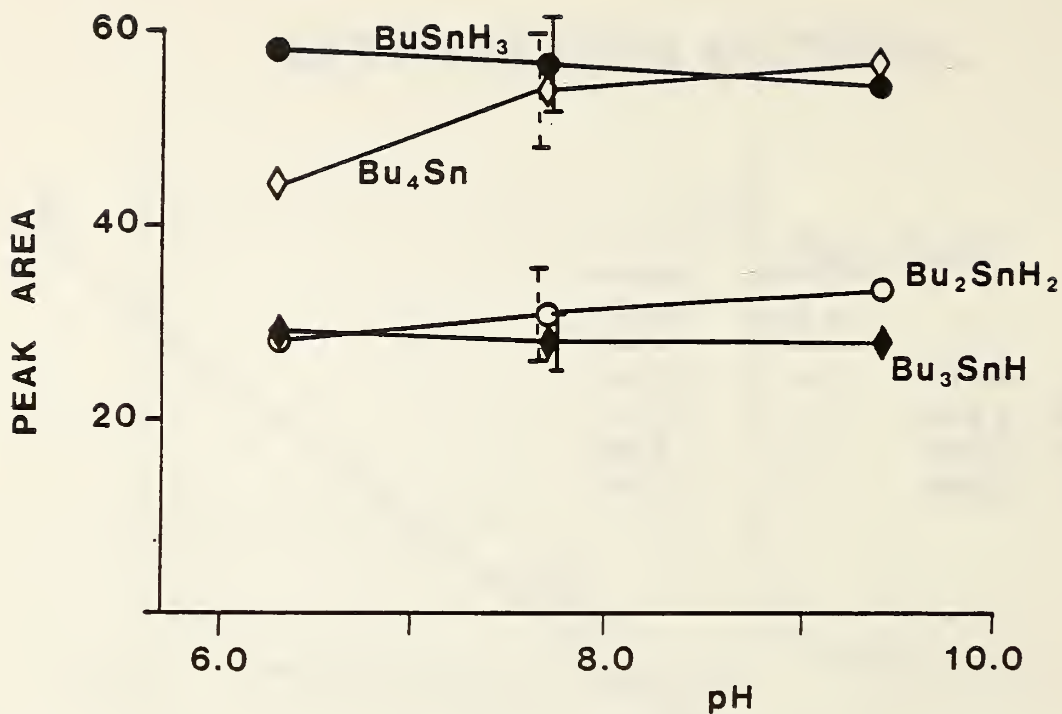
1. Effect of extraction and hydridization sequence on detection of butyltin species spiked (1.0 µg/L final aqueous concentration for $Bu_{2-4}SnCl_{4-n}$, 4µg/L for $BuSnCl_3$) into Chesapeake Bay water (site 858c). "Extract/hydride" denotes that the extraction step was performed first followed by hydridization of the extract.

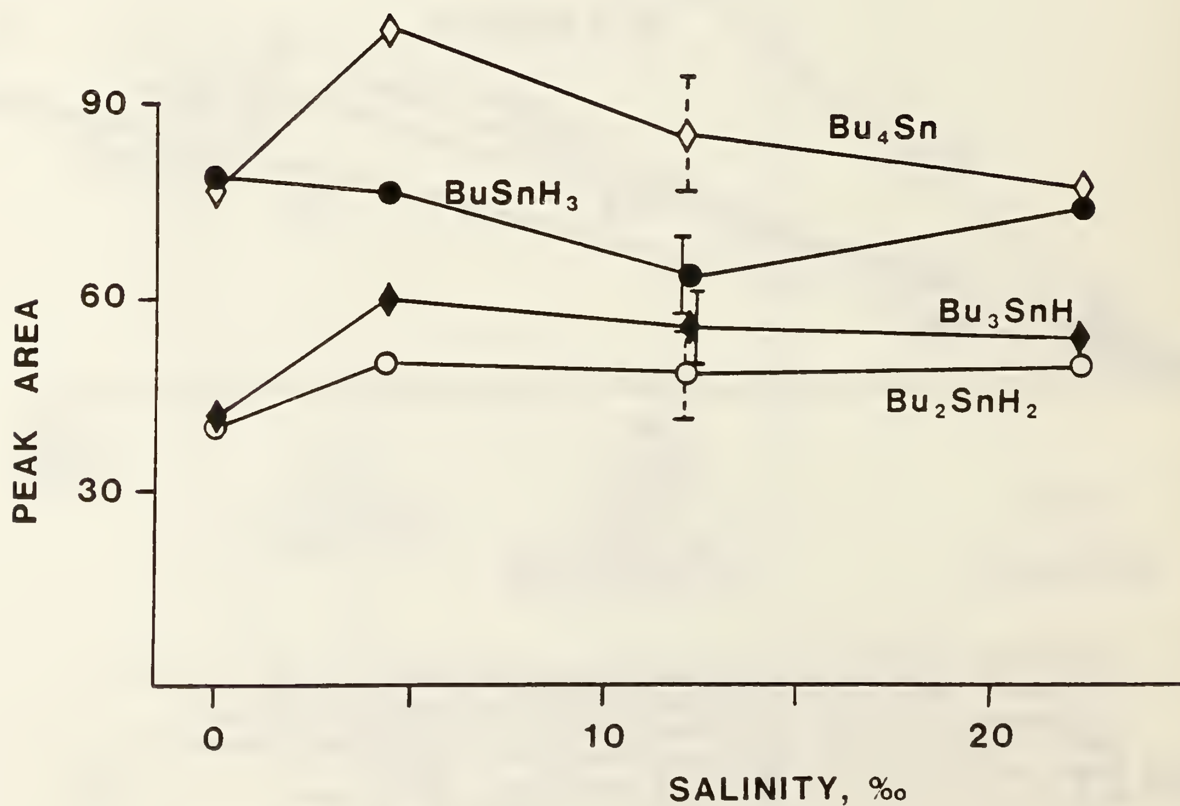


2. Analysis of chromatographically prepared tributyltin research material. The bottom chromatogram shows the material spiked with Bu_nSnH_{4-n} at 3% (n=1-3) to 12% (n=1) of the concentration of the tributyltin level.

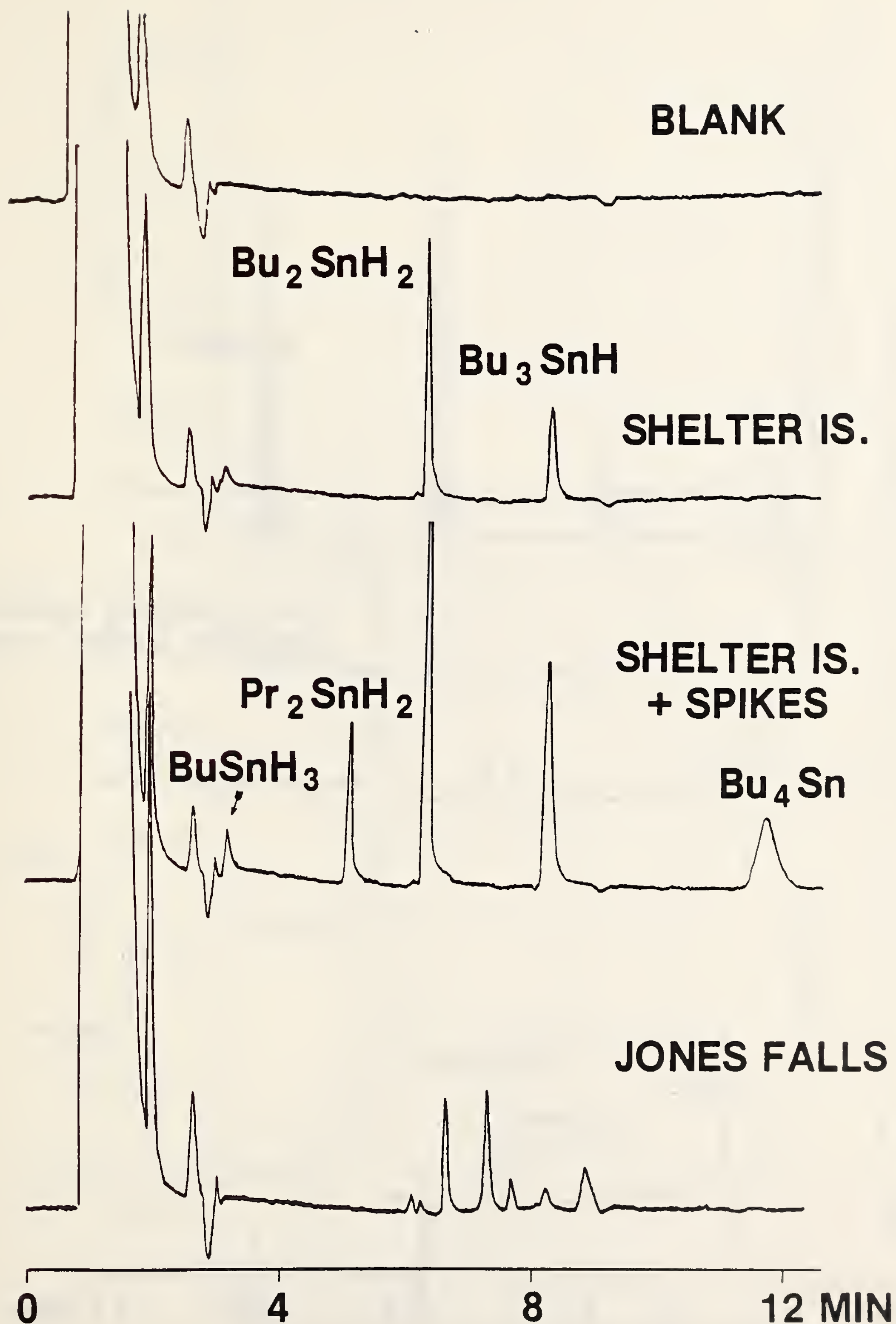


3. Calibration curves for $Bu_{1-4}Sn$ species spiked in Chesapeake Bay (site 701) water. Here 100 mL water was extracted and hydridized and the dichloromethane extract concentrated to 50 µL. Data points represent means of duplicate 5 µL injections except for the lowest concentrations which are means of 5 injections. In all cases, variance was ≤10%. Detection limits are given for the 95% confidence interval (DL^{95}, ref. 32).

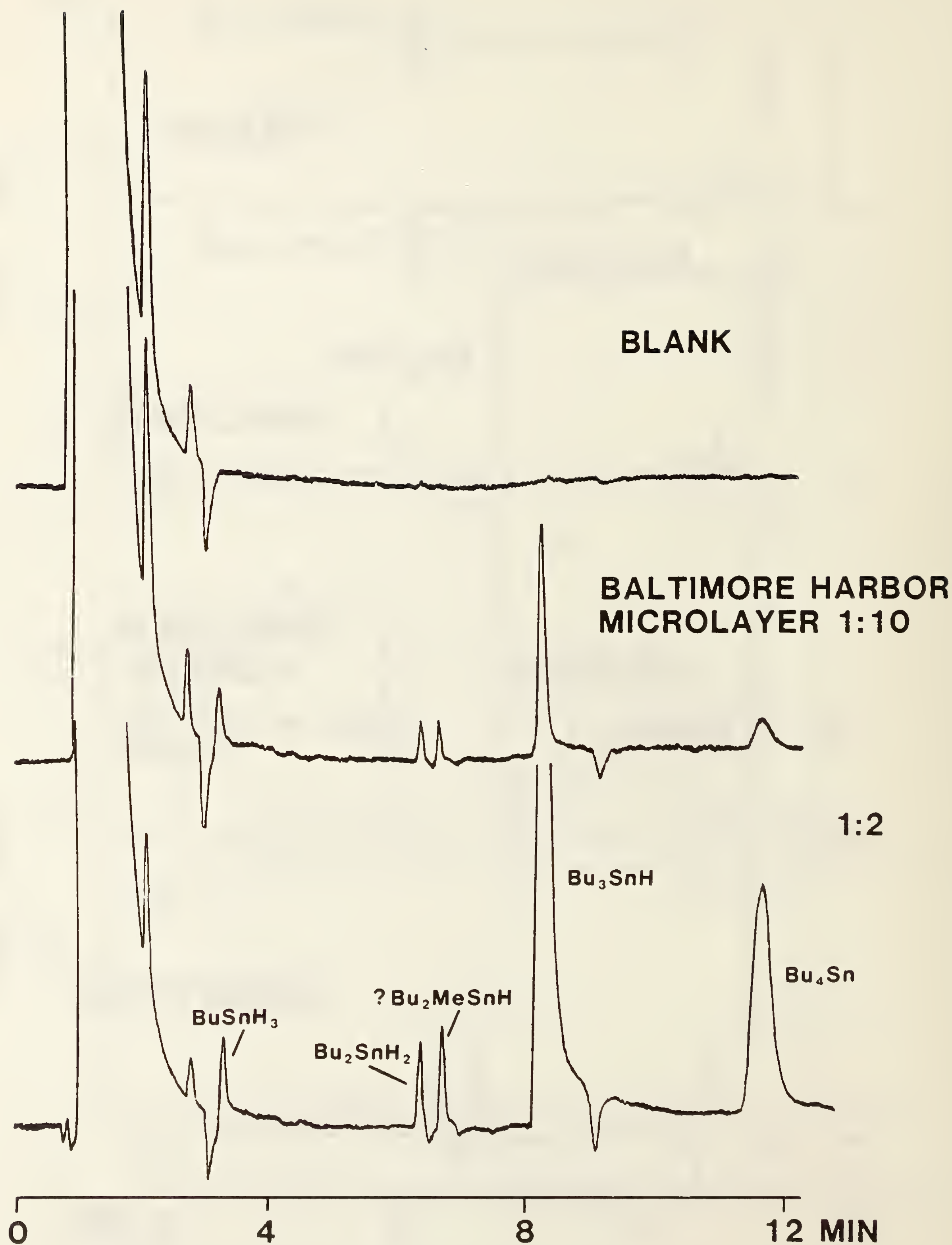




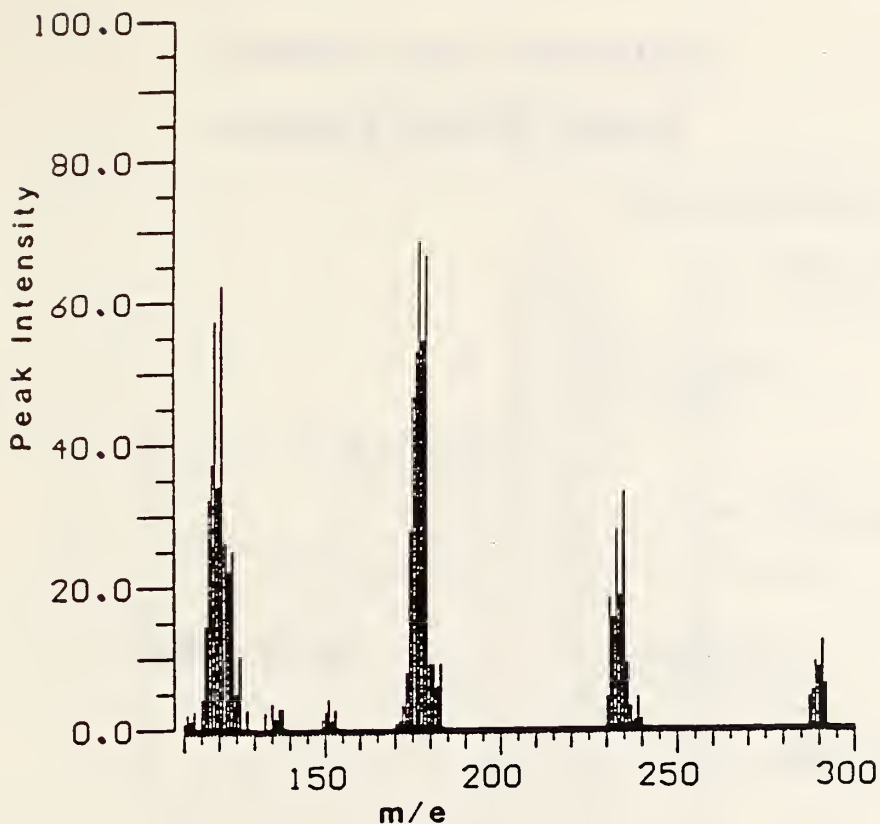
4. Effect of pH (top) and salinity (bottom) on detection of butyltin species spiked (1 µg/L) into Chesapeake Bay waters. Water from site 858c was used in pH experiments. Waters of differing salinities in Chesapeake Bay were collected and analyzed in the salinity experiments. Zero salinity denotes deionized water. Error bars denote ± one standard deviation.

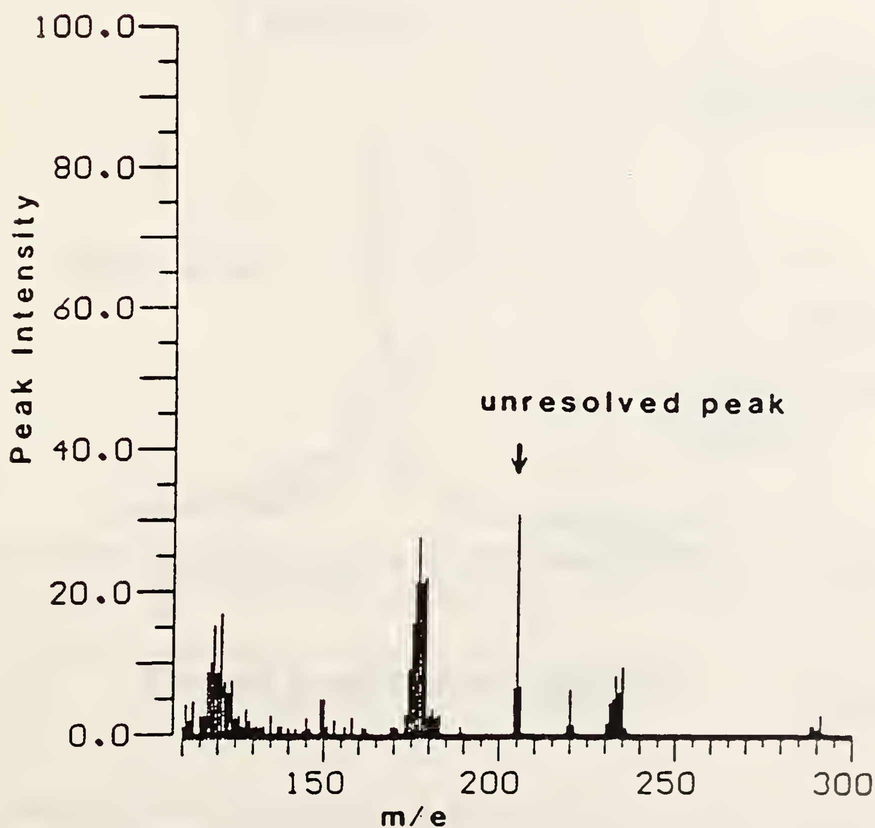


5. Butyltin species detected in samples form Baltimore Harbor (Jones Falls) and San Diego Bay (Shelter Is.). The spikes in the Shelter Is. chromatogram represent 50 ng/L $Bu_{2-4}SnCl_{4-n}$ and 210 ng/L $BuSnCl_3$. Dipropyltindichloride (internal standard) was added at 50 ng/L.

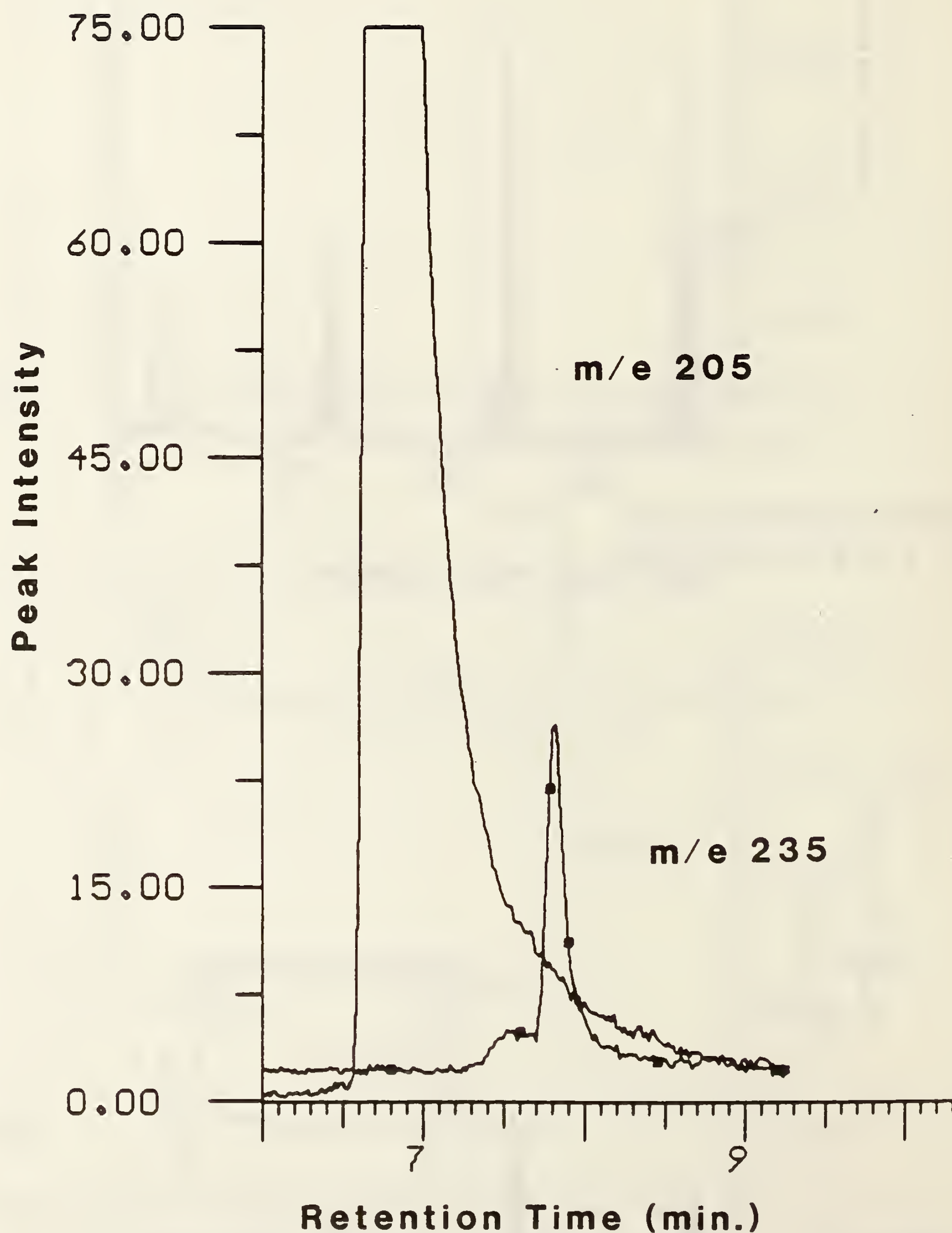


6. Chromatograms of Baltimore Harbor microlayer samples diluted 1:10 and 1:2 with deionized water prior to extraction.

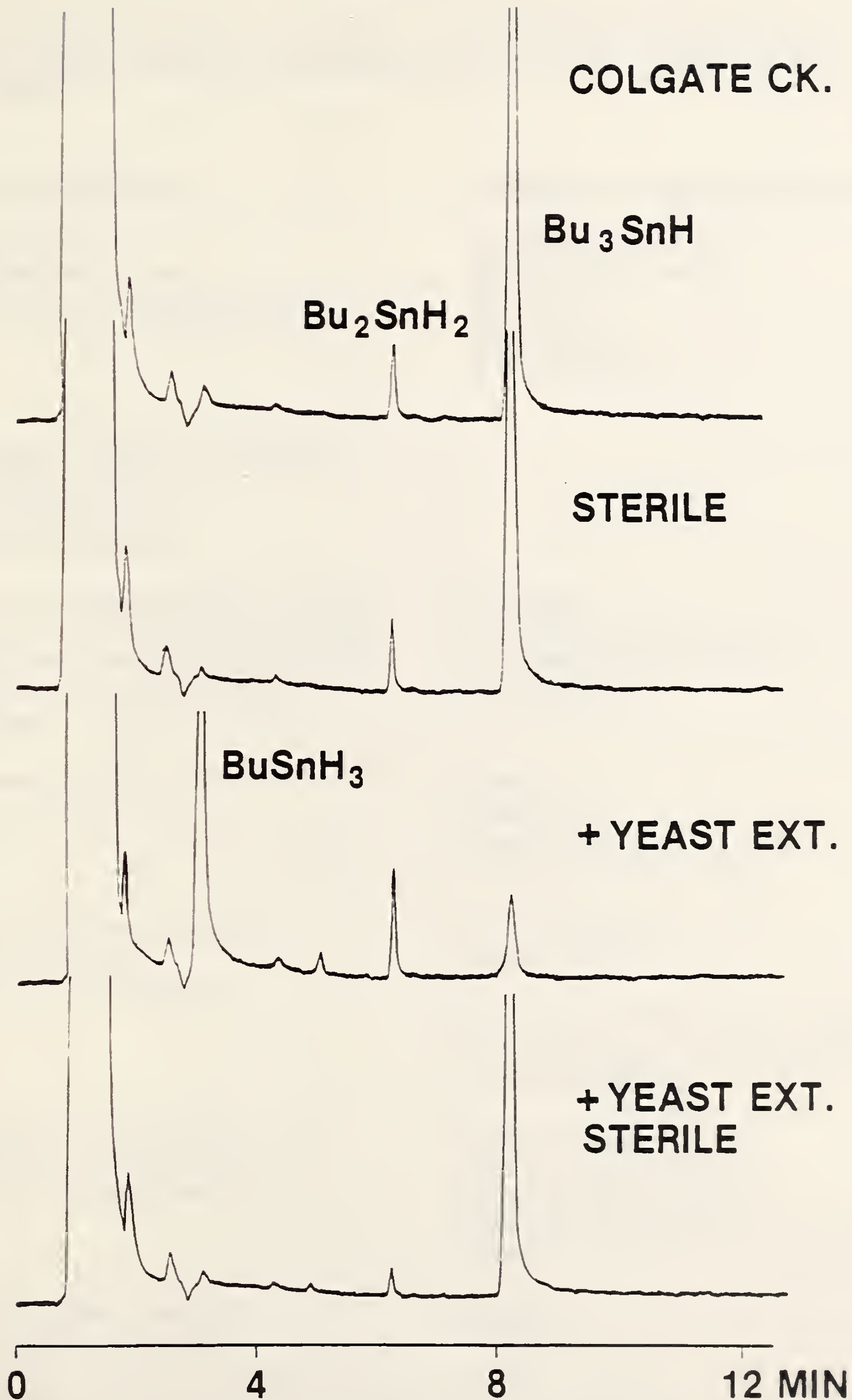




7. Mass spectrum of authentic tetrabutyltin (top) and tetrabutyltin found in dry dock sample (bottom) containing tributyltin antifouling paint. Peak at m/e = 205 is large non-butyltin containing peak which is not fully resolved chromatographically (see Fig. 8).



8. Selected ion chromatogram demonstrating large unresolved peak with m/e = 205 and retention time of ~ 6.8 min, with peak tailing into the 7.8 min tetrabutyltin peak(M/e = 235).



9. Degradation of tributyltin spiked (0.1 mg/L, final concentration) into Baltimore Harbor (Colgate CK) surface waters. Four 250 mL bottles received 45 mL Colgate CK water and 5 mL of aqueous tributyltin research material (1.0 mg/L). Two of the bottles were sterilized (121° C, 15 min) prior to addition of tributyltin. One sterile and one non sterile bottle received 1.0 mL sterile 25% yeast extract (0.05% final concentration). After three months incubation in the dark, the bottles were analyzed for butyltin concentrations.

APPENDIX A

PROTOCOL FOR THE ULTRA-TRACE SPECIATION OF BUTYLTINS IN SEAWATER BY GC-FPD USING SIMULTANEOUS HYDRIDIZATION/EXTRACTION WITH THE TRACOR MODEL 570 DUAL GC-FPD SYSTEM[a]

Equipment and Supplies	Examples of Suppliers and Notes
Gas chromatograph with FPD detector and Spectra-Physics integrators	Tracor Instruments 6500 Tracor Lane Bldg 27 Austin, TX 512-929-2051
Gases nitrogen-zero grade for carrier air-breathing quality oxygen-tech. grade hydrogen-tech. grade	
Regulators for above gases, including a regulator with a stainless steel diaphragm for use with high purity nitrogen such as the Matheson 3800 series	Matheson 6655 Amberton Drive-unit 0 Baltimore, MD 21229 301-796-0517
Glass columns to fit above 6ft x 1/4 in O.D. 2mm I.D.	Tracor or Supelco Supelco Park Bellefonte, PA 16823
Optical interference filters 600-2000 nm cut on 610 nm (10nm band pass)	Ditric Optical Hudson, MA One set of these will be provided GC instrument, you may wish to purchase spares
Column packing: 1.5% OV-1001 on Chromosorb G HP (100/120), 3% OV-101 on Chromosorb G HP	Varian Instrument Co. 650 E. Areqes Drive Sunnyvale, CA 94084 Available from Supelco on Special Order
Glass Wool (silane treated)	Supelco

[a]Certain commercial products or equipment are mentioned in order to adequately describe experimental procedures. In no case does such identification imply endorsement by the National Bureau of Standards, nor does it imply that the material is necessarily the best available for the purpose.

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Glass Wool (silane treated)	Supelco

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Item	Source
GC septa	Tracor or Supelco
Syringes for manual injection 10 microliter size	Precision Sampling Corp. Available through Supelco
Dichloromethane spectro-grade	Such as Omnisolve, available through suppliers such as Fisher
Sodium borohydride 98% pellets (ea. pellet approx. 0.4g)	Aldrich Chemical Co. Milwaukee, WI 53201
Organotin compounds for use in quantitation: monobutyltin chloride dibutyltin dichloride tributyltin chloride tetrabutyltin dipropyltin dichloride	Alfa Products 925 Andover St. Danvers, MA 01923 Organometallics, Inc. Route 11 East Hampstead, NH 03826
Spectro-grade methanol for making stock solutions	Omini-solve or similar
Separatory funnels with Teflon screw-cap and Teflon stopcock (250 ml size)	Wheaton Scientific Special Order ATTN: Maryalice Robinson
Pasteur pipets and bulb	Probably available in your Chem. Stock room
Micropipets	Such as Eppendorf, Pipetman, Lange-Levy, etc. depending on the preference of your chemists
Volumetric flasks, 50, 25, 10 ml	Standard item in Chem. Stores
Bench top centrifuge	Eppendorf type
Tubes to fit above (polypropylene)	Cole Parmer 7425 N. Oak Park Dr. Chicago, IL 60648
Burrell wrist-action shaker	Burrell Corp. Pittsburgh, PA
Sample evaporator (Be sure air used for this is free of oil and water)	We use home-made device, but for large sample through-put, you might consider a commercial evaporator-concentrator such as in Supelco 1985 catalog, p 179
Nitric Acid	Should be available in your Chemistry storeroom

Laboratory detergent and brushes	Should be available in your Chemistry storeroom
Deionized water/Articfical seawater	For use in blanks and calibration curves
Ring Stands and Clamps	
Kimwipes, or similar wipers	
Glass bottles with Teflon-lined caps, for storage of stock solutions (50 to 100 ml)	
Aluminum foil	

Analytical Procedure for the Tracor model 570

Gas Chromatograph-Flame Photometric System

CAUTION!!

GC-FPD unit should be equipped with 600-2000 nm cut on filter for tin detection in RIGHT detector (front photomultiplier) and 394 nm filter for sulfur detection in LEFT detector (rear photomultiplier). Be sure to unplug power supply wires on both PM tubes or turn off main power to GC before removing PM tube. to check or change filters or the PM tubes will be permanently damaged. There is no separate power switch for the photomultiplier tubes on the Tracor G. C.

GC-FPD set-up and check

1. Turn on nitrogen carrier gas and adjust regulator to deliver 50 psi.

2. Nitrogen flow rate = 20mL/min. This is approximately 17 divisions on the built-in flow meter on GC. Check with bubble flow meter attached to flame exhaust tube before turning on flame gases. The nitrogen flow is controlled by adjusting a knob labeled "SPLITTER GAS" on the left side of the detector, under the flip-up cover on the top of the G. C.

3. Turn on main power to GC-FPD (if unit is not on stand-by) and set detector to 200°C and injection port to 150°C. Wait until detector is up to operating temperature before trying to light flame.

4. Turn on hydrogen and air tanks, adjust regulators to 50 psi. For easy lighting of flame, set air to 150 mL/min and hydrogen to 100 mL/min. This ratio may require adjustment during flame lighting in order to ignite detector. Flame is ignited by turning hydrogen flow OFF with knob on detector, then using toggle switch to heat ignitor. While holding ignitor switch ON, turn hydrogen flow ON with knob. Flame ignition can be detected by holding a cold metal object (viz., a small wrench) to the outlet of the exhaust tube and observing condensation. The flame will probably not light the first time, and slight adjustment in gas ratios may facilitate flame lighting. Repeated inability to light flame could be due to deposits in exhaust tube. (See trouble shooting guide.)

5. For operation, set hydrogen at 170 mL/min and air to 75 mL/min on the flow controllers.

6. Check that external attenuation = 100 (on GC front panel) and internal attenuation = 1 (set with Spectra physics integrator).

7. Make sure that the oven fan in ON, and adjust CRT screen brightness.

8. Call up desired temperature program from microprocessor memory. When oven reaches required temperature the unit is ready to run.

9. The temperature program used for the 3% OV-101 column is - (See Fig. 1)

Initial over temperature: 40°C

Following injection hold 40°C for 2 min

then increase to 170°C at 40°C/min

hold for 1 min then to 180°C at 40°C/min

hold for 2 min then to 200°C at 40°C/min and hold for 3.5 min

Under these conditions the retention times are approximately:

Compound	R.T. (min)
Monobutyltin	2.90 ± 0.05
Dipropyltin	4.78 ± 0.05
Dibutyltin	5.91 ± 0.07
Tributyltin	8.46 ± 0.1
Tetrabutyltin	11.87 ± 0.2

Note: This is control method 06 in G. C. microprocessor memory.

The temperature program use with the 1.5% column is (See Fig. 2)

Initial temperature: 40°C

After injection hold 40°C for 2 min

then increase to 170°C at 40°C/min, hold for 2.5 min

then go to 190°C at 40°C/min

Under these conditions the retention times are approx:

Compound	R.T. (min)
Monobutyltin	2.28 ± 0.05
Dipropyltin	4.34 ± 0.05
Dibutyltin	5.48 ± 0.07
Tributyltin	7.71 ± 0.1
Tetrabutyltin	10.78 ± 0.2

Note: This is Control Method 02. The even number control methods control the RIGHT detector and the odd

number methods control the LEFT. In its present configuration, this particular instrument has only a right detector, no left detector is installed.

Column temperatures over 200°C are not advised because the butyltin hydrides tend to decompose above that temperature. The maximum temperature for the column packings is 350°C.

The retention times noted are approximate and will vary slightly from column to column due to differences in tightness of column packing, etc. These retention times should be checked using known compounds each time a new column is installed and periodically over the life of a column.

Packing and Conditioning Columns

The analytical columns can easily be packed by plugging the column outlet with glass wool, applying a slight vacuum and using a Pasteur pipet bulb with the top cut off as a funnel to transfer the packing material to the column. Gentle tapping of the column with a pencil or similar object will insure uniform packing. When the column is filled to within about 1 inch of the inlet, remove the vacuum and plug the inlet with glass wool. The column is now ready to be conditioned. This is accomplished by installing the inlet end of the column into the injection port of the GC with the carrier gas flowing (do not connect the column outlet to the detector) and running a slow temperature gradient as recommended by the packing manufacturer. For the OV packing, the recommended procedure is to purge the column at ambient temperature for at least 30 min with the carrier gas flowing, then program the oven to heat from 50°C to the highest anticipated operating temperature of the column at 2°C/min, then hold this temperature overnight. The new column is then ready for use.

The packing material seems to have some active sites which bind the organotin compounds irreversibly. To passivate these sites on a newly packed column, make several injections (3-4 is usually sufficient) of organotin analyte prior to the first injection used for quantitation. These procedures are necessary only for newly packed columns. After extensive use with environmental samples, the inlet glass wool and first several centimeters of the column packing material may become discolored (usually green) and inefficient. It is possible to gently remove the discolored portion, then repack and replug with glass wool. This repaired

column should be conditioned and passivated as a new column. Eventually the packing material loses efficiency after extensive use (>200 injections). This is usually manifested in exaggerated peak tailing and poor peak shape. The exhausted material can be blown out and the glass column repacked.

For best resolution of monobutyltin trihydride analyte from the solvent peak, use the 3% OV-101. For a slightly faster G. C. run, but poorer monobutyltin peak, the 1.5% is satisfactory. (See Figs. 1 and 2). The reduced loading packing (1.5%) has the advantage of less column bleed and possibly fewer problems with heavy build up of material in detector exhaust tube.

Hydridization/Extraction Procedure

Reagent Preparation

1. Make stock solutions of mono-, di-, and tri-n-butyltin chloride, tetrabutyltin and di-n-propyltin dichloride in Spectro grade methanol at concentrations of approximately 1000 ppm Sn in 50 mL volumetric flasks. Transfer to acid washed borosilicate glass bottles and cover bottles with aluminum foil. Maintained in the dark and tightly capped, these standards have a shelf life of 3 months or more.

2. The working standards are prepared from the above methanol stock solutions by dilution of 1:1000 in deionized water, to give a working solution of approx. 1 ppm. For example, dilution of 50 microliters of 1000 ppm methanol stock into 50 mL of water yields a working solution of 1 ppm Sn. For working a the 1 ppb level this working solution can be used directly (0.100 mL of this dilution into a 100 mL sample gives final Sn concentration of 1 ppb). For work at the parts-per-trillion level, a second dilution of 1:10 is advisable. Diluting 1 ppm solution 1.0 mL to 10 mL gives a working solution of 100 ppb. A 0.050 mL spike into a 200 mL sample gives a final concentration of 25 parts-per-trillion Sn.

Note: Prepare the aqueous working solutions daily.

3. Prepare 4% sodium borohydride solution in deionized water. Each pellet weighs approximately 0.4 gram, so one pellet dissolved in 10 ml water is a 4% solution. Prepare daily.

Analysis Sequence

The following procedure is for 200 mL sample size, using a 250 mL Wheaton screw top separatory funnel. For larger or smaller sample volumes the reagents are scaled up or down accordingly.

1. Place water sample in separatory funnel. Add dipropyltin dicloride solution spike to give final concentration in the range that you expect to find in the particular sample being analyzed. For most environmental waters, a final concentration of 25 or 50 ng Sn/L for the internal spike is satisfactory. The same spike is added to all sample and calibration runs, and peak areas are normalized to the internal standard. Shake funnel to disperse spike.

2. Add 6.0 mL high purity dichloromethane and 2.0 mL 4% sodium borohydride solution. Cap and shake by hand for about 30 sec. Open cap and VENT, as considerable pressure builds up quickly. Recap funnel and shake on mechanical shaker for ten minutes. Venting during mechanical shaking is not necessary.

3. Remove funnel from shaker, open cap, allow 5 min. for phase separation with caps loosened. Recovery is aided by hitting the funnels with the heel of the hand to recover the layer of dichloromethane that is often found floating on the sample's surface. Remove the lower (dichloromethane) layer into centrifuge tubes. In sample with considerable algal concentration or other particulate matter there may be an emulsion layer composed of particate matter, water and solvent. This can be easily broken by centrifugation for about 1 min. The upper water/particulate layer can then be pipetted off.

4. Add 3.0 mL dichloromethane to the sample, hand shake and vent as before. Shake mechanically for 10 min.

5. While second extraction is being done, the first extract can be evaporated under a gentle stream of dry air. DO NOT EVAPORATE TO DRYNESS.

6. After the 10 min. shake, and following 5 min. equilibration, the second solvent extract is added to the centrifuge tubes, combining the extracts. For 100 mL samples both extracts will fit into the 1.5 mL centrifuge tube. For larger sample volume (250 mL) two tubes, or more, are required. For 1 L samples, 15 mL glass centrifuge tubes have been used successfully.

7. The combined dichloromethane extracts are then concentrated by evaporation under flowing air. For water samples of 1 ppb tributyltin (TBT) concentration, the solvent extract need only be evaporated to about 1 mL. For samples in the 10-50 parts-per-trillion range, evaporation to 50-100 microliters is required. The final volume of the solvent need not be measured exactly, as the use of the internal standard compensates for small variations in volume, mechanical losses during work up, etc.

8. A five microliter portion of the sample extract is then injected into the gas chromatograph.

9. The syringe should be flushed with dichloromethane immediately after use, to prevent deterioration and contamination of the syringe. Sample carry over in the syringe can be a problem, especially if going from samples of high to low TBT concentration. Injection of 5 microliters of dichloromethane as a syringe blank at the beginning of the day is useful to insure that there is no syringe carry-over from previous uses.

10. Appropriate blanks should be analyzed daily, including a sampling blank (i.e., a distilled water or artificial seawater sample that

was handled and stored in the same way as the environmental sample), and one or more reagent blanks (i.e., distilled water or artificial sea water which has not been exposed to the sampling procedure, but is carried through the entire analytical work up). These blanks are absolutely necessary to insure that no source of butyltin contamination has been introduced into the analytical environment through incompletely cleaned glassware or other sources. When working at these ultra-trace concentrations contamination of the samples is very troublesome and frequent analysis of blanks is the only way to be sure that the measured concentrations represent what is found in the sample, not what may have been added accidentally during or after sampling.

11. Calibration curves are generally done at the end of the day, to insure that there is no carry-over of the butyltins used for calibration into sample analyzed subsequently. A 3 or 4 point calibration curve is usually sufficient, using the internal standard as well as all butyltin species seen in the sample. Calibration curves can be run in deionized water, artificial sea water or clean sea water. The choice of matrix depends on the type of sample and availability. The use of the internal standard allows for the use of a calibration curve, rather than doing a standard addition technique for each sample, but it is possible that there are interferents (positive and negative) that exist at specific sampling sites. For this reason it is recommended that comparison of results obtained by standard addition and calibration curve methods be done for each site to be monitored, to insure that site specific interferents are not a problem.

Maintenance and Trouble-shooting Guide for GC-FPD System

A white solid material composed of column bleed and combusted analyte tends to accumulate in the detector. This material decreases detector sensitivity and can cause severe problems, such as inability to light flame. Several procedures should be followed to minimize these difficulties.

1. The analytical column should be periodically "baked" at elevated temperature (170-200°C) with carrier gas flowing for several hours (overnight) to elute any highly retained materials. During this baking, the detector should be burning or the column outlet should be removed from the detector, to prevent build-up of refractory materials on the detector.

2. The exhaust tube which exits the detector should be removed daily and rinsed with water to remove any accumulated material which could impair gas flow through column and/or detector. Heat tape applied to the exhaust tube, although not tried at NBS, may prevent condensates.

3. The detector should be removed periodically and cleaned with acetone as described in the operator's manual for the detector. The interval between cleanings is difficult to predict as it depends on the number and type of samples being analyzed. A monthly or bi-monthly cleaning schedule will probably be sufficient.

4. The o-rings of the detector must be replaced periodically as they become brittle and crack with extended use. The operator's manual recommends replacement at intervals of six months, but more frequent changes are probably advisable as preventive maintenance.

5. The windows (heat shields) inside the detector can become cloudy and impair sensitivity. These windows should be inspected each time the o-rings are changed and replaced if they begin to look cloudy.

6. If there is a catastrophic loss of sensitivity, the first thing to check is whether the flame gas mixture is correct because response is highly dependent on flame conditions. Next check pneumatic problems, in particular a blockage in the flame exhaust pipe. If this does not solve the problem, remove the detector and flush with acetone as described in FPD manual. If this does not work, there is probably a leak caused by a bad o-ring or a clouded heat shield. Replace o-rings (and heat shield, if necessary). If none of these measures are successful, it may be that the photomultiplier tube is going bad, as PM tubes show diminished response with aging. In our experience, PM tubes have lasted several years and none of the problems we have experienced have been caused by the PM tube.

7. Unusual peaks are sometimes seen since this method is tin selective, not tin specific. These may be S, As, Pb or other compounds which emit in the observed wavelengths when burned in a hydrogen rich flame. These peaks often occur in the 5-7 minute retention time range, but do not generally interfere with butyltin peaks. By using simultaneous Tandem detection with two integrator/plotters, it is possible to determine if the peak is caused by tin or by another element. One detector is equipped with a 600-2000 nm filter selective for Sn and the other is equipped with, for example, a 394 nm filter which is sulfur selective. Comparison of ratios of authentic Sn compound peak areas on the tin and sulfur filters will reveal if the unknown peak is Sn containing. Measurements were made comparing the Sn/S peak area ratios for two sulfur

compounds. For dimethyldisulfide, the ratio is 0.029 ± 0.008 and for 2-mercaptoethanol, the ratio is 0.018 ± 0.002. A FID detector is also present on the Tracor instrument, if simultaneous FID-FPD is desired.

Cleaning Glassware

Butyltin compounds absorb onto glass and Teflon surfaces and rigorous cleaning procedures are required to prevent cross-contamination from sample to sample. This is especially critical when going from high to low concentration samples. After use the funnels should be shaken with methanol for about 5 minutes, then washed with hot soapy water and a brush. After the glassware is rinsed, it is completely disassembled and leached with warm 10% nitric acid overnight, followed by copious deionized water rinses.

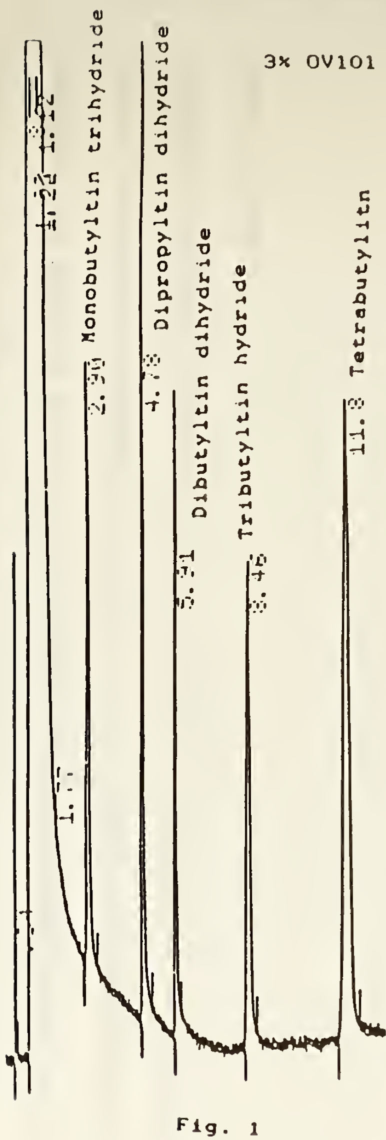

Fig. 1

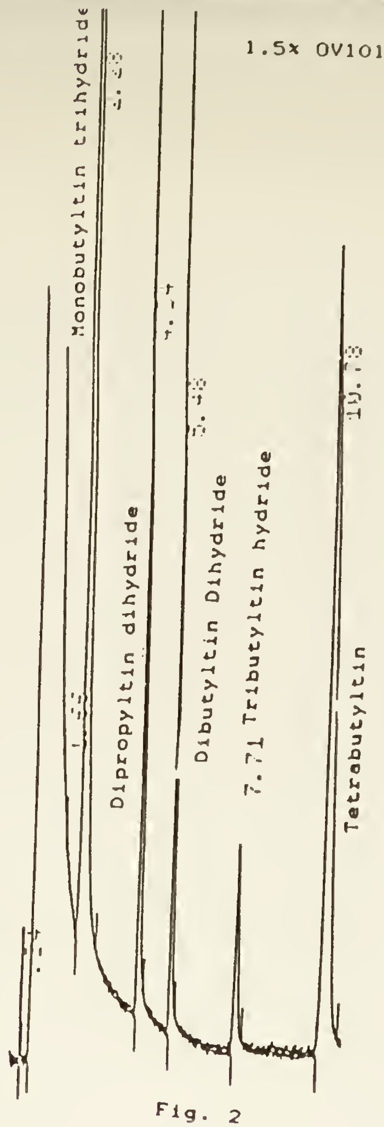

Fig. 2

See text for complete description of analytical conditions.

NBS-114A (REV. 2-80)

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4. TITLE AND SUBTITLE

A Comprehensive Method for the Determination of Aquatic Butyltin Species at Ultratrace Levels Using Simultaneous Hydridization/Extraction With GC-FPD

5. AUTHOR(S) G.J. Olson, F.E. Brinckman, C. Matthias, J.M. Bellama

6. PERFORMING ORGANIZATION (If joint or other than NBS, see instructions)

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10. SUPPLEMENTARY NOTES

☐ Document describes a computer program; SF-185, FIPS Software Summary, is attached.

11. ABSTRACT (A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here)

A method for the analysis of aquatic butyltin and mixed methylbutyltin species using simultaneous hydridization with sodium borohydride and extraction into dichloromethane is described. The detection limits are 7 ng Sn/L for tetra-butyltin, 7 ng Sn/L for tri-n-butyltin, 3 ng Sn/L for di-n-butyltin, and 16 ng Sn/L for mono-n-butyltin. The presence of tetrabutyltin in harbor waters is reported.

12. KEY WORDS (Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)

alkyltin compounds; chemical speciation; Chesapeake Bay; element selective detectors; environment; extraction; gas chromatography; organometallic compounds; organotin biocides

13. AVAILABILITY

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