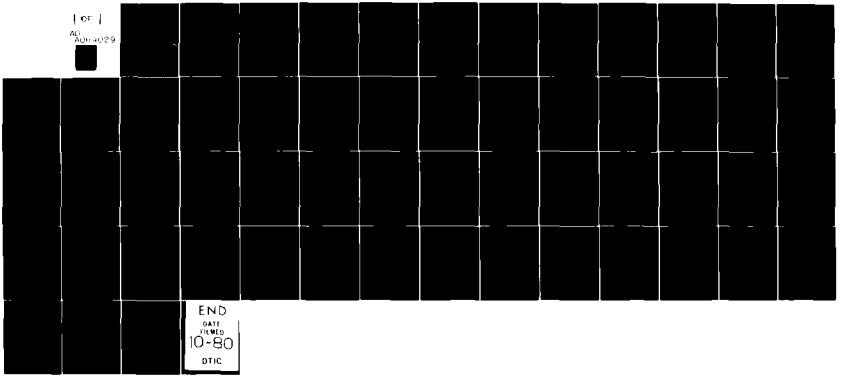
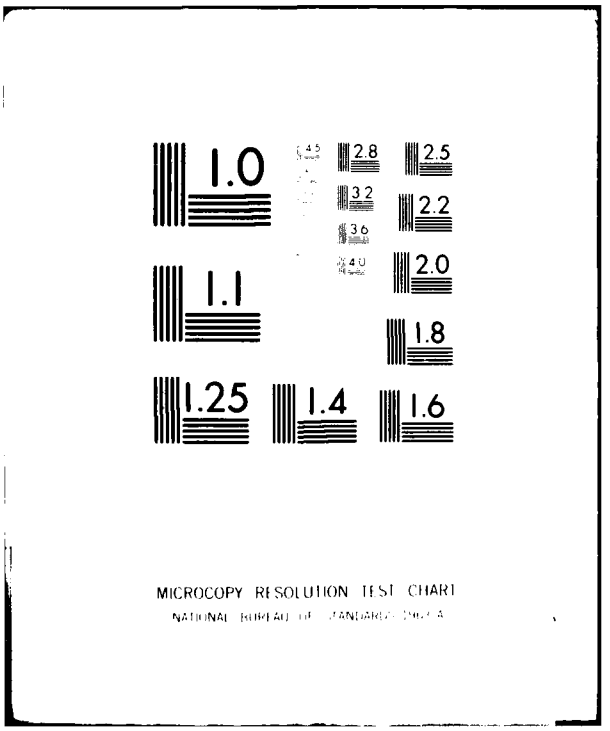
AD-A089 029 NATIONAL BUREAU OF STANDARDS WASHINGTON DC CHEMICAL --ETC F/G 7/4
SPECIATION OF TRACE DI- AND TRIORGANOTINS IN WATER BY ION EXCHA--ETC(U)
AUG 80 K L JEWETT, F E BRINCKMAN
UNCLASSIFIED NBS-5610406

NL

1 OF 1
AD A089029



END
DATE FILMED
10-80
DTIC


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A089 029
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) Speciation of Trace Di- and Triorganotins in Water by Ion Exchange HPLC-GFAA, (14) NBS	5. TYPE OF REPORT & PERIOD COVERED Interim technical report,
6. PERFORMING ORG. REPORT NUMBER 5610406	
7. AUTHOR(s) K.L. Jewett and F.E. Brinckman	8. CONTRACT OR GRANT NUMBER(s) HR 356-689
9. PERFORMING ORGANIZATION NAME AND ADDRESS Chemical & Biodegradation Processes Group ~~Chemical Stability & Corrosion Division~~ National Bureau of Standards, Washington, D.C. 20234	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Department of the Navy Office of Naval Research Arlington, VA 22217	12. REPORT DATE Aug. 5, 1980
	13. NUMBER OF PAGES 55
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for Public Release, distribution unlimited. Reproduction is permitted for any purposes of the United States Government.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Distribution of this document is unlimited

THIS DOCUMENT IS BEST QUALITY PRACTICABLE. THE COPY FURNISHED TO DDC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

18. SUPPLEMENTARY NOTES

Prepared for publication in the Journal of Chromatographic Science

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Biocides, Complexation, Diorganotin Compounds, Element-Specific Detection, Graphite Furnace Atomic Absorption, High-Pressure Liquid Chromatography, Ion Exchange, Leaching, Nanogram Sensitivityk Organotin Cations, Speciation, Triorganotin Compounds.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Based on their behavior as stable cations in saline solutions, direct trace speciation of a broad range of organotins, representing current industrial or environmental interests, was performed by combination of an tin-specific graphite furnace atomic absorption detector with HPLC employing reverse bonded-phase strong cations exchange columns. Column and system performance and detection limits (5-30 ng as tin) vary predictably with individual substituents on organotins, but are easily optimized, for example, for identifying marine antifoulant leachates.

DD FORM 1473 JAN 73 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

DISCLAIMER NOTICE

THIS DOCUMENT IS BEST QUALITY PRACTICABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

SPECIATION OF TRACE DI- AND TRIORGANOTINS IN WATER BY ION EXCHANGE HPLC-GFAA*

K. L. Jewett** and F. E. Brinckman
Chemical and Biodegradation Processes Group
National Bureau of Standards
Washington, D.C. 20234

Brief Abstract

Based on their behavior as stable cations in saline solutions, direct trace speciation of a broad range of organotins, representing current industrial or environmental interests, was performed by combination of an tin-specific graphite furnace atomic absorption detector with HPLC employing reverse bonded-phase strong cations exchange columns. Column and system performance and detection limits (5-30 ng as tin) vary predictably with individual substituents on organotins, but are easily optimized, for example, for identifying marine antifoulant leachates.

SUBMITTED FOR PUBLICATION

SPECIATION OF TRACE DI- AND TRIORGANOTINS IN WATER BY ION EXCHANGE HPLC-GFAA*

K. L. Jewett** and F. E. Brinckman
Chemical and Biodegradation Processes Group
National Bureau of Standards
Washington, D.C. 20234

ABSTRACT

A broad range of organotins representing current industrial and environmental interests have been speciated in trace quantities by a combination of an element-specific graphite furnace atomic absorption detector coupled with HPLC employing commercial bonded-phase strong cation exchange (SCX) columns. Optimization of SCX column parameters was characterized in terms of efficiency and resolution, to provide examples for separation of organotins, $R_nSn^{(4-n)+}$, by class (n = 2,3), functionality (R = acyl, alkyl, alicyclic), and as geometric isomers (R = n-Butyl vs i-butyl; benzyl vs 4-tolyl). This permitted a novel application of molecular substituent parameters available from literature in a linear relationship to the free energy term ln k'. Means for predicting optimal chromatographic conditions or for identifying unknown R groups were shown. SCX column performance varies for individual organotin analytes, as do HPLC-GFAA system detection limits (95 percent confidence limit) in the range 5-30 ng (as Sn). Applications of the method to current problems involving direct speciation of organotins in field samples from marine antifoulant leachates are described.

KEY WORDS: Biocides, Complexation, Diorganotin Compounds, Element-Specific Detection, Graphite Furnace Atomic Absorption, High-Pressure Liquid Chromatography, Ion Exchange, Leaching, Nanogram Sensitivity, Organotin Cations, Speciation, Triorganotin Compounds.

SPECIATION OF TRACE DI- AND TRIORGANOTINS IN WATER BY ION EXCHANGE HPLC-GFAA*

K. L. Jewett** and F. E. Brinckman
Chemical and Biodegradation Processes Group
National Bureau of Standards
Washington, D.C. 20234

INTRODUCTION

Organotin compounds are achieving world-wide prominance in many industrial and agricultural applications of environmental importance. The general class of triorganotins are favored as selective biocides (1), notably as marine antifoulants, pesticides, or wood preservatives because of their demonstrated effectiveness and potential for molecular tailoring (2,3). Many studies show that both the number and kind of organic groups, R, covalently bound to tin in the organotin species $R_nSn^{(4-n)+}$, affect the toxicity displayed towards various biota (3,4). Moreover, for a given R group, triorganotin moieties are considerably more toxic than the corresponding diorganotin functions to most organisms. This fact is of special significance since the latter class now enjoys commercial success as catalysts and stabilizers in plastics for bulk and specialty purposes, including food containers (5). Consequently, as a result of expanding use and diversity in molecular forms of di- and triorganotin materials, the need for rapid, reliable and general methods capable of trace speciation for a broad range of R in both of these classes is apparent.

*Presented in part at the 178th National Meeting of the American Chemical Society, Washington, D.C., September 9-14, 1979.

**Author to whom correspondence should be addressed.

Direct methods for isolation and characterization of trace organotins in environmental media are not available. Current methods rely upon digestion or extraction, combined with various chemical means for derivation by complex formation or by formation of neutral covalent species. Most procedures seek to form volatile, hydrophobic organotin analytes representative of the original tin-containing substrate. This provides a means for concurrent preconcentration from the sampled medium, typically an aqueous solution. Consequently, although little work is reported on the direct solution derivatization and environmental speciation methods, notably by TLC (6,7), considerable effort has been devoted to applications of GC or evaporation separation schemes employing tin-specific detectors for quantification of such volatile organotin derivatives. Exhaustive hydridation (8-10), methylation (11,12), and bromination (13) are among procedures used to volatilize and separate individual organotins. These species are subsequently carried into flame photometric detectors selective for SnH (9,12), or into electron capture (8), conductivity (13), atomic absorption (10,14), or mass spectrometer (11) detectors.

An essential feature common to all of the foregoing analytical approaches stems from the recognition that most organotins, except the neutral tetraorganotins, are highly solvated by environmental media such as saline fluids. This occurs to an extent primarily dictated by the degree of substitution, n, and kind of organic groups in the $R_nSn^{(4-n)+}$ species. Moreover, for those organotin species not strongly complexed by natural ligands present in environmental media, the organotin cations behave as classic solvated metal ions when n = 1-3 (15,16). For such stable organotin cations, equilibria of fundamental significance to ion exchange or ion-pair chromatography occur:

$$R_nSn^{(4-n)+} + xL^{m-} = R_nSnL_x^{(4-n-xm)+} \qquad \text{Eq. 1}$$

(n = 1-3, m = 0-2, x = 1-3)

Quantitative determination of the distribution of such neutral or charged organotin solvates in aqueous solutions is now limited to those few cases where stability constants are measured, e.g., R = methyl or ethyl and L = acetate, chloride or hydroxide. Convenient computer programs are readily available for rapid computation of such complex equilibria (16) as additional data appear.

In continuation of our studies on biogenesis of methyltins (17) and associated aquatic organotin chemistry (16), we sought to develop the best direct means for solution speciation applicable to these compounds as well as to the broadest range of R groups characteristic of anthropogenic organotins (2,3,5). A useful method in this work also must provide means for direct speciation of di- and triorganotin mixtures at trace concentrations, since ample evidence exists that these are related by environmental degradation pathways (16,18).

The recent advances in ion chromatography which rely upon tandem columns of both anion and cation ion exchange properties (19) or a single column of hybrid properties (20) offer attractive possibilities for non-selective conductivity detectors. Such detectors are, however, biased by concentration variations needed for efficient ion elution. Introduction (21) of a graphite furnace atomic absorption (GFAA) detector, automatically coupled to a HPLC employing conventional ion exchange columns, permits both the requisite degree of element selectivity and sensitivity while completely eliminating background signal fluctuations caused by flow control and gradient elution. Thus, successful speciation of trace aquatic organoarsenicals in our laboratory and else-

where (22,23), using various anion exchange columns with the HPLC-GFAA method, suggested that the simple cation exchange chemistry implied by Equation 1 could be adapted to the direct organotin separation problem. Utilizing a commercially available reverse bonded-phase SCX column, we have demonstrated use of HPLC-GFAA to provide reliable and extensive prospects for direct speciation of both trace di- and triorganotin species in water, broadly representative of current industrial and environmental interest.

EXPERIMENTAL

Chemicals and Materials

Organotins were obtained from commercial sources and used without further purification; all inorganic salts were analytical grade. Stock solutions of organotins, nominally at 1,000 ppm (μg/mL as tin), were prepared with spectro-grade methanol. These were diluted to appropriate working concentrations (0.1 - 2 ppm) with deionized water (18 MΩ-cm resistivity) or methanol on a daily basis prior to chromatographic runs. Eluent solutions were prepared by first dissolving the required quantity of (buffer) salt(s) into deionized water, then adding sufficient methanol to yield the desired water-methanol ratio on a volume basis.

For those cases where speciation of geometric isomers or organotins was involved, the individual R groups (viz., n-butyl and t-butyl or o-tolyl and benzyl) were authenticated by detailed interpretation of ^{13}C and ^{13}C-^{1}H FT-NMR spectra. Appropriate R_2Sn or R_3Sn compounds, as received, were dissolved into methanol (20 mm O.D. tubes) and run, tributyltin oxide (TBTO) being run as a neat liquid. Spectra were acquired on a Brüker Model CPX-200 Spectrometer

operating at 50.3 MHz. Five to 1,000 transients were collected depending upon the individual relaxation properties of each organotin compound. Positive structural identifications were made from relative signal intensities, ^{13}C chemical shifts, and multiplicities of ^{1}H- and ^{119}Sn-coupled peaks. No carbon-containing impurities were seen at the one percent level in the organotins, except for $(t\text{-butyl})_2SnCl_2$ which contained about five percent unknown aliphatic component. The NMR data obtained agreed closely with literature assignments, where available (24), for the same or closely similar compounds.

Chromatographic Instrumentation and Operating Parameters

Experiments were performed with a commercial dual-piston solvent pump automatically coupled to an element-specific detector provided by a graphite furnace atomic absorption spectrophotometer (GFAA). The complete HPLC-GFAA system with auto-sampling interface and digital readout peripherals has been reported in detail (21). For the present studies, we employed an Altex (Beckman Instruments, Inc., Berkeley, California) Model 100 pump coupled through a Perkin-Elmer (Norwalk, Connecticut) AS-1 Auto-Sampler interfaced with their Model 460 spectrometer fitted with the HGA-2200 graphite furnace. Working solutions of individual or mixed organotin compounds were injected conventionally via a Rheodyne (Berkeley, California) Model 7120 high-pressure valve in 50-200 µL quantities. Typically, runs were performed under isocratic conditions and programmed flow was performed by an Altex (Beckman) Model 420 microprocessor controller.

Since the GFAA detector is insensitive to variations in eluent composition (14,21-23), we inserted an Altex Model 153 ultra-violet detector (254 nm) between the HPLC pump and the AA unit in order to continuously provide necessary information concerning passage of solvent fronts after injection (t_o) and equilibration status of columns.

At least 50-60 mL of each new eluent was passed through the Whatman (Clifton, New Jersey) Partisil-10 SCX analytical columns (10 μm particle size, 4.6 mm I.D. x 25 cm) prior to chromatographic runs. This afforded sufficient equilibration to achieve a stable base line in the UV detector operating at its highest (0.005 AU full scale) sensitivity. Use of recommended (25) guard column and silica pre-column gave more consistent results.

Two standardized operating conditions for the GFAA detector were adopted:

		A	B
Furnace thermal program:	dry	80° for 10s	100° for 10s
	char	100° for 10s	500° for 10s
	atomize	3000° for 1s 2500° for 6s	3000° for 1s 2500° for 6s
Furnace purge gas:		argon 200 mL min^{-1} stopped-flow mode	
Microprocessor:		auto-zero background mode integration period = 8s	
Auto-Sampler:		Pipetting interval = 50s Sample volume = 20 μL	
Spectrophotometer settings:		Analytical wavelength 224.6 nm Slit width 0.7 nm D_2 continuum lamp on	

Normally, furnace program A was employed. In cases where large quantities of smoke were produced by pyrolysis of highly carbonaceous buffers (i.e., citrate), program B proved more reliable and yielded greater sensitivity. Graphite tubes were coated with pyrolytic carbon as provided by the manufacturer (Perkin Elmer).

Orion Model 701 and 701A pH meters, employing a combination microprobe electrode, were used to obtain $[H^+]$ data both for stock eluent solutions and for eluents delivered from the HPLC system.

Data Processing

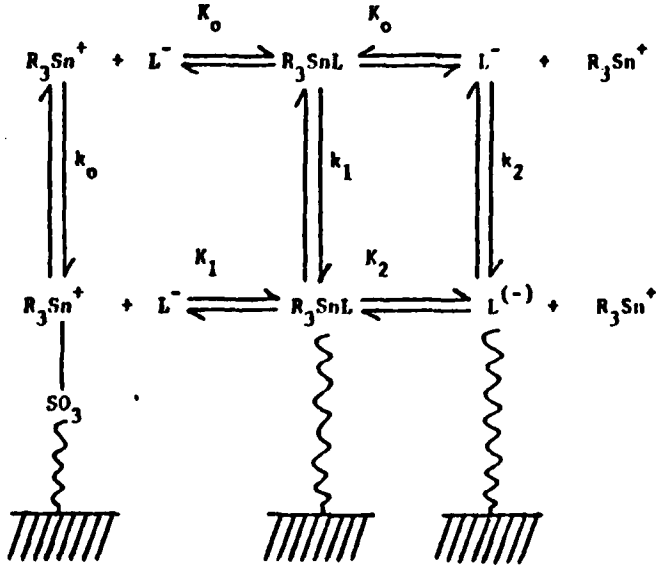
The GFAA detector operates in a periodic fashion to sample the laminar HPLC column eluent at pre-selected intervals of about 45-60 s. Consequently we generated element-specific concentration data for tin content in a histogramic format. A Shimadzu (Columbia, Maryland) Model C-R1A integrator-printer provided programmable conversion of histogrammic data to peak areas by summing GFAA peak outputs to give chromatographic peak areas. We have previously shown (21,22) that HPLC peak areas can be reliably generated either by digital planimetry of the "peak" derived from joining the tops of the GFAA "multiplet" or by summing the heights of the individual peaks forming the GFAA multiplet, to reliably give equivalent chromatographic peak areas.

RESULTS AND DISCUSSION

Column Properties and Mechanism of Organotin Separation

Introduction of commercial microparticulate silica gels as substrates for uniform and high density incorporation of substituted organosiloxanes offers nearly unlimited applications for high performance liquid chromatography. Not only do such chemically bonded column packings resist a wide range of pH in electrolytes, but their demonstrated rapid equilibration rates allow flexible elution programming at high ionic strengths without impairing column performance by swelling or irreversible changes in active sites on the stationary phase.

For an ideal reverse bonded-phase (RBP) strong cation exchanger, exemplified by the Partisil SCX siloxane-bonded benzenesulphonic acid function, we can regard the individual organotin ion as a classical cation. The comparable relation between the basic (anionic) species, a major complexing ion (or buffer) forming the supporting electrolyte, and active RBP anionic exchange sites has been treated fully in principle by Horváth et al. (26). A variant of that approach appears suited to qualitatively assessing organotin species and column properties found in the present work:



SCHEME 1

The simplest example conceptualized in Scheme 1 involves a monoacid R_3Sn^+ and a singly charged anion L^- interacting mutually or competitively with the substrate.

Principal stability constants for R_3Sn^+ or its ion pair R_3SnL in the mobile phase and at different kinds of active sites on the stationary phase are respectively represented by K_o or K_1, and, analogously for the electrolyte (buffer) ligand L^-, by K_2. Corresponding capacity (retardation) factors (k') contributing to or diminishing the apparent efficiency of the column, defined (27) by,

$$k' = t_R'/t_o, \quad \text{where } t_R' = (t_R - t_o)$$

are denoted by k_o, k_1 and k_2, respectively. The principal homogeneous equilibrium constant K_o for the mobile organotin eluate is exactly specified by Equation 1, barring influences of competing neutral ligands (such as methanol in the solvent). Thus K_o presumes either an "inert" neutral or an ion-pair product is formed (16). Where K_o and k_o can be expected to dominate the ion exchange column elution process, and this is not generally certain, the relative order and extent of retention t_R' for a mixture of organotin cations could be inferred from independently available or estimated stability constants with a given ligand L^-.

Organotin salts typically reported as "insoluble" in water (~ 0.1-100 ppm) (11,18), are considerably more (10^2) soluble in lower alcohols. Therefore, under the experimental constraints consequently imposed by optimizing their solubility in methanol-water mobile phases, in concert with improved column efficiences or capacities and necessary ionic strengths to achieve reasonable separation times and sensitivities, the individual effects of pH or ligand L^- selectivity were not measured. Nonetheless, it will be seen that these factors also variably affect k_o, k_1 or k_2 to some extent. Overall, the several Partisil-10 SCX columns used in the work, although from different lots, displayed qualitatively similar retention properties with their nominal quantitative variances (RSD) all in the ranges tabulated.

In Figure 1 are shown representative dual chromatograms of an aqueous sample containing both triphenyl- and tri-n-butyltin chlorides in equimolar concentrations. These compounds are representative of the distinctive differences between organotins in commercial use. The conventional UV detector trace illustrates the presence of the phenyl chromophore active at 254 nm, and, correspondingly, the absence of any active chromophore for the butyltin species. This last fact is common for all non-aromatic derivatives of organotins, thereby giving impetus to general metal-specific HPLC detection schemes for trace alkylmetals or alkylmetalloids (21). Also to be noted is the considerably greater sensitivity available with the GFAA detector (operating at about 1/50 full sensitivity) as compared with the UV detector operating at maximum sensitivity.

Similar chromatograms were generated for a series of tri-n-butyltin compounds bearing different anionic groups in order to test the validity of Equation 1 with the SCX column and its relevance to Scheme 1. Pertinent data are summarized in Table I where it is seen that the small variance (RSD = 3.3 percent) in k' for such a wide range of tributyltin derivatives indicates essentially the same retention properties. Clearly, the Bu_3Sn eluate species acts as a normal cation, in accord with Equation 1, in the sense that the original nature of its labile gegenion is unimportant to the main separation mechanism on the SCX column. Also included in Table I are capacity factors for three neutral, covalent butyltin derivatives, TBTO, tributylstannane, and tetrabutyltin. The last two both display markedly reduced retention on the ion exchange column, as anticipated for non-ionic molecules bearing no ligand labilized by aqueous medium. Nonetheless, their measurable k' values (~ 0.5) imply that either slight partition or RBP adsorption occurs, further implying that the apparent SCX column capacity ratios involve some additive combin-

stions of k_o plus k_1 or k_2. Possibly for some R_3Sn^+ salts, especially strong complexation by L^- (large K_o) accompanied by large k_1 would also simulate such non-ionic exchange behavior (21).

This measure of such SCX column performance by covalent R_4Sn was suggested by considering non-specific interactions of the organotin analyte with non-functional components of the ion-active surface, that is, interactions with the organic linkages bridging the gel siloxo-backbone and the active sulfonato function. Of course, RBP sulfonato groups do not occupy all the available hydroxy-siloxo sites on the gel, hence some possibility for conventional "partition" separation of organotins also exists, but this may be subject to steric effects with bulky organotin ions. To a degree, then, the column can perform as a "normal" hydrocarbon-like RBP packing, similar, for example to a C_2 or dimethylsiloxane bonded phase. For some triorganotin monoacids at least, incorporating phenyl, butyl and propyl moieties, molecular or ion-pair separation behavior rather than ion exchange was observed in pure methanol for hydrocarbon RBP C_2, C_8 or C_{18} substrates (21). Further, this continuum of behavior was recently exploited in the preparation of a "hybrid" or combination RBP-ion exchange stationary phase which satisfactorily separated both neutral, non-ionizable organometals from their homologous ionic organometal forms (28).

With the "true" strong cation exchange (SCX) column used in the present work, partial non-ionic column separation, this being a measure of the relative magnitudes of k_o and k_1, could be qualitatively evaluated by varying solvent properties. More important for our purposes, the clearcut involvement of a "free" tributyltin cation separation on the SCX column (Table I) indicated that such evaluation of the variations of mobile phase composition and ionic strength could yield optimized conditions for speciation of mixtures of various commercial organotins.

Table I

SPECIATION[a] OF TRIBUTYLTIN CATION AND RELATED SPECIES FROM VARIOUS R_3Sn-X SOURCES

-X	k'[b]
-OAc	3.33 ± 0.05
-F	3.46 ± 0.10
-Cl	3.36 ± 0.04
-Br	3.42 ± 0.11
$-SO_4(-SnBu_3)$	3.15 ± 0.04
$-O-SnBu_3$[c]	3.22[d] ± 0.24
-H	3.37[d] ± 0.13
	0.58[e] ± 0.10
-Bu	0.45 ± 0.08

k' = 3.33[f] ± 0.11

RSD = 3.30 percent

[a] Isocratic at 1.00 mL min^{-1}, 0.06 M NH_4OAc in methanol-water (30:70).

[b] Replicate runs, mean ± ave. dev.

[c] TBTO, tributyltin oxide.

[d] Fresh and aged aqueous solutions or methanol solutions containing 1-2 ppm as tin.

[e] Undissociated Bu_3SnH.

[f] Mean ± std. dev. excluding last two k' values.

Varying mobile phase methanol-water composition in theory (27,29) can either increase or decrease retention of R_3Sn species on an ion exchange bed depending upon several factors. The neutral methanol ligand could permeate the SCX matrix, thereby serving, by exchange site deactivation, to reduce k_0, and possibly k_1 or k_2. More likely is the prospect that ionization of the eluent R_3SnL according to Equation 1, or Scheme 1 is repressed (15,16). In any case, decreasing methanol in the solvent yields a familiar (29) hyperbolic relationship between k' and two very different triorganotins, where R = phenyl or n-butyl, as illustrated in Figure 2. With pure methanol, where either organotin should exist predominantly in an unionized form (16), absorption or partition separation mechanisms can prevail. A residual column retardation factor (27) involving k_1 and k_2 reaches a minimum at $k' \leq 2$ for tributyl- and triphenyltin, and even shows a measurable increase for the more hydrophobic aryltin (31,32). As the relative amount of water in the mobile phase increases, the column separation factor, $\alpha = k'_{Bu}/k'_{\phi}$ (27), increases from 0.7 to over 2. To a degree, under these conditions, the dissociation or hydration of R_3SnL is influenced by pH (16), but the small decrease (0.4 unit) in apparent pH (32) over the entire range of water increase shown in Figure 2, suggests that this cannot be a major controlling factor. More significant could be the reduced dissociation of electrolyte NH_4OAc in the richer methanolic mobile phases with corresponding reduction in available L^- or true ionic strength.

Effects on k' by variation of ionic strength represent a best test for the mechanism of retention by an ionic exchanger (31). If the mechanism of retention is pure ion exchange, basic chromatographic theory requires that the thermodynamically derived term k' should be linearly proportional to reciprocal ionic strength (26,27,31).

Figure 3 depicts our evaluation of the relationship between k' and $1/\mu$, again comparing triphenyl and tributyltin cations. A linear relationship is approximately obeyed ($r = 0.899$ to 0.995) for all the combinations tested over a fifteen-fold change in apparent ionic strength, with NH_4NO_3 showing nearly ideal behavior. Two other important features are noted: first, considerable electrolyte selectivity or influence on k' for either organotin occurs; second, positive intercepts result in the plots which confirm (29,32) that residual non-ionic retention processes still transpire at very high ionic strengths. Strong negative curvatures in the k' versus $1/\mu$ plots for NH_4OAc and $NaNO_3$ occur under these conditions, and additional non-linear (log-log) regression analyses indicate that the intercepts of the curves for butyl and phenyl species converge at $k' \sim 1.1 \pm 0.3$ and 1.9 ± 0.4, respectively. These results agree satisfactorily with those intercepts predicted by the more regularly behaved NH_4NO_3 curves, and are consistent with the k' measured (0.5 ± 0.1) for neutral, covalent tri- or tetrabutylstannanes (Table I). Thus a picture is completed of residual SCX column separation processes for several kinds of unionized triorganotin species, which are dependent upon combinations of k_o, k_1 or k_2, in turn dependent upon solvent composition.

Qualitatively, tributyl- and triphenyltin ions were found to behave as regular cations in appropriate methanol-water mobile phases, and it was shown that these are undoubtedly separated on the SCX column by an ion exchange mechanism in the range of $\mu = 0.02 - 0.005$ M for commonly used 1:1 electrolytes. At very low ionic strengths, the selectivity of the three salts used converges with triphenyltin to a common capacity factor $k' \sim 5$. This suggests that NO_3^- and OAc^- do not differ significantly in their stability constants, K_c, with this weakly acidic cation and that exchanger binding by Na^+ or H_o^+ in competition with $\phi_3Sn^+(k_o)$ is more important at higher ionic strengths. In

contrast, more typical behavior (29,32) with tributyltin ion showing increased selectivity with decreased μ was found. The relative magnitude of retention in the presence of each electrolyte is in the same order for both organotin cations. The two nitrates both conform to the expectation (27) that the tin analyte ion retention should increase with weaker electrolyte stationary phase binding in the order $NH_4^+ < Na^+$. The reversal in this trend for greatest retention in methanolic NH_4OAc must be reckoned in terms of both a greater extent of specific complexation of the stronger Bu_3Sn^+ acid by the bidentate acetate ligand in the mobile phase (3,15) combined with the lowest pH (7.58 ± 0.03) range for all the methanolic electrolytes. These factors in combination favor dissociation (K_o) and ion exchange capacity (k_o) according to Scheme 1 over such a large electrolyte concentration gradient.

Chromatographic separations of dibasic R_2Sn^{2+} species also follow the trends observed for R_3Sn^+ cations. On the microparticulate SCX column, use of singly charged acetate or nitrate salts was ineffective for achieving both reasonably narrow bandwidths and k' values sufficiently small for practical-analysis of diorganotins or the strongly acidic triorganotins with small alkyl groups. Since k' is inversely proportional to $1/\mu$, regular retention trends might be predicted (27) for these last categories of organotins, if they exhibited regular ion exchange behavior. In the next section, we consider these factors, along with prospects for predicting relative k' values for both R_2Sn^{2+} and R_3Sn^+ classes, based upon independently available molecular substituent properties.

System Performance of MPLC-GFAA With SCX Columns--Predictable Speciation and Reliable Quantitation of Aqueous Organotin Mixtures

Optimal application of element-specific detectors to chromatographs depends on comparable confidence in the molecular separation scheme. In

complex environmental samples, non-specific HPLC detectors cannot discern among a large number of bands which contains a selected element, such as tin. hence analytical confidence relies totally upon reproducibility (and, indeed, predictability) of t_R, V_R or k'. Although the tin-containing molecules can be confidently identified with the GFAA detector, a truly practical system nonetheless must also provide a high degree of repeatability in order that both known (authentic) and unknown tin-containing bands can be quantitated with assurance.

Stability of organotins.

Care was taken from the outset to evaluate both the stability of fresh and aged stock organotin solutions and their subsequent stability on the SCX columns. Our concern was not with the expected redistribution of labile anions on the organotin cations, but rather with any prospects for scission of important covalent Sn-C, Sn-H or Sn-O bonds which contribute to the distinctive chemical and biological, as well as chromatographic, properties of such analytes. In Table I, several additional k' values are reported which resulted not from the original organotin sampled as received, but from chemical decomposition of various sorts incurred by conventional handling.

Thus, for tributylstannane (Table I), two k' values are reported, one of which is coincident with a second peak assigned to the "free" tributyltin cations. The appearance of this latter peak was correlated ($r = 0.92 - 0.99$) with a first-order growth rate at the expense of the first peak, dependent upon the stannane's storage time in methanolic ($t_{1/2} \sim 149$ min) or in aqueous ($t_{1/2} \sim 42$ min) stock solutions. The decomposition was not apparently dependent upon residence time, e.g., flow rate, in the SCX column. These

results are consistent with and explained by the known protolysis of alkylstannanes in protic media, such as alcohols or water.

$$R_3SnH + H_2O = R_3Sn^{+} + OH^{-} + H_2\uparrow$$

These protolysis rates are considerably accelerated in water and in the presence of carboxylates (33).

Tributyltin oxide, $Bu_3Sn\text{-}O\text{-}SnBu_3$ (TBTO) is a liquid organotin biocide in widespread commercial use (1-3,5). Known to be moisture-sensitive yet highly insoluble in water (11,33), its analysis and bioassay are usually performed with solutions prepared by intermediate dissolving in miscible polar organic solvents, such as methanol and acetone, followed by needed dilution into water. Our data for TBTO summarized in Table I, whether for freshly prepared and aged methanolic or aqueous solutions, indicates that only free tributyltin cation was eluted. We confirmed the stannoxane molecular structure for our stock TBTO by comparing its ^{13}C and $^{13}C\{^{1}H\}$ FT-NMR spectra as a neat liquid with literature spectra (24,34). Comparison by NMR of TBTO dissolved in methanol indicated that cleavage of Sn-O-Sn did not occur, confirming that hydrolysis (33) occurred during passage through the HPLC-GFAA,

$$Bu_3SnOSnBu_3 + H_2O = 2Bu_3SnOH$$

These results are of general significance to workers involved with use of reactive organometal biocides unstable in test calibration solutions or during chromatography, and point to the utility of a direct speciation method for critical bioassays. Similar, repeated examination by HPLC-GFAA of both tri- and diorganotin salts in methanolic and aqueous solutions (100-1,000 ppm) over

storage periods in glass from five minutes to one year indicated no chemical alteration in the specified organotin cations moiety in terms of significant deviations in k' (RSD ~ 5 percent).

Scope and Prediction of Separations.

Heretofore, TLC methods provided the only comprehensive, albeit indirect, means for separating mixtures of both R_3Sn^+ and R_2Sn^{2+} species in a single analysis (6,7,35). Using SCX columns discussed in the foregoing section, we examined approaches to provide a direct means for speciating mixtures containing many biocidal triorganotins in single analyses. Figure 4 depicts one set of chromatographic conditions that successfully resolve a broad group of R_3Sn^+ species. The relevant parameters are summarized in Table II.

Qualitatively, the order of elution of R_3Sn^+ in a purely ion exchange separation should depend upon the availability of the charged cation, i.e., magnitude of K_o in Equation 1. Thus, barring steric effects by individual R groups that inhibit K_o or k_o (Scheme 1), we would infer that relative electronic effects of R in the R_3Sn^+ ion will be primarily responsible for relative retention on the SCX bed. The elution order seen in Figure 4 generally conforms to the usual chemical inferences which predict that R_3Sn^+ acidity increases in the order, Bu<Pr<Et<Me<ϕ (33). Even cyclohexyl (c-Hx) appears to conform to this trend, but phenyl behaves anomalously. Ideally, a quantitative relationship between k' and selected molecular substituent properties derived from measurements physically-independent of chromatography is required to permit accurate prediction of elution of known ions, or characterization of unknowns from elution phenomena. Further, this should be valid for different operators with the same or different stationary phases or different mobile phases.

Table II

SPECIATION OF TRIORGANOTINS BY HPLC-GFAA ON AN ION EXCHANGE COLUMN[a]

Cation[b]	k'[c]	Mean Peak Area[c,d]	Peak Area/ng Sn[c,e]	N, M^{-1}[c,f]
Ph_3Sn^+	2.98 ± 0.03	55,567 ± 8,518	741 ± 114	891 ± 143
Bu_3Sn^+	4.63 ± 0.12	61,273 ± 12,388	817 ± 164	1,361 ± 149
Pr_3Sn^+	8.77 ± 0.25	51,072 ± 2,296	681 ± 31	2,307 ± 602
Et_3Sn^+	15.20 ± 0.29	29,664 ± 14,510	396 ± 192	5,649 ± 556
Me_3Sn^+	47.26 ± 2.28	43,092 ± 3,786[g]	86 ± 8[g]	5,109 ± 86
C-Hx_3Sn^+	6.09 ± 0.13	12,599 ± 5,524	175 ± 74	1,348 ± 106

[a]Whatman 10 μ Partisil SCX, 0.03 M NH_4OAc in methanol-water (70:30) at 1.5 to 2.0 mL min^{-1}.

[b]First four cations, 75 ng each as mixed R_3SnCl solution in methanol 100 μL injections.

[c]Mean ± ave. dev. for replicate runs.

[d]Units of μV·s employing sum of peak heights method (21).

eGFAA detector program A .

[f]Based upon approximate plate equation $N = 5.54 (t_R/w_{1/2})^2$ (27).

[g]Injected as 500 ng samples.

[h]Run as single component, 75 ng as hydroxide in methanol solution quadruplicate runs.

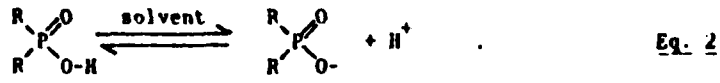
Much attention has recently been devoted to exploring both the underlying theoretical and empirical prospects for such dimensionless "retention indices" (36). Since preliminary retention factors obtained for both R_3Sn^+ and R_2Sn^{2+} cations suggested regularities that were not simply related to those substituent constants often correlated with equilibria of the type examplified by Equation 1, we examined this important consideration in further detail.

The logarithm of the capacity factor, ln k', is proportional to the free energy change associated with the chromatographic partitioning process (26,37), especially Ko as defined in Scheme 1. With this view, we surveyed the linear proportionality of ln k' for R_3Sn^+ versus a large number of "quantitative structure-activity relationships" (QSAR) compiled by Hansch and Leo (38). A similar approach was successful for linear correlations between substituents on closely related homologous series of organic molecules separated either on paper chromatography or RBP HPLC (39).

For the relationship,

$$\ln k' = m(\text{QSAR}) + \text{constant} \quad ,$$

only poor to fair correlations obtain for alkyl R_3Sn^+ with those conventional molecular substituent constants (38) that emphasize diffuse stereoelectronic forces (molar refraction, r = 0.898); pure inductive effects, (σ_I = -0.415); specific steric effects, (ES, r = 0.772); or hydrophobic (partition) effects, (π, r = 0.917). Inspection of Scheme 1 suggests that contributions (possibly additive) of R to the stabilization, or abundance of, charge on R_3Sn^+ or R_2Sn^{2+} are better represented by an analogous series of ionization reactions widely studied for organophosphonic acids in water or water-alcohol solutions (40,41):



Eq. 2

Equation 2 bears many similarities to Equation 1, principally because no change in oxidation state occurs in the dissociation, the integrity of covalent R-heteroatom bonds is preserved, a unit change of ± one electron charge is involved, and data for a very broad range of alkyl, alicyclic, aryl, olefinic and heteroatom substituents are available. Against this, it should be recognized that substantial rehybridization of s-p-d orbitals occurs during aquation and dissociation of organotin salts upon solution in polar media (3, 15,16), and that modes of bonding interactions between R and central charged tin can change. For the organophosphonate, where anionic charge is not localized on the central P atom, but rather on the more electronegative oxygen, this should be less important (41). Excellent linear correlations between pK_a for Equation 2 are available (38,40) in the form,

$$pK_a = pK_o + m\sigma^{\Phi} \quad ,$$

for all of the R groups examined on organotins in this paper. The new Hammett-type QSAR σ^{Φ} represents combinations of both inductive (σ_I) and p_π or d_π resonance (σ_R) effects which can occur with energetically available p or d orbitals in R-P or R-Sn bonding (3,33). Thus, not only does σ^{Φ} present a suitably selective diagnostic for predicting organophosphonate anion formation, the linearity cited also holds for the alcoholic solutions of the type required in organotin ion exchange chromatography.

Figure 5 (top) indicates the excellent fit ($r = 0.992$) for 23 individual k' values obtained in NH_4OAc for the five alkyl R_3Sn^+ species listed in Table II.

Clearly, the σ^{ϕ} constants available in literature provide potential predictors for chromatographic retention factors in the n-alkyl (and possibly cyclo-alkyl) R_3Sn series, but equally clear was the failure to fit k' for triphenyltin cation. Presumably, in the aryltin case, a marked change in the σ_I and σ_R contributions to σ^{ϕ} occurs during ionization of the phosphonate relative to those involved with ionization of R_3Sn^+. For alkyl substituents, either on P or Sn, inductive effects should predominate during ionization processes, with similar effects on both elements, and this was borne out experimentally in the present case.

Major questions yet remain in order to fully apply the potential of such a widely available QSAR as σ^{ϕ} to the other classes of organotins of concern to chromatographic analysis. In particular, we sought to more fully correlate R substituent effects for aryl groups in R_3Sn and both alkyl and aryl groups in R_2Sn species. If possible, a relationship predicting k' for both R_3Sn and R_2Sn eluents was desired. Table III summarizes measured and predicted capacity factors for a number of organotin ions featuring steric and electronic differences presented by isomeric R functions on both R_3Sn and R_2Sn classes in several mobile phases using acetate or citrate electrolytes as examples of uni- and divalent counter ions. The appropriate correlation data in Table III are also plotted in Figure 5.

Two types of behavior are observed. The alkyl R_3Sn^+ homologs display (Figure 5, upper plot) excellent positive correlations with σ^{ϕ}, even in diverse electrolytes such as 0.03 M acetate and 0.03 M citrate. The behavior of aryl substituents (including benzyl) on R_3Sn^+ is more similar to that seen (Figure 5, lower plots) for either alkyl or aryl substituents on R_2Sn^{2+}, e.g., negative correlations with σ^{ϕ}. Solvent strength plays an expected role in that increased ionic strengths diminish the magnitude of slopes of all of the correl-

Table III

CORRELATION MATRIX FOR SCX CAPACITY FACTORS AND Σ^{ϕ}

	Me	Et	n-Pr	n-Bu	c-Hx	ϕ	4-MeϕCH$_2$	ϕCH$_2$	i-Bu	t-Bu
σ^{ϕ}[a]	-0.96	-1.10	-1.18	-1.22	-1.19	-0.48	-0.60	-0.69	-1.30	-1.65
R_3Sn Class[b]:										
k' obs[c]	47.3	15.0	8.70	4.54	6.10	3.26				
k' calc[c]	49.5	14.5	7.23	5.09	6.62	----				
k' obs[d]	8.03	2.79	2.05	0.88	----	1.52				
k' calc[d]	8.24	2.89	1.59	1.18	----	----				
R_2Sn Class[b]:										
k' obs[e]				5.07					0.87	11.7
k' calc[e]				5.51					6.72	12.3
k' obs[f]				2.53					3.47	5.60
k' calc[f]				2.68					3.22	9.70
k' obs[g]						0.85	1.29	4.35		
k' calc[g]						0.74	1.82	3.58		
k' obs[h]						0.54	0.86	3.21		
k' calc[h]						0.46	1.24	2.60		
k' obs[i]						0.49	0.64	2.06		
k' calc[i]						0.42	0.92	1.68		

[a] From (41,42)

[b] All separations run isocratically in methanol-water (70:30) at 1.0 to 3.0 mL min^{-1}.

[c] In 0.03 M NH_4OAc, n = 23, r = 0.992, ln k' calc = $8.74\sigma^{\phi}$ + 12.29.

[d] In 0.03 M $(NH_4)_2$ citrate, n = 11, r = 0.969, ln k' calc = $7.47\sigma^{\phi}$ + 9.28.

[e] In 0.06 M $(NH_4)_2$ citrate, n = 7, r = -0.939, ln k' calc = $-2.39\sigma^{\phi}$ - 1.21.

[f] In 0.12 M $(NH_4)_2$ citrate, n = 3, r = -0.986, ln k' calc = $-2.29\sigma^{\phi}$ - 1.81.

[g] In 0.005 M $(NH_4)_2$ citrate, n = 3, r = -0.936, ln k' calc = $-7.53\sigma^{\phi}$ - 3.92.

[h] In 0.0075 M $(NH_4)_2$ citrate, n = 3, r = -0.938, ln k' calc = $-8.22\sigma^{\phi}$ - 4.71.

[i] In 0.01 M $(NH_4)_2$ citrate, n = 3, r = -0.914, ln k' calc = $-6.64\sigma^{\phi}$ - 4.86.

ation curves (Table V), as predicted from ln k' ∝ 1/μ behavior discussed in the previous section. More notable is the observation that, when such reduction occurs, each family of curves characteristic for R and R_nSn maintains nearly a parallel relationship. This trend is consistent with the previous idea that a uniform column separation mechanism prevails for each class of analyte mainly in terms of K_o and k_o (Scheme 1).

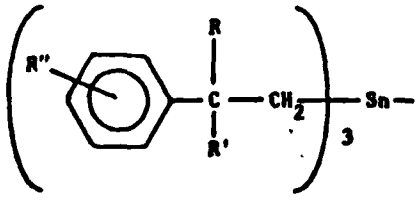
With nearly 200 k' values collected, it was not possible to establish any useful correlations between R_3Sn^+ and R_2Sn^{2+} eluates that allow prediction of retention factors for one class from the experimental data collected on the other class. The available σ^ϕ for each R group probably cannot discriminate in a simple additive manner the effects on k' as the number of R groups in tin varies. Evidence (40) for different values of σ^ϕ depending on the number of given R groups bonded to phosphorus indicates the likelihood of this problem. Nonetheless, Table III shows that within the respective classes of organotins, their k' values can be presently predicted from independent literature values of σ^ϕ to better than (RSD) 12 percent for R_3Sn series and eight percent or 25 percent for the alkyl or aryl R_2Sn series, respectively. These variances are expected to diminish with additional k' values for new R groups.

An important consideration meriting future study, will be application of σ^ϕ literature values to prediction of k' for mixtures of organotins, or as an index for identifying unknown peaks occurring in well-defined chromatograms spiked with authentic organotins as internal calibration standards. Figure 6 illustrates this point for isomeric R_2Sn cations bearing either alkyl or aryl substituents. From Table III data, σ^ϕ values for 4-tolyl (4-MeΦ) and benzyl (ϕCH_2) groups are, respectively, -0.60 and -0.69. Comparison of k'_{obs} in 0.005 M to 0.01 M citrate concentrations show that separation factors (27), $\alpha = k'_{4\text{-}Me\phi}/k'_{Bz} > 3.2$. Since acceptable separation is achieved with column efficiencies (N) available in 0.01 M citrate, we expect that other aryl

substituents differing in σ^{ϕ} by more than ± 0.1 unit (such as p-$CH_3OC_6H_4$, σ^{ϕ} = -0.59 or p-ClC_6H_4, σ^{ϕ} = -0.29) could be separated as well as in the case depicted in Figure 6. Similarly, for a dialkyltin species which differs in the geometry of R, the satisfactory separation factor (~ 1.6), also illustrated in Figure 6 for n-Bu and i-Bu derivatives, can be predicted to provide successful speciation of sec-Bu (σ^{ϕ} = -1.36) (41) in a n-Bu or t-Bu mixture under comparable chromatographic conditions. However, only marginal separation of sec-butyl from i-Bu is expected, though the order of retention would likely be discerned.

The case for R_3Sn^+ speciation and identification appears to be better established with more R groups and less data scatter. From data in Table III, we deduce that both the retention order and resolution could be confidently inferred to within about ± 0.03 unit of σ^{ϕ} in the more favorable 0.03 M acetate mobile phase. This implies a separation factor of ~ 1.3. In fact, this prospect was tested in a subsequent separation involving ϕ_3Sn, n-Bu_3Sn, and c-Hx_3Sn species, where σ^{ϕ} values are respectively -1.22 and -1.19 for the alkyl substituents. The calculated separation factor α $k'_{c\text{-}Hx}/k'_{n\text{-}Bu}$ was 1.30, that observed was 1.29 ± 0.01.

These results point to significant challenges and opportunities for chromatographers interested in QSAR separation phenomena applied to predicting behavior of organometals and organometalloids in ion exchange separations. Of special interest in this connection will be predictable speciation of more complex molecular variations of commercial organotins newly introduced as biocides, viz.,



(42,43) and their environmental residues. In such highly branched R groups on organotins, the possibilities for "fractional" or additive applications of σ^0 QSAR values will likely prove important (38,44).

HPLC-GFAA Repeatability and Organotin Detection Limits

For quantitation of trace organotins by HPLC, not only does the analyst require precision in reproducing retention times (t_R) or k', he is concerned with comparable reliability in estimating peak areas of eluted analytes. Figure 7 illustrates a set of replicate chromatograms for samples taken from a solution containing equimolar concentrations (1 ppm) of di(4-tolyl)tin and dibenzyltin cations. These compounds were selected because of the nearly ideal separation factor ($\alpha \sim 3.5$) (27) obtained at the moderate SCX column efficiencies available ($\underline{N} \sim 115, 320$) respectively, and because the benzyltin moiety exhibits an unusually large absorbance which provides UV detector sensitivity nearly comparable to that of the GFAA detector. With the benzyltin peak, it was therefore possible to make direct comparisons between the repeatability of UV and GFAA peak areas. The numerical results are summarized in Table IV. With respect to column retention, both R_2Sn analytes elute within (RSD) ± five percent of the mean k'; this variance is also characteristic for replicate runs conducted with the R_3Sn series (Tables I and III). On the other hand, slightly wider variations occur for replicate determinations of individual peak areas, but not as large as the variation in apparent sensitivity found in Table II for separation of many peaks with widely different k' and $\underline{N}$ values.

The problem is basically a kinetic one. With constant, isocratic flow, the GFAA detector is a concentration detector with fixed sensitivity, hence variations in flow rate alter its apparent sensitivity, e.g. flow ~ 1/sensi-

Table IV

REPEATABILITY OF CHROMATOGRAPHIC PARAMETERS FOR SEPARATION[a] OF DI(4-TOLYL)- AND DI(BENZYL)-TIN CATIONS[b]

	Peak Areas in Arbitrary Units			k'	
	$(4\text{-tolyl})_2Sn^{2+}$	$(benzyl)_2Sn^{2+}$	[c]	$(4\text{-tolyl})_2Sn^{2+}$	$(benzyl)_2Sn^{2+}$
Run	GFAA	GFAA	UV	———	———
1	13,602	14,132	3.662	0.885	3.191
2	14,884	14,707	3.018	0.908	3.086
3	15,544	16,628	3.163	0.862	3.246
4	15,663	17,351	3.240	0.969	3.308
MEAN	14,923	15,705	3.273	0.906	3.208
σ	945	1,531	0.283	0.046	0.094
RSD, %	6.33	9.75	8.58	5.08	2.94

[a] With 0.0075 M diammonium citrate in water-methanol (70:30 v/v), isocratic at 1.00 mL min-1.

[b] Both compounds injected as 100 ng Sn.

[c] Correlation between GFAA and UV signals for runs 2-3 r = 0.947, for runs 1-4 r = -0.404.

tivity (21). More significant is the fact that the GFAA autosampler periodically removes a small segment (usually 20 μL) of the eluent at relatively large intervals, Δt, typically every 50 seconds. In consequence, at a given flow rate of one mL min^{-1}, for example, only 100 x (20 μL)/ (5/6 x 1000 uL) = 2.4 percent of total eluent is sampled. Resulting chromatographic GFAA peak areas must therefore be rationalized for shape (skewness and half-widths) and boundaries (areas and maxima) through interpolation of relatively few points, e.g., $t_w/\Delta t \sim 3\text{-}15$ (where t_w is peak width at base). Incautious measurements of GFAA chromatograms can lead to errors of ± Δt (21) in t_R or t_w (27) (or the peak width at half-height, $t_{1/2}$) with consequent RSD of 5-10 percent for k' = six or three, respectively, or of 9-20 percent for corresponding $\underline{N}$ = 1500 and 900, respectively. These deviations represent the magnitude of variances seen in Tables II and IV, for example. Decreased flow rates offer the desired effects of increasing both GFAA sensitivity and improving resolution of peak shapes and boundaries, but these must be consistent with other requirements (27) for optimal chromatographic separation.

For the most precise quantitative work, it is apparent from Table IV that separations involving only several analytes with favorable separation factors ($\alpha > 1.5$) are preferable. For survey studies, necessarily involving separation of many peaks, as in Table II, peak to peak quantitation requires careful calibration or reruns of selected portions of the chromatogram at lower flow rates. One other factor is important and peculiar to organometal chromatography. The organotins are especially susceptible to volatilization during the GFAA thermal cycle with considerable loss of apparent sensitivity. Were this not important in going from relatively involatile tributyltin to highly volatile trimethyltin deposits in the graphite furnace tube, the variation in peak area per unit mass (μV·s/ng) examples in Table II would more closely follow trends in k', or better, $\underline{N}$.

Because we are interested in speciating and quantitating present day commercial organotin biocides, we developed suitable chromatographic conditions based upon the foregoing discussion of optimal separation factors, and repeatable peak area measurements. A typical chromatogram showing such a separation of triphenyl-, tri-n-butyl- and tri-c-hexyltin cations, all in widespread commercial use (1-3,8,13), is portrayed in Figure 8. The measurement bars under each analyte signify the consistent range of GFAA peaks summed for peak area measurements. Six chromatograms were acquired for the triad of organotin standards at various concentrations between 0.125 - 0.75 ppm, to give the raw data and linear regressions analyses (with correlation coefficents, r) depicted in Figure 9. One data point for the c-hexyl derivative was a gross outlier, but another chromatogram produced an acceptable value in accord with the trend. The calibration curves thus produced are linear over nearly an order of magnitude and indicate reasonable zero intercepts. The detection limits δ (95 percent confidence level) of 5-26 ng shown reflect both the relative slopes (sensitivity) for each organotin species and the reliability or scatter in the measured peak areas (14).

Comments made before about intrinsic differences in apparent sensitivities GFAA for organometals are apparent in the significantly different slopes obtained for each species. Since we employ essentially a "fixed" thermal program for the entire HPLC-GFAA run, no individual eluate is detected under truly optimized conditions; the situation is analogous to, but less troublesome than, the enormous range of molecular extinction coefficients which dictate effective sensitivities for varied analytes in HPLC-UV (cf. Figure 6). Under the chromatographic conditions selected (relatively high flow rate to reduce analysis time), we conclude that variable but useful detection limits were obtained for all three of the commercial organotins indicated in Figure 9, and

that, to a large extent, the practicality of this particular speciation method resulted from their favorable separation factors: $\alpha_{21} = k'_{Bu}/k'_{Q} = 1.47 \pm 0.01$ and $\alpha_{32} = 1.29 \pm 0.01$.

Applications of HPLC-GFAA to Speciation of Organotins in Environmental Materials

A very interesting and fast-growing application of organotin-containing materials relies upon introduction of specific R groups tailored to yield selective toxicity and maximum service lifetime through attachment of biocidal R_3Sn moieties to organic or inorganic polymer backbones (45,46). The efficacy and predicted life of such controlled release host materials for use as marine antifouling coatings (1,2) or as moluscicides controlling snail-borne Schistosomiasis disease (47), heretofore have relied upon measuring leach rates and corresponding toxic effects on organisms by total tin analyses. We examined a novel marine antifoulant organometal polymer (OMP) developed by the U. S. Navy (45,48). This monolithic polymer resin involves co-polymerization of methyl methacrylate, tri-n-butyltin methacrylate, and tri-n-propyltin methacrylate in 1:1:1 proportions. Our concern was to assess the composition and leach rate of released organotin biocides during exposure to aquatic media. Figure 10 typifies our results with a chromatogram taken directly of the leachate solution at the point in time where the rate of release has approached zeroth order kinetics (49). The aqueous sample, therefore, represented a relatively integrated picture of the overall release chemistry, barring minor absorption of the organotin leachates by glass vessel walls or the polymer matrix itself. Compared with a calibration chromatogram, the leachate sample is seen to contain substantially more propyl than butyltin. This apparently greater

release rate for the more acidic Pr_3Sn^+ is consistent with greater ease of ionization, or nucleophilic cleavage by water, of the Sn-O bonds that fix the organotin to the polymer backbone as predicted by the above mentioned QSAR values for the respective R groups (Table III), e.g.,

$$R_3Sn\text{-}O\text{-}\overset{O}{\overset{\|}{C}}\text{-polymer} + HOH \longrightarrow R_3Sn^+ + OH^- + H\text{-}O\text{-}\overset{O}{\overset{\|}{C}}\text{-polymer} .$$

Controlled release polymer matrices incorporating bioactive organometals are just now emerging as means to deliver selective toxicants or nutrients to biological systems (50). The need to speciate the precise form of the released molecule, necessarily appearing only at very low concentrations, and its rate of release for reliable bioassay studies will offer important challenges for HPLC-GFAA, not only for tin, but for other elements as well.

In another connection, we have developed means for speciating the types and amounts of organotin leachates occurring during shipyard operations involving removal of commercial marine antifouling paints from ships hulls. Unlike the oceanic service environment where only relatively small amounts of bioactive organotins are released from the paints, the shipyard procedures involve physical removal of the coating by sandblasting, chipping, scraping and heating, during which time, debris is continuously removed by water sluicing. We examined the aqueous leachates from typical sandblasting grits exposed to distilled/deionized water for two days with mild agitation. The supernatant solution was not preconcentrated or chemically altered but was only ultracentrifuged (15 kG) to remove fine particles or detritus prior to injection into the HPLC-GFAA system. Figure 11 illustrates a typical result where we found that 5.8 ppm of dibutyltin and 3.0 ppm of tributyltin were leachable from the grit, a molar ratio of 1.9:1.

This is a significant result because of concerns for ultimate disposal of toxic Bu_3Sn under conditions of occupational exposure, and because it provides a graphic example of the ultimate environmental fate of the original antifoulant tributyltin paint matrix following weathering in the ocean and removal on-shore. Since such organotins are bacteriostats (2-4), this example of direct HPLC-GFAA speciation also demonstrates an effective means for field monitoring of aqueous effluents before and after biological treatment by sludge or sanitary digestion/disposal units supporting such shipyard or related commercial operations involving organotin biocides.

ACKNOWLEDGMENTS

We thank Dr. Rolf B. Johannesen for obtaining FT-NMR spectra. We are indebted to Prof. C. Horváth for permitting us to read a manuscript prior to publication. Support of this research by the Office of Naval Research is gratefully acknoledged. We thank the U.S. Naval Ship Research and Development Center (Annapolis) for samples. Certain commercial equipment, instruments or materials are identified in this paper in order to specify, adequately, the experimental conditions and procedures. In no case does such identification imply recommendation of or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

REFERENCES

1. C. J. Evans and P. J. Smith, Organotin-based antifouling systems. J. Oil Col. Chem. Assoc. 58: 160-68 (1975).
2. J. J. Zuckerman, ed. Organotin Compounds: New Chemistry and Applications, Adv. in Chem. Ser. No. 157. Amer. Chem. Soc., Washington, D.C., 1976.
3. P. Smith and L. Smith. Organotin compounds and applications. Chem. Britain 11: 208-12, 226 (1975).
4. J. S. Thayer. Organometallic compounds and living organisms. J. Organometal. Chem. 76: 265-95 (1974).
5. C. J. Evans. Developments in the organotin industry. Tin and Its Uses. 100: 3-6; 101: 12-15 (1974). Available as Internat. Tin Res. Inst. (London) Publ. 491.
6. H. Akagi, R. Taskehita and Y. Sakagami. Separation and detection of inorganotin and organotin compounds by thin layer chromatography. Bull. Inst. Publ. Health (Japan). 19: 165-92 (1970).
7. C. J. Riggle, D. L. Szontz and A. P. Graffeo. The analysis of organotins in the environment. Proc. 4th Int. Conf. Sens. Environ. Pollut. Amer. Chem. Soc., Washington, D.C., 1978, pp. 761-64.
8. C. J. Soderquist and D. G. Crosby. Determination of triphenyltin hydroxide and its degradation products in water. Anal. Chem. 50: 1435-39 (1978).
9. R. S. Braman and M. A. Tompkins. Separation and determination of nanogram amounts of inorganic tin and methyltin compounds in the environment. Anal. Chem. 51: 12-19 (1979).

10. V. F. Hodge, S. L. Siedel and E. D. Goldberg. Determination of tin(IV) and organotin compounds in natural waters, coastal sediments and macro algae by atomic absorption spectrometry. Anal. Chem. 51: 1256-59 (1979).

11. H. A. Meinema, T. Burger-Wiersma, G. Versluis-de Haan and E. Ch. Gevers. Determination of trace amounts of butyltin compounds in aqueous systems by gas chromatography/mass spectrometry. Environ. Sci. Technol. 12: 288-93 (1978).

12. B. W. Wright, M. L. Lee and G. M. Booth. Determination of triphenyltin hydroxide derivatives by capillary GC and tin-selective FPD. J. High-Resolution Chromatogr. & Chromatogr. Comm. 2: 189-90 (1979).

13. W. O. Gauer, J. N. Seiber and D. G. Crosby. Determination of organotin residues from Plictran in fruit crops by gas-liquid chromatography. J., Agr. Food Chem. 22: 252-54 (1974).

14. G. E. Parris, W. R. Blair and F. E. Brinckman. Chemical and physical considerations in the use of atomic absorption detectors coupled to with a gas chromatograph for determination of trace organometallic gases. Anal. Chem. 49: 378-86 (1977).

15. R. S. Tobias. σ-Bonded organometallic cations in aqueous solutions and crystals. Organometal. Chem. Rev. 1: 93-129 (1966).

16. K. L. Jewett, F. E. Brinckman and J. M. Bellama. Influence of environmental parameters on transmethlation between aquated metal ions. In Organometals and Organometalloids: Occurence and Fate in the Environment. F. E. Brinckman and J. M. Bellama, Eds. ACS Sympos. Ser. No. 82. Amer. Chem. Soc., Washington, D.C., 1978, pp. 158-187.

17. F. E. Brinckman. Bio- and environmental chemistry on tin. 34th Southwester Regional Mtg. Amer. Chem. Soc., Corpus Christ, TX, November 29--Dec. 1, 1978. See. R. Zingaro. How certain trace elements behave. Environ. Sci. Technol. 13: 282-87 (1979).

18. C. J. Soderquist and D. G. Crosby. Degradaton of triphenyltin hydroxide in water. J. Agr. Food Chem. 28: 111-17 (1980).

19. d. Small, T. S. Stevens and W. C. Bauman. Novel ion exchange chromatographic method using conductimetric detection. Anal. Chem. 47: 1801-09 (1975).

20. K. Harrison. Anion analysis by HPLC. Abstr. Pittsburgh Conf. Anal. Chem. Appl. Spectr., Cleveland, Ohio, March 5-9, 1979, paper 301.

21. F. E. Brinckman, W. R. Blair, K. L. Jewett and W. P. Iverson. Applicaton of a liquid chromatograph coupled with a flameless atomic absorption detector for speciaion of trace organometallic compounds. J. Chromatogr. Sci. 15: 493-503 (1977).

22. F. E. Brinckman, K. L. Jewett, W. P. Iverson, K. J. Irgolic, K. C. Ehrhart and R. A. Stockton. Graphite furnace atomic absorption spectrophotometers (GFAA) as automated element-specific detectors for high prssure liquid chromatography (HPLC): the determination of arsenite, arsenate, methylarsonic acid, and dimethylarsinic acid. J. Chromatogr. 191: 31-46 (1980).

23. E. A. Woolson and N. Aharonson. Speciation of arsenical pesticide residues and some of their metabolites by high pressure liquid chromatography--graphite furnace atomic absorption spectrometry. J. Assoc. Official Anal. Chem., in press.

24. S. R. Heller and G. W. A. Milne. The NIH-EPA chemical information system. Environ. Sci. Technol. 13: 798-803 (1979).

25. F. M. Rabel. The value of a precolumn in liquid chromatography. Amer. Lab. 12: 81-3 (1980).

26. C. Horváth, W. Melander and A. Nahum. Measurement of association constants for complexes by reverse-phase high-performance liquid chromatography. J. Chromatogr. 186: 371-403 (1979).

27. L. R. Snyder and J. J. Kirkland. Introduction to Modern Liquid Chromatography. 2nd ed. John Wiley & Sons, New York, 1979.

28. J. C. Liao and C. R. Vogt. Bonded reverse phase ion exchange columns for the liquid chromatographic separations of neutral and ionic organic compounds. J. Chromatogr. Sci. 17: 237-44 (1979).

29. P. J. Twitchett, A. E. P. Gorvin and A. C. Moffat. High pressure liquid chromatography of drugs. J. Chromatogr. 120: 359-68 (1976).

30. R. Eksteen, J. C. Kraak and P. Linssen. Conditions for rapid separations of nucleobases and nucleosides by high-pressure anion-exchange chromatography. J. Chromatogr. 148: 413-27 (1978).

31. J. H. Khox and J. Jurand. Application of high-speed liquid chromatography to the analysis of morphine, heroin, 6-(O-acetyl)morphine and methadone. J. Chromatogr. 87: 85-108 (1973).

32. M. Paabo, R. A. Robinson and R. G. Bates. Reference buffer solutions for pH measurements in 50 percent methanol. J. Amer. Chem. Soc. 87: 415-18 (1965).

33. W. P. Neumann. The Organic Chemistry of Tin. John Wiley & Sons, New York, 1970.

34. T. N. Mitchell. Carbon-13 NMR investigations on organotin compounds. J. Organometal. Chem. 59: 189-97 (1973).

35. T. S. Vasundhara and D. B. Parihar. Microdetermination of organotin compounds by TLC and spectrophotometry using haematoxylin as reagent. Fresenius Z. Anal. Chem. 294: 403 (1979).

36. J. K. Baker. Estimation of high pressure liquid chromatographic retention indices. Anal. Chem. 51: 1693-97 (1979).

37. C. Horváth, W. Melander and I. Molnár. Solvophobic interactions in liquid chromatography with non-polar stationary phases. J. Chromatogr. 125: 129-56 (1976).

38. C. Hansch and A. Leo. Substituent Constants for Correlation Analysis in Chemistry and Biology. John Wiley & Sons, New York, 1979.

39. B-K. Chen and C. Horváth. Evaluation of substituent contributions to chromatographic retention: quantitative structure-retention relationships. J. Chromatogr. 171: 15-28 (1979).

40. T. A. Mastryukova and M. I. Kabachnik. The Application of the Hammett equation with the constants σ^{ph} in the chemistry of organophosphorus compounds. Russian Chem. Rev. (Engl. Trans.). 38: 795-811 (1969).

41. T. A. Mastryukova and M. I. Kabachnik. Correlation constants in the chemistry of organophosphorus compounds. J. Org. Chem. 36: 1201-05 (1971).

42. C. J. Evans. A new organotin miticide. Tin and Its Uses. 110: 6-7 (1976).

43. M. Sano, M. Furukawa, M. Kournai and I. Tomita. Flameless atomic absorption spectrophotometric determination of Vendex, an organic tin miticide, in apples, oranges and tea leaves. J. Assoc. Off. Anal. Chem. 62: 764-68 (1979).

44. J. K. Baker, R. E. Skelton, T. N. Riley and J. R. Bagley. Estimation of high pressure liquid chromatographic retention indices of narcotic analysis and related drugs. J. Chromatogr. Sci. 18: 153-58 (1980).

45. W. L. Yeager and V. J. Castelli. Antifouling applications of various tin-containing organometallic polymers. Organometallic Polymers. C. E. Carraher, J. E. Sheats and C. V. Pittman, Eds. Academic Press, New York, 1978, pp. 175-80.

46. A. P. Gysegem, G. H. Law, G. A. Rivas and R. E. Foscanto. Synthesis and characterization of a novel organometallic polymer system for controlled release antifouling marine coatings. Abstr. Papers, *176 ACS Nat'l Mtg., Miami Beach. Florida*, September 10-15, 1978, ORPL 112.

47. C. J. Evans. Organotins against Bilharzia. Tin and Its Uses. 88: 7-10 (1971).

48. E. J. Parks, F. E. Brinckman and W. R. Blair. Applications of a graphite furnace atomic absorption detector automatically coupled to a high-performance liquid chromatograph for speciation of metal-containing macromolecules. J. Chromatogr. 185: 563-72 (1979).

49. H. B. Scher, Ed. Controlled Release Pesticides. ACS Symposium Series No. 53. Amer. Chem. Soc., Washington, D.C., 1977.

FIGURE CAPTIONS

1. Dual chromatograms illustrating speciation of a 1:1 solution of triphenyltin (ϕ_3Sn) and tri-n-butyltin (Bu_3Sn) cations (500 ng as Sn) are compared in tin-specific (GFAA, bottom) and conventional UV (top) modes. Utility of UV monitoring for solvent fronts and chromophores is evident as are relative sensitivities of two detectors. Conditions: Whatman Partisil-10 SCX; 0.01 M NH_4OAc in methanol-water (70:30); isocratic flow, 1.0 mL min^{-1}; furnace program A.
2. Effects of varying eluent methanol-water composition on SCX column capacity factor k' for triphenyltin and tributyltin ions are compared at constant ionic strength. Flow rate, 1.0 mL min^{-1}; 0.03 M NH_4OAc; pH range 7.54 to 7.18 (at 50:50 composition). Relative size of data points indicates 2x average deviation of replicate chromatograms.
3. Effects of varying apparent total ionic strength μ on SCX column capacity factor k' for tributyltin (upper plot) and triphenyltin (lower plot) are compared in isocratic mobile phases. Flow rate, 1.00 mL min^{-1}; all salts indicated in methanol-water (70:30). The respective chromatograms were run on solutions containing 100 ng (as Sn) of each organotin employing GFAA program A. Relative sizes of the data points signify 2x average deviation of replicate runs.
4. Complete separation of a series of R_3Sn^+ (75 ng each as tin, except 500 ng Me_3Sn^+) in a single chromatogram is demonstrated on the SCX column. GFAA detector program A was employed. This, in combination with fixed GFAA sampling rate of effluent yields apparent sensitivity decreases with increasing volatility (decreasing R size). Conditions: isocratic flow program shown, 1.50-2.00 mL min^{-1};

0.03 M NH_4OAc in methanol-water (70:30); organotins were injected as chlorides in methanol, 100 µL sample. Bars shown below each peak denote those GFAA peaks summed for eluate peak areas; these and other relevant chromatographic parameters are summarized in Table IV.

5. A linear relationship is shown in plots between the substituent constant σ^{ϕ} (40,41) for R groups on various organotins against the logarithm of the observed capacity factor k' (see Table III). Upper plot compares R_3Sn series separated in 0.03 M acetate (-O-) or 0.03 M citrate (-⊕-) (excluding cyclohexyl) mobile phases, with the anomalous behavior apparent for triphenyltin (Ⓒ) in both media. Lower plots compare separation both of butyl and aryl R_2Sn isomer series, all in citrate eluent at 0.06 M (-O-) or 0.12 M (-⊕-) for butyl species and 0.005 M (--⊕--), 0.0075 M (--O--) or 0.01 M (—⊕—) for the aryl isomer species. Relative vertical sizes of the data points signify 2x average deviation of replicate runs.

6. Comparison of dual UV-GFAA chromatograms of isomeric aryl (upper) and butyl R_2Sn (lower) R_2Sn compounds show nearly baseline separation. Though formally a substituted methyltin derivative, the benzyl group behaves as a regular aromatic substituent and shows the strongest absorbance (at 254 nm) of any aryltin thus far studied. Conditions: 100 ng (as Sn) of each analyte; isocratic in methanol-water (70:30) at 1.00 mL min^{-1}; (top) 0.0075 M $(NH_4)_2$ citrate and (bottom) 0.06 M $(NH_4)_2$ citrate.

7. Quadruplicate chromatograms for samples from an equimolar (1 ppm) solution of $(4\text{-tolyl})_2Sn$ and $(benzyl)_2Sn$ cations utilizing GFAA and UV detectors for comparing repeatability. Conditions as for Figure 6, top; numerical results are summarized in Table IV.

8. Representative chromatogram of calibration solution containing 75 ng each of triphenyltin (), tri-n-butyltin (Bu) and tricyclohexyltin () injected as a 200 µL sample. Conditions: 1.00 mL min^{-1}; 0.03 M NH_4OAc in methanol-water (70:30) isocratic; GFAA thermal program A. Solid bars beneath chromatogram represent range or number of GFAA "peaks" summed to generate eluate peak areas.

9. Calibration curves for solutions of commercial triorganotins (R = phenyl, ; n-butyl, Bu; and c-hexyl,) speciated by HPLC-GFAA are compared by linear regression to give excellent correlation coefficients (r) and useful detection limits (δ) at ng levels.

10. Aqueous leachate from an organotin polymer formulation, OMP-1, was speciated directly by HPLC-GFAA after release rate of tin reached zeroth order. Compared against a calibration solution (top) containing 200 ng tri-n-butyltin and 1,200 ng tri-n-propyltin cations, the apparent release of propyltin toxicant considerably overshadows that of the butyltin OMP component. Conditions: 1.00 mL min^{-1}; 0.06 M NH_4OAc in methanol-water (70:30), isocratic; GFAA program A.

11. Tap water leachate from a shipyard sandblasting grit used to remove weathered antifouling paints from ships hulls was directly speciated by HPLC-GFAA using 203 µL injections of sample. After two days contact with mild agitation, comparison of calibration solutions such as illustrated at top for 100 ng each of di- and tri-n-butyltin cations, with leachate samples (bottom example) indicated that about 6 and 3 ppm, respectively, of the organotins were released. Conditions: isocratic programmed flow, 0.50 mL min^{-1} for 15 min then 1.50 mL min^{-1} for remainder of run (vertical dashed line indicates flow change); 0.06 M $(NH_4)_2$ citrate in methanol-water (70:30); GFAA program B.

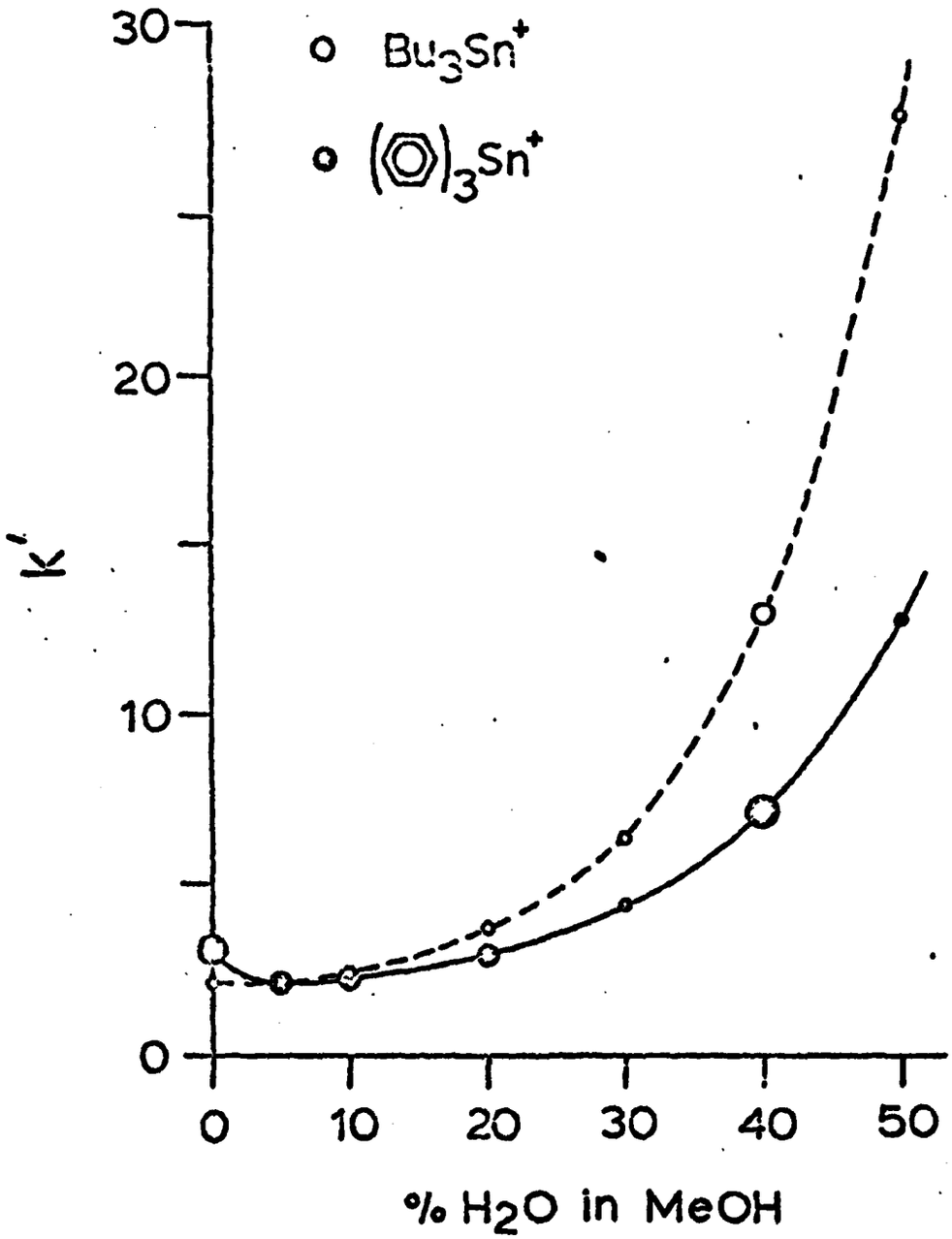


FIGURE 2

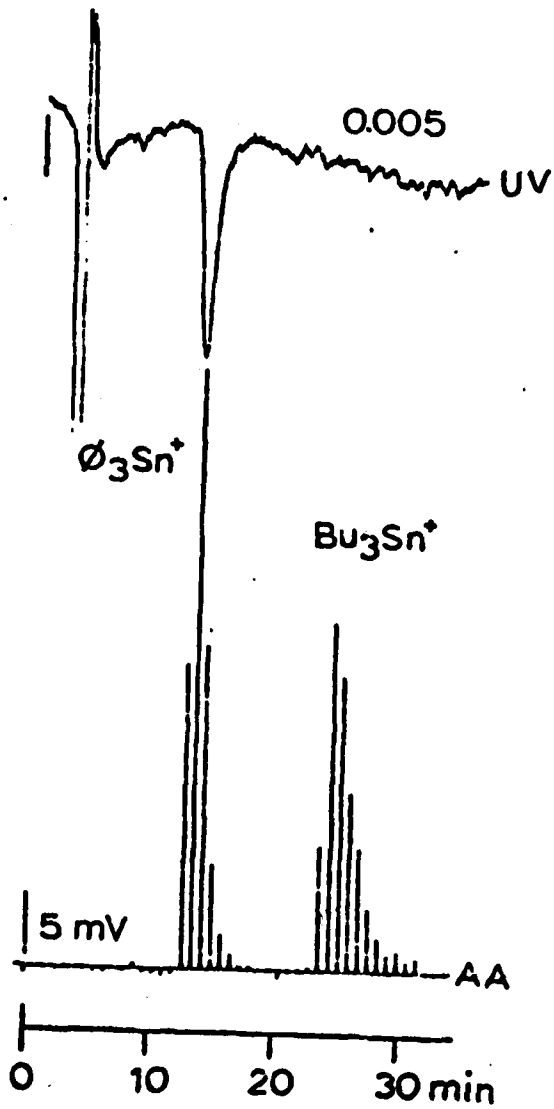


FIGURE 1

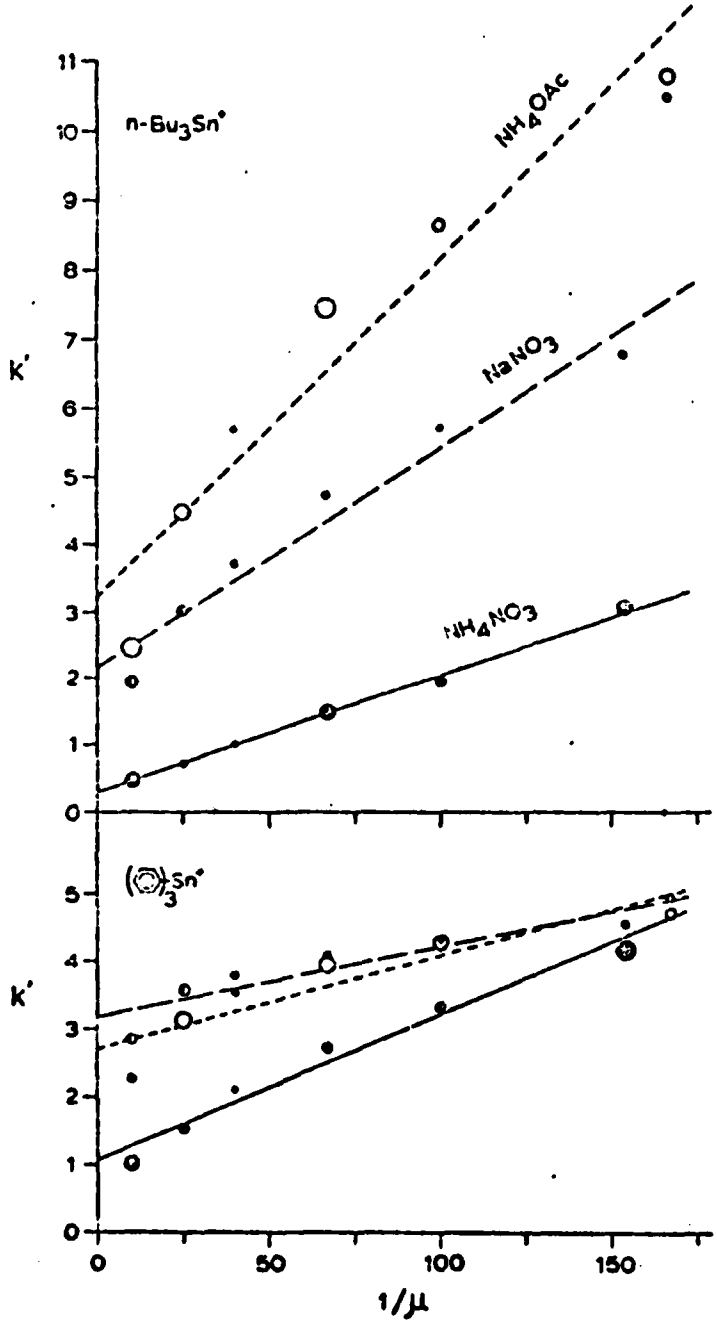


FIGURE 3

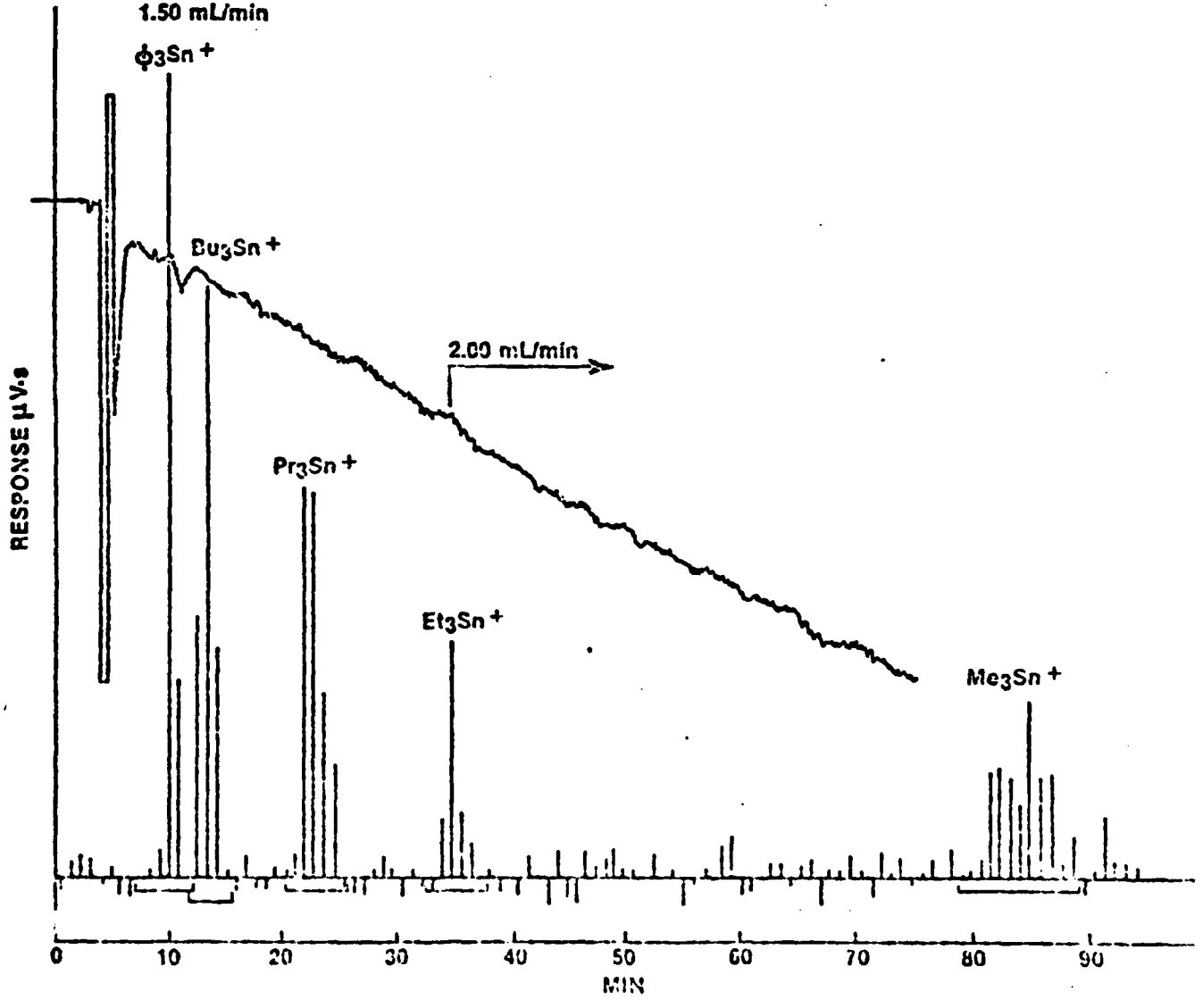

1.50 mL/min
ϕ3Sn +
Bu3Sn +
2.00 mL/min
Pr3Sn +
Et3Sn +
Me3Sn +
RESPONSE µV·s
0
10
20
30
40
50
60
70
80
90
MIN

FIGURE 4

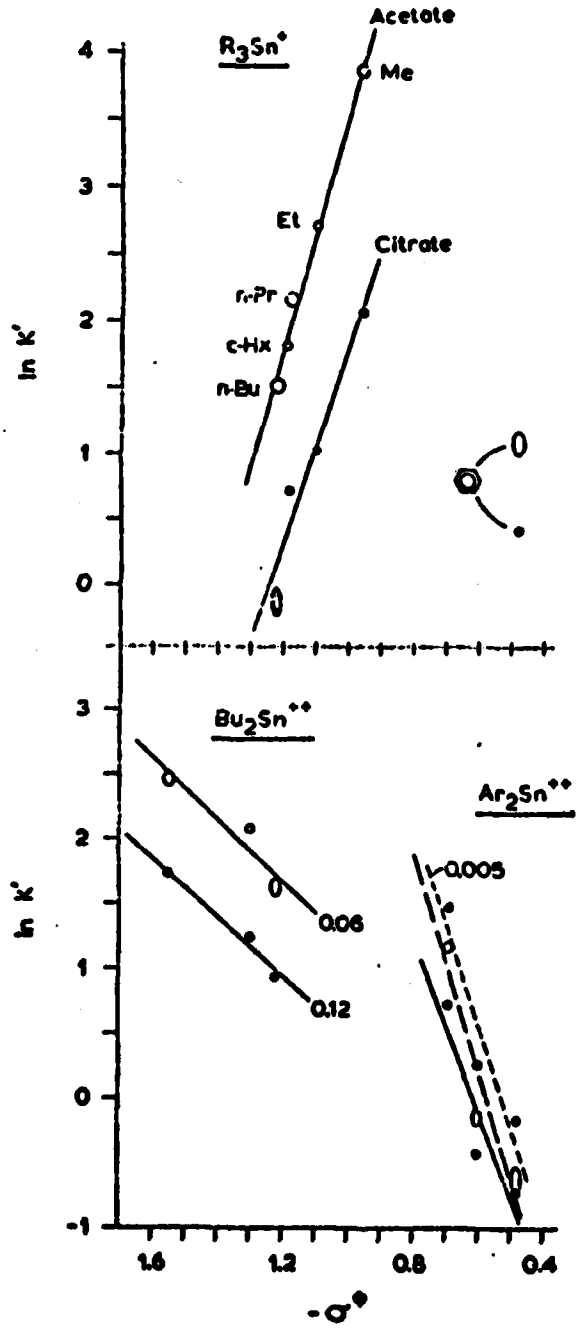

Acetate
R_3Sn^+
Me
Et
Citrate
n-Pr
c-Hx
n-Bu
ln k'
Bu_2Sn^{++}
Ar_2Sn^{++}
0.005
0.06
0.12
1.6
1.2
0.8
0.4
$-\sigma^*$

FIGURE 5

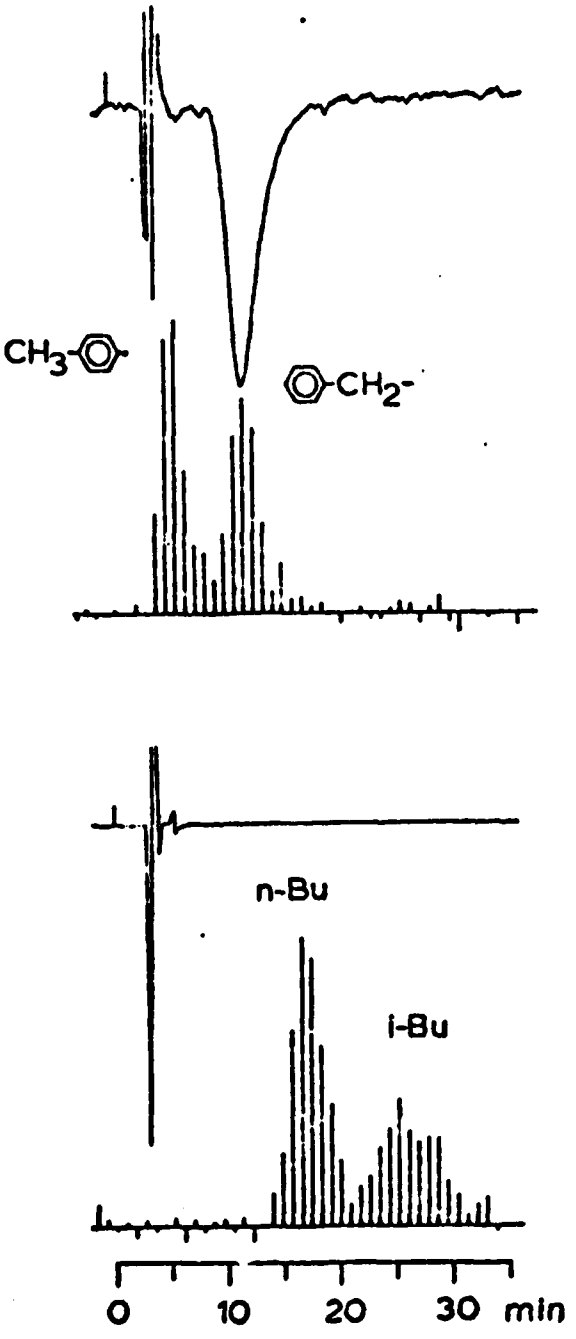


FIGURE 6

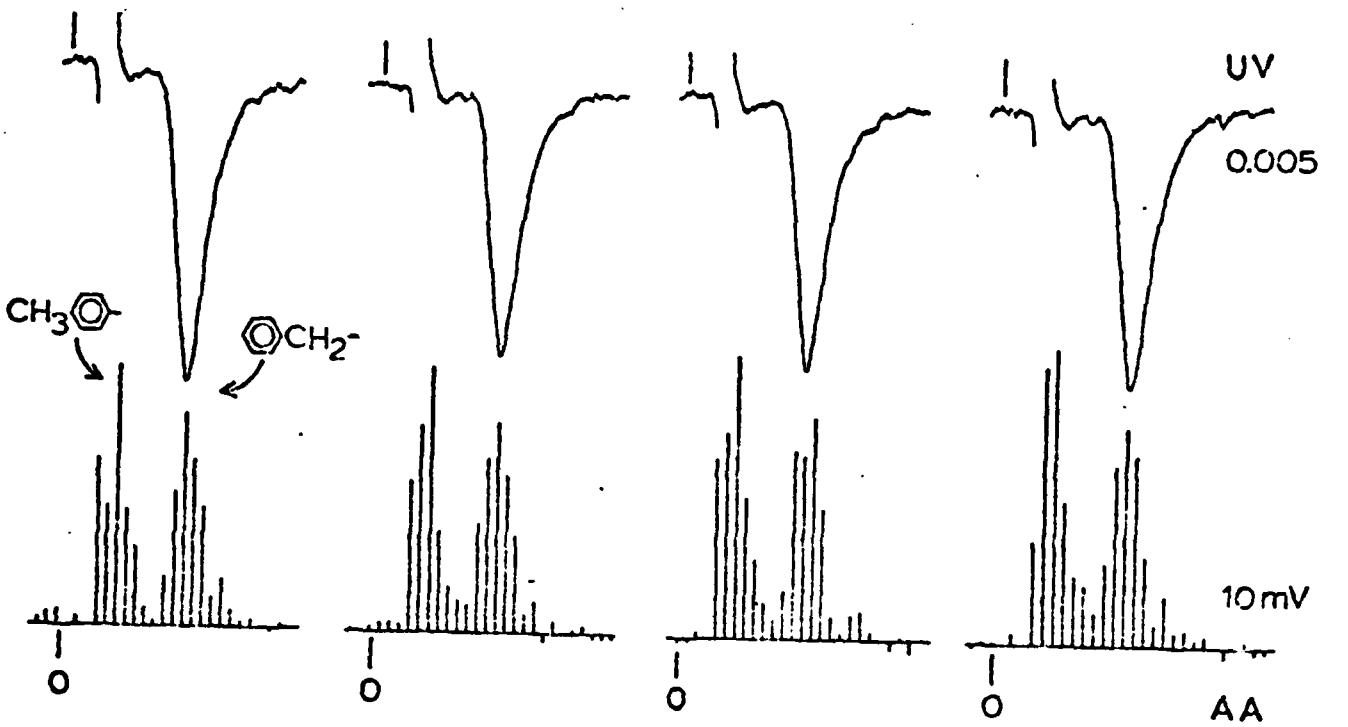


FIGURE 7

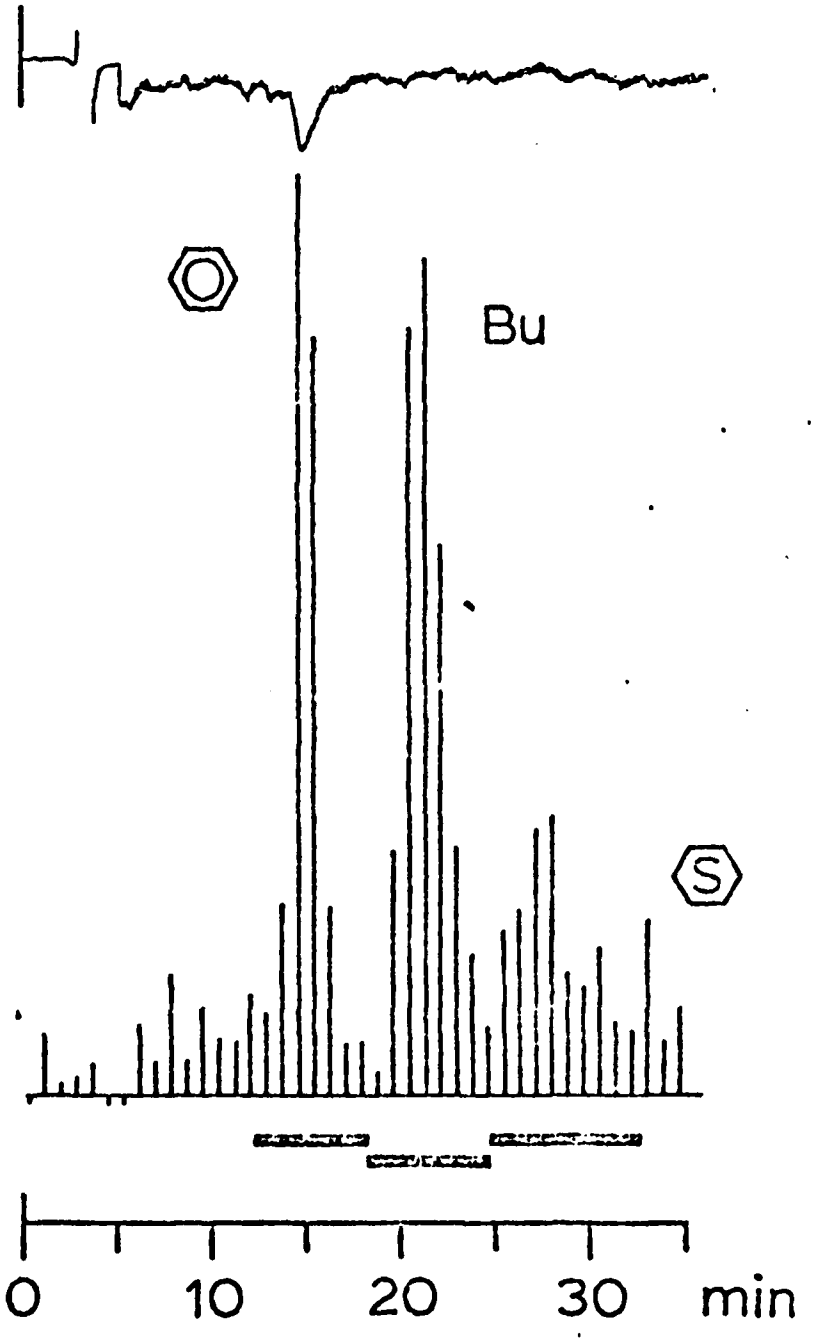


FIGURE 8

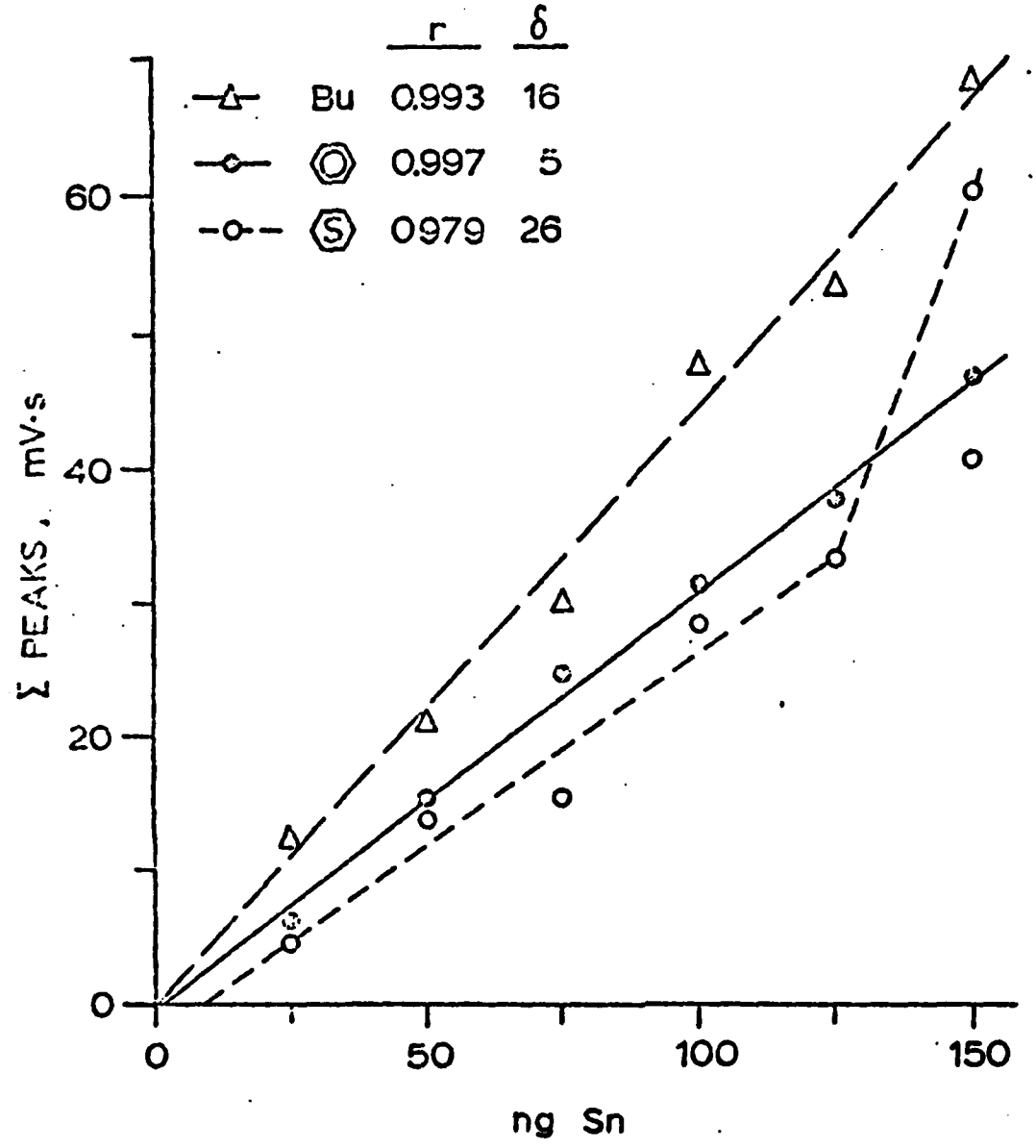

r δ
Bu 0.993 16
0.997 5
S 0979 26
60
40
20
0
Σ PEAKS, mV·s
0
50
100
150
ng Sn

FIGURE 9

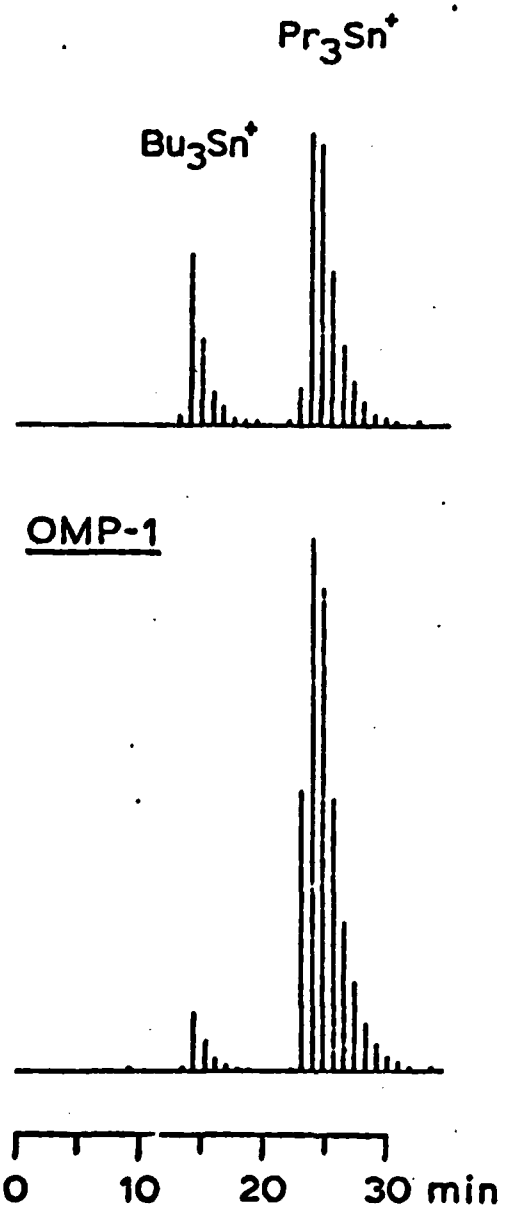


FIGURE 10

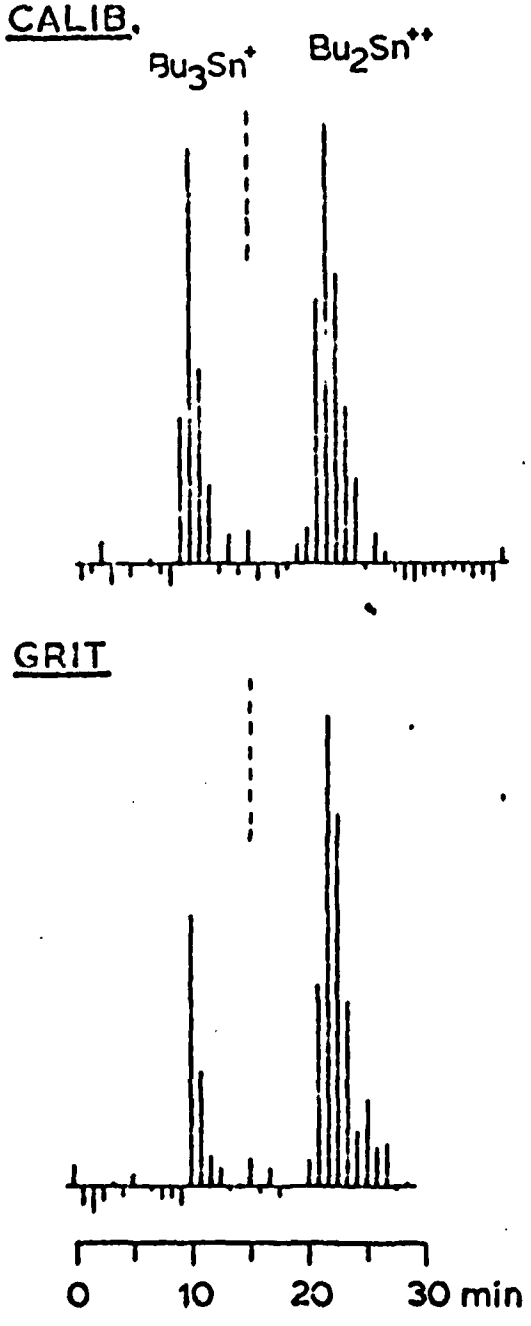


FIGURE 11