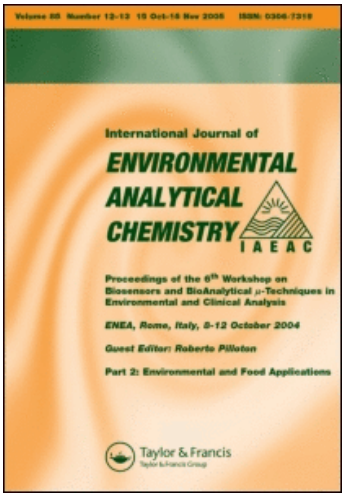

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Purge and Trap Flame Photometric Gas Chromatography Technique for the Speciation of Trace Organotin and Organosulfur Compounds in a Human Urine Standard Reference Material (SRM)

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Ultratrace levels of organotin species and an organosulfur compound were detected in a National Bureau of Standards (NBS) human urine Standard Reference Material, SRM 2670, and a previously certified urine SRM 2672, using a purge and trap system coupled to a gas chromatograph equipped with a flame photometric detector. Samples of the SRM were treated with sodium borohydride to form volatile tin hydrides. Species detected included dimethyltin (1.04 ng/ml), butyltin (0.03 ng/ml), and dimethyldisulfide (2.73 ng/ml) in the new stock of freeze dried human urine SRM 2670 being prepared for issue by NBS and methyltin (1.0 ng/ml), butyltin (1.5 ng/ml), and inorganic tin (28.1 ng/ml) in the old stock of SRM 2672. This analytical technique should have useful applications in studies that are needed to develop a toxicological data base and monitoring programs for human organotin exposure.

KEY WORDS: Element specific speciation, flame photometric detection, standard reference materials, organotin compounds, organosulfur compounds, alkyltins, urine, gas chromatography.

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INTRODUCTION

The industrial use of organotin compounds as stabilizers for plastics, catalysts, and biocides has increased dramatically in recent years.[1] The United States Environmental Protection Agency (EPA) has recently added seven major commercial alkyltin compounds (i.e., dimethyl and dibutyltin esters) to its list for priority consideration under the Toxic Substances Control Act (TOSCA).[2] Consequently, there is growing concern regarding accurate measurements of human exposure and the environmental fate of these toxic organometals.

Presently, very little is known regarding the levels and species of tin taken up or excreted by humans, mainly because of analytical limitations both for quantitation and speciation of trace organotins in body fluids. Manzo *et al.*[3] suggested that 10–20 ng/ml tin was a normal level for human urine, but they indicated that little data was available on normal and toxic levels of tin in body fluids. As man finds increasing use for organotin compounds, analytical techniques, routinely capable of speciating tin compounds at ultratrace ($<$ng) concentrations in urine, and appropriate supporting clinical standard reference materials (SRMs) are necessary for toxicological data bases and monitoring.

Braman and Tompkins first presented evidence for organotin compounds in human urine taken from a relatively small population. They analyzed for tin species in human urine by using a hydride generation technique to form volatile organotin hydrides which were collected in a frozen U-tube trap.[4] The trap was gradually heated to separate the various tin compounds (by their boiling points), and these volatile stannanes were quantitated by using a tin-selective flame emission detector.[5] The bulk of the tin so determined in urine samples was inorganic; however, monomethyltin (up to 0.32 μg/l), dimethyltin (up to 0.17 μg/l), and trimethyltin (up to 0.13 μg/l) species were detected. The detection limits in the study were 0.02 μg/l. Iwai *et al.*[6] studied tetra- and tri-alkyltin (R_4Sn, R_3Sn) species uptake, circulation, and excretion in rats. Alkyltins were extracted from samples of tissue, blood, bile, feces, and urine with ethyl acetate following HCl acidification. Extracts were eluted on a silica gel column with hexane, for R_4Sn, or hexane-ethyl acetate (4:1), for R_3SnCl, and eluates were injected into a gas chromatograph for detection with a flame ionization (R_4Sn) or

electron capture (R_3SnCl) detector, respectively. R_3SnCl (R = butyl, ethyl) compounds were detected in rat urine following subcutaneous injections of R_4Sn or R_3SnCl compounds.

Other studies have been performed on excretion of tin in urine of mammals following organotin exposure, however the actual molecular tin species in urine were not determined. White rats excreted ^{119}Sn in urine and feces following administration of a single oral dose of tri[cyclo]hexyltin(^{119}Sn) hydroxide.[7] Neutron activation analysis was used to measure levels of tin in the urine of two farmers poisoned by triphenyltin acetate that was to be used as an agricultural microbiocide.[3]

In this paper we describe the first use of an automated tin-selective purge and trap (P/T) flame photometric gas chromatography technique (GC-FPD) to directly measure organotin species at ultratrace concentrations in human urine. For this work we chose a potentially important reference matrix, a rehydrated freeze-dried urine SRM 2670 (elevated and normal) being prepared for issue by the Office of Standard Reference Materials at the National Bureau of Standards. Additionally, we analyzed an older, out of stock freeze-dried urine SRM 2672 certified by the NBS Office of Standard Reference Materials for total mercury.

Organosulfur compounds in urine are also selectively detectable using our procedure and data for these are reported along with organotin speciation results.

EXPERIMENTAL

Glassware used in these experiments was leached with 10% nitric acid solutions for 8–12 h and rinsed repeatedly with deionized water.[8] Organotin and organosulfur compounds were obtained from commercial suppliers and were used without further purification. The standards were prepared as concentrated (ppm) solutions in methanol and were diluted in deionized water daily to appropriate working concentrations. A Hewlett Packard Corp. (Palo Alto, CA) (HP) model 5730 gas chromatograph (GC) equipped with a HP flame photometric detector (FPD) coupled to a HP model 7675A purge and trap sampler (P/T) (Figure 1) was used to measure tin and sulfur species (see Ref. 8 for full details and detection limits for

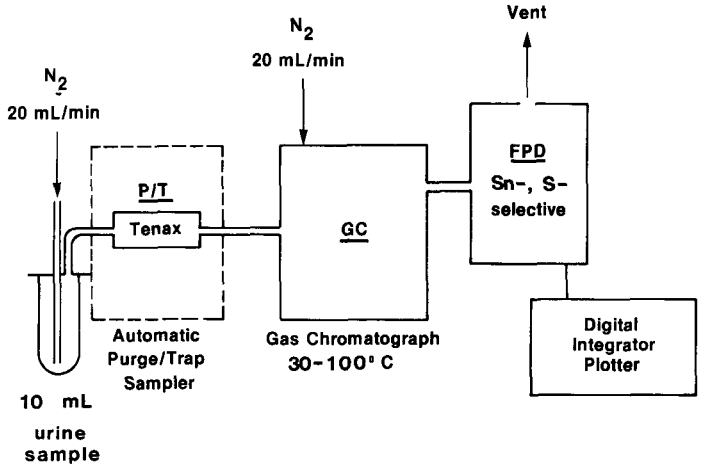

FIGURE 1 Block diagram of the P/T GC-FPD system.

the system). A 6 ft × $\frac{1}{4}$ in. (2 mm i.d.) glass column containing 10% SP2100 plus 3% SP2401 coated on 80/100 Supelcoport (Supelco, Inc., Bellefonte, PA) was used with high purity nitrogen as a carrier gas. The column was generally run with a temperature program of 30°C for 4 min followed by heating at 32°C/min to 100°C, and the detector was maintained at 200°C. The sample (10 ml in a screw-capped 16 × 150 mm glass test tube) was purged with high purity nitrogen at 20 ml/min for 10 min, volatile compounds being trapped on 60/80 mesh Tenax GC (Alltech Associates, Deerfield, IL) at ambient temperature. Following purging the sample was desorbed at 200°C for 5 min, followed by a 3 min vent period in which the trap was heated 50°C above the desorb temperature. The auxiliary temperature was maintained at 150°C. The analyses were run in either a sulfur- or a tin-selective mode.[8] The sulfur mode employed a 394 nm interference filter (supplied by HP with the GC) with the FPD, and flame gas flows of 50 ml/min for hydrogen and air, and 10 ml/min for oxygen. In the tin-selective mode a 600 to 2000 nm bandpass filter (Ditric Optics, Inc., Hudson, MA) was used along with a hydrogen-rich flame (hydrogen 110 ml/min, air 70 ml/min). The output signal from the flame photometric detector was recorded on a HP model 3390A recorder/integrator and a strip chart recorder.

Freeze-dried human urine samples were obtained from the Office of Standard Reference Materials at the National Bureau of Standards as SRM 2672 (supply exhausted), and as a new SRM 2670 (elevated and normal) being readied for distribution. The lyophylized material was aseptically rehydrated with sterile, deionized water immediately prior to use in accord with the certificate instructions.

RESULTS AND DISCUSSION

Nanogram amounts of tin and sulfur species were detectable in aqueous solutions using the P/T-GC-FPD system. To be detected using P/T-GC-FPD, these compounds must have sufficient hydrophobicity and volatility to be efficiently purged from solution within practicable time periods.[8] Certain covalent tin and sulfur compounds already volatile at room temperature (i.e., tetramethyltin, dimethylsulfide and dimethyldisulfide) satisfy these conditions. However, relatively non-volatile polar compounds such as methyl- or butyltin chlorides are highly solvated (aquated) in aqueous or biological media[8-10] and must be derivatized, for example by addition of sodium borohydride,[4] to form the corresponding volatile methyl- or butyltin hydrides:

$$(CH_3)_2Sn^{2+}(aq) + BH_4^-(aq) \rightarrow (CH_3)_2SnH_2(g).$$

The detectability of some organotin and organosulfur compounds in deionized water with and without the addition of sodium borohydride is shown in Figure 2. The vigorous hydride treatment shows little effect on the recovery of neutral, volatile molecules. Volatile methylsulfur compounds were detected with or without the addition or sodium borohydride (top two chromatograms), whereas methyltin trichloride, dimethyltin dichloride, trimethyltin chloride, and butyltin trichloride required conversion to the corresponding volatile hydride species for detection (bottom two chromatograms). P/T-GC-FPD analyses with and without borohydride treatment represent an important adjunct to our protocol since the possible presence of both volatile permethyl and hydridic species of many elements can be ascertained in environmental and biological samples.[8,10] In the case of lyophilyzed urine SRMs little likelihood

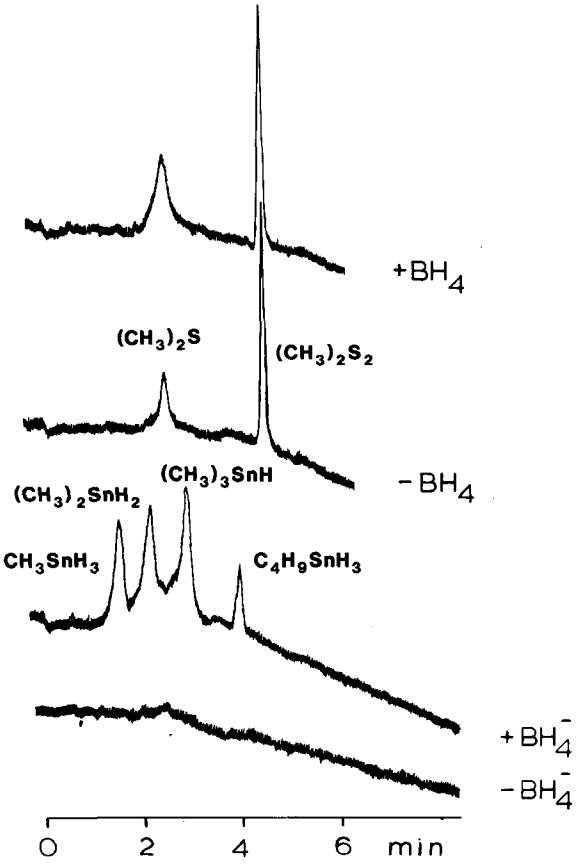

FIGURE 2 Effect of sodium borohydride on the detection of volatile sulfur and non-volatile tin compounds. The top two chromatograms show deionized water (10 ml) spiked with $(CH_3)_2S$ (16.9 ng) and $(CH_3)_2S_2$ (21.3 ng) with or without 200 μl 4% (w/v) sodium borohydride. The bottom two chromatograms represent water spiked with an organotin mixture (peaks left to right: CH_3SnCl, 17.8 ng; $(CH_3)_2SnCl_2$, 1.9 ng; $(CH_3)_3SnCl$, 1.5 ng; C_4H_9SnCl, 0.3 ng). All chromatograms were run in tin mode (see Materials and Methods section).

of tetramethyltin remaining after processing was anticipated, nor was any detected. The situation for fresh urine or other biotic fluids remains open.

The P/T-GC-FPD system was made selective for tin or sulfur compounds by employing appropriate interference filters and by

varying FPD flame chemistry (see Materials and Methods section). Figure 3 shows that the detectability of tin compounds in a mixture of tin and sulfur compounds was greatly attenuated in the sulfur mode (top chromatogram), whereas both tin and sulfur compounds were detected in the tin mode (bottom chromatogram).

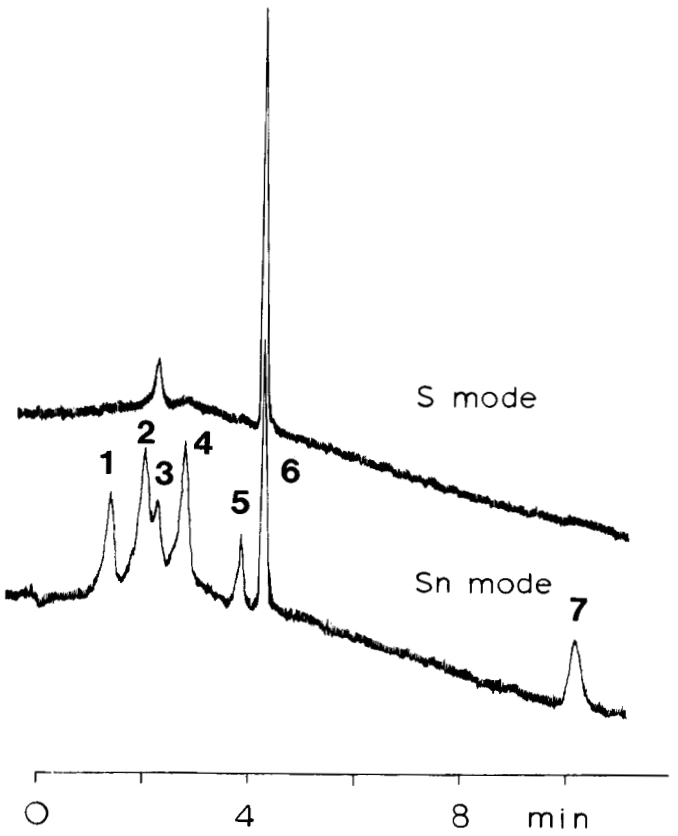

FIGURE 3 Comparison of Sn and sulfur analysis modes for detection of organotin and organosulfur compounds. A mixture of authentic tin and sulfur compounds in 10 ml deionized water plus 200 μl 4% sodium borohydride was analyzed. Bottom chromatogram: 1, CH_3SnCl_3 (17.8 ng); 2, $(CH_3)_2SnCl_2$ (1.9 ng); 3, $(CH_3)_2S$ (16.9 ng); 4, $(CH_3)_3SnCl$ (1.5 ng); 5, $C_4H_9SnCl_3$ (0.3 ng); 6, $(CH_3)_2S_2$ (21.3 ng); 7, $(C_4H_9)_2SnCl_2$ (2.0 ng).

Representative chromatograms of rehydrated NBS freeze-dried urine SRM 2670 (normal) are shown in Figure 4. In the sulfur mode only two peaks appeared, one large peak with a retention time identical with dimethyldisulfide, and a small peak corresponding with dimethylsulfide. In the tin mode a peak appeared with a retention time close to dimethyltin. Another peak with a retention time corresponding to monobutyltin also appeared. Additionally, several peaks eluting after dimethyldisulfide (which also appears in the tin

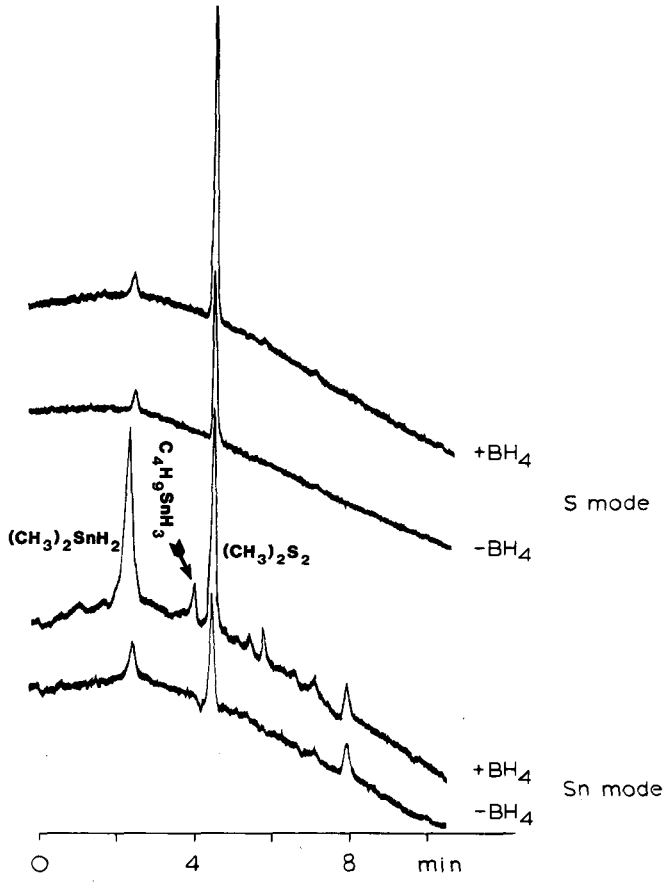

FIGURE 4 Chromatograms of rehydrated NBS freeze dried urine SRM analyzed in sulfur and tin modes with or without addition of sodium borohydride.

mode) were detected. These may represent mixed butylmethyltin species arising from transalkylation reactions induced during the analysis or by a hitherto unreported biological pathway.[11] When borohydride was omitted in subsequent chromatograms, the tin peaks corresponding to dimethyltin and monobutyltin disappeared and dimethylsulfide, dimethyldisulfide, and an unidentified peak appearing at about 8 min retention time remained.

Dimethyltin and dimethylsulfide could be chromatographically separated, but had similar retention times, with dimethyltin eluting just prior to dimethylsulfide. The retention time of the suspected dimethyltin peak in the urine SRM was actually between those of dimethyltin and dimethylsulfide. However, the elevated level of the peak (Figure 4) when analyzed in the tin mode with borohydride reduction, its disappearance when analyzed without borohydride reduction, and its disappearance in sulfur mode all strongly indicated that this was the tin compound. Moreover, Figure 5 shows chromatograms run isothermally at 25°C to better separate dimethyltin and dimethylsulfide. The top two chromatograms were SRM urine run in the S mode with and without a spike (175 ng) of dimethylsulfide. The bottom three chromatograms were run in the tin mode with and without spikes of dimethyltin and dimethylsulfide. Retention times for authentic dimethylsulfide averaged 2.99 $\pm$0.02 min, and for dimethyltin 2.57$\pm$0.01 min. The retention time for the suspected dimethyltin peak averaged 2.75$\pm$0.03 min. As in Figure 3, the suspected dimethyltin peak disappeared in the sulfur mode, hence it is clearly not dimethylsulfide, since dimethylsulfide is seen as a distinctly separate peak when added to the SRM urine (second chromatogram from bottom in Figure 5). The suspected dimethyltin compound, however, was not resolvable in chromatograms using urine with authentic dimethyltin and dimethylsulfide spikes (Figure 5, bottom) or with a single dimethyltin spike (data not shown). Further resolution of this question is being sought in our laboratory by combining our P/T-GC-FPD system in tandem with a quadrupole mass spectrometer.[8]

Figure 6 shows a method of additions quantitation of organotin and sulfur compounds in the urine SRM 2670 (normal). Dimethyltin was detected (95% confidence interval) at a level of 1.04$\pm$0.11 ng/ml, monobutyltin at 0.03$\pm$0.01 ng/ml, and dimethyldisulfide at 2.73 $\pm$0.84 ng/ml. A human urine SRM 2672 issued previously by NBS,

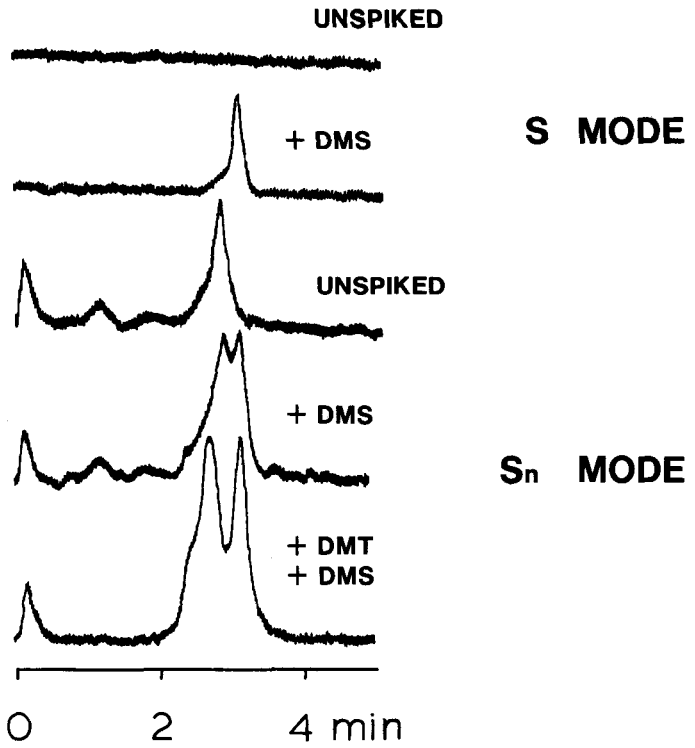

FIGURE 5 Chromatograms of rehydrated NBS freeze dried urine SRM in sulfur (top two chromatograms) and tin (bottom three chromatograms) modes using a 25°C column temperature. Spikes (aqueous) of authentic dimethylsulfide (DMS) and/or dimethyltin dichloride (DMT) were added to the urine along with sodium borohydride just prior to purging.

representing a different aggregate source population was also analyzed with the P/T-GC-FPD system and contained a different distribution of tin species (Figure 7). In that previous SRM, methyltin and inorganic tin species were detected. Both the older and newer urine SRMs contained a butyltin species, however the previous SRM contained much higher butyltin levels than the current one. These differences in organotin composition of the two urine SRMs may represent real differences in the dietary or environmental exposure of the source populations to organotins, as well as

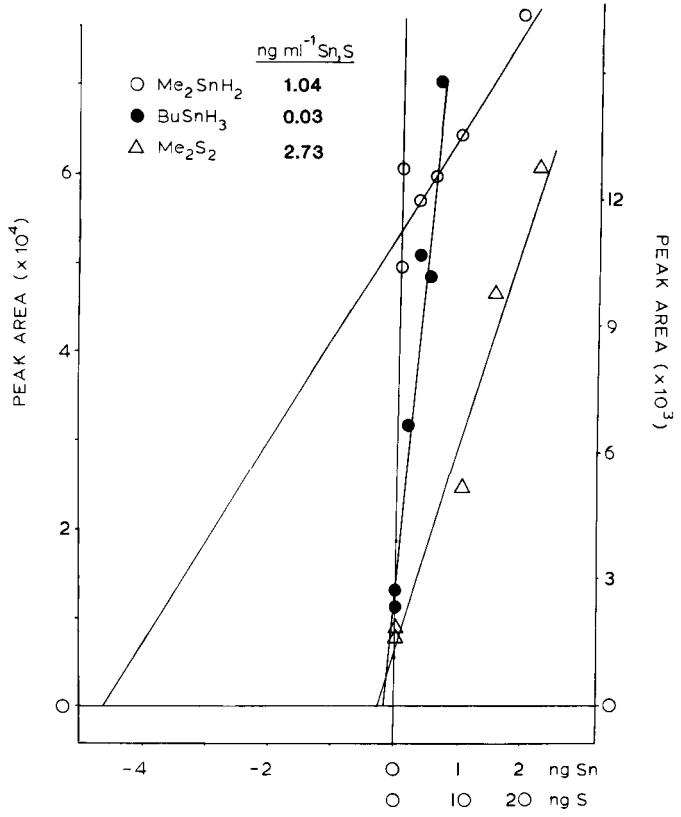

FIGURE 6 Quantitation of organotin and sulfur compounds in the current NBS urine SRM by method of additions.

small contributions from processing large urine batches for the NBS SRMs. The presence of methyltins in urine could result from human exposure to methyltins used as stabilizers in plastics (for example in PVC pipe),[1,2] or by human biomethylation of substantial dietary intake of inorganic tin in a fashion similar to that demonstrated for arsenic ingestion.[12] Inorganic tin is a constituent of many fluoridated toothpastes and is added to soft drinks as an antioxidant. Biomethylation of inorganic tin by bacteria occurs in the marine environment;[8,11] however, it is not known if tin biomethylation

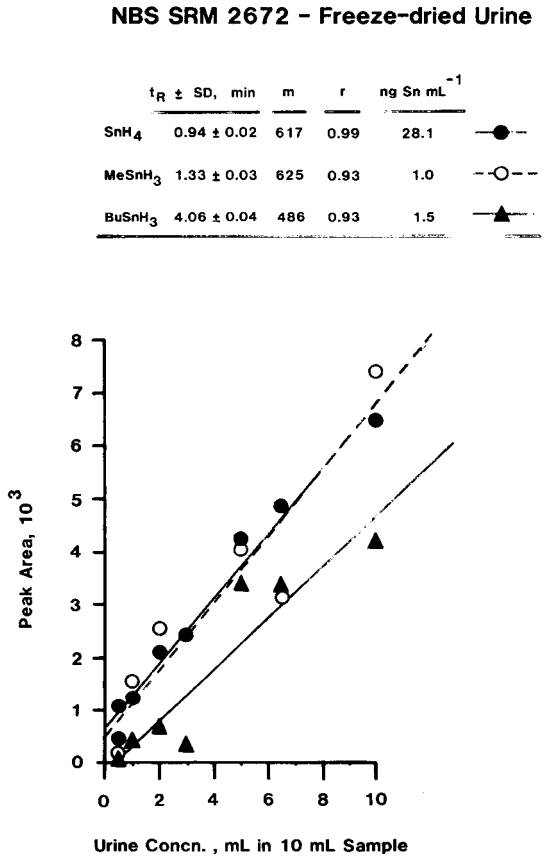

FIGURE 7 Quantitation of tin species in previous stock of NBS urine SRM (2672).

reactions occur in the human body. There are no known biobutylation reactions, suggesting that butyltin species we detect in human urine may be present as a result of dietary or environmental exposure to anthropogenic butlytin compounds widely dispersed by modern technology.

The levels of tin and sulfur species reported for the NBS urine SRMs in this paper may not represent actual levels of these species in urine from the different aggregate source populations. The SRMs were subjected to freeze-drying during preparation. Consequently,

some species, especially volatile ones, could have been partially or completely lost. In addition, transalkylation reactions *in situ* must be contemplated.[10,11] Nonetheless, our present demonstration of the wide applicability of this technique to the development of speciated biological SRMs and its use for the examination of clinically controlled fresh urine samples should be revealing.

Acknowledgments

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