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Demethylation of Trimethylantimony Species in Aqueous Solution during Analysis by Hydride Generation/Gas Chromatography with AAS and ICP MS Detection

I. Koch,[1] J. Feldmann,[1] J. Lintschinger,[1] S. V. Serves,[1] W. R. Cullen[1]* and K. J. Reimer[2]

[1] Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z1

[2] Environmental Sciences Group, Royal Military College, Kingston, Ontario, Canada K7K 5L0

The method of hydride generation for the speciation of antimony compounds was examined with respect to the problem of molecular 'rearrangement'. Specifically, demethylation of trimethylstilbine during the analysis of trimethylantimony dichloride (Me_3SbCl_2) was studied. Previously published observations that enhanced demethylation takes place as a result of inadequate preconditioning of the analytical apparatus were found to be not reproducible. However, demethylation was enhanced as the pH decreased when using two different analytical methods: semi-continuous flow hydride generation–gas chromatography–atomic absorption spectrometry (HG–GC–AAS), and batch-type hydride generation–gas chromatography–inductively coupled plasma mass spectrometry (HG–GC–ICP MS). Applications of the hydride generation method to environmental samples revealed differences in analytical results at high and low pH, and enhanced demethylation taking place because of the matrix in a fungal extract sample. The authors recommend that researchers using the method of hydride generation for antimony compounds carefully test the reaction conditions with standard compounds and use the method of standard addition only. © 1997 John Wiley & Sons, Ltd.

* Correspondence to: W. R. Cullen, Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z1.

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INTRODUCTION

Antimony is a metalloid with a chemical behavior similar to that of arsenic. Its presence in the environment is increasing due to the extensive use of antimony compounds as flame retardants in plastics and textiles, as additives in metal alloys, as doping agents in semiconductors, and as antiparasitic drugs.[1] As a result, antimony is listed by the US Environmental Protection Agency as a priority pollutant.[2] Like arsenic, various forms of antimony are toxic, and to different degrees, making speciation necessary in order to assess the impact of antimony on the environment.

The species of antimony that have previously been detected in the aqueous environment are: inorganic antimony in the III and V oxidation states[3–5] and compounds that can be derivatized to form methyl-, dimethyl- and trimethyl-stibine ($MeSbH_2$, Me_2SbH and Me_3Sb).[3, 6] The analytical methods used in these studies invariably involve hydride generation from an aqueous sample with the aid of sodium borohydride ($NaBH_4$) at various levels of acidity, followed by separation and detection of the resulting stibines. The oxidation states of inorganic antimony species in

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a sample can be determined by adjusting the pH of the hydride generation reaction, since only the III state forms stibine (SbH_3) at neutral pH, and both oxidation states form SbH_3 at low pH. A problem has been found by various researchers during the hydride generation of methylated stibines.[6,7] When trimethylantimony dichloride (Me_3SbCl_2), trimethylantimony dihydroxide ($Me_3Sb(OH)_2$) and dimethylantimony dihydroperoxychloride ($Me_2SbCl(O_2H)_2$) were reacted to form their corresponding stibine, four peaks appeared for each compound (SbH_3, $MeSbH_2$, Me_2SbH, Me_3Sb), rather than the anticipated one (Me_3Sb or Me_2SbH). Dodd *et al.*[6] postulated that this 'rearrangement' occurred when the reaction apparatus had not been conditioned properly and found that the problem disappeared when the apparatus was rinsed with the reagents ($NaBH_4$ solution and acid solution) for 3 min or more.

The aim of this study is to demonstrate some problems of the hydride generation derivatization technique for antimony, when used for speciation. The study cannot give an explanation for these problems. However, we will present some examples of rearrangement/demethylation which can lead to misinterpretation of analytical results.

EXPERIMENTAL

Apparatus

Two methods consisting of different hydride generation and detection systems were used. The apparatus for Method A comprised a semi-continuous flow hydride generation (HG) system developed for arsenic analysis[8] coupled to an atomic absorption spectrometer (AA1275, Varian) using an Sb lamp (Varian) at 217.6 nm wavelength. One modification was made to the apparatus by using a gas–liquid separator[9] that resulted in less analyte carry-over. The apparatus consisted of Tygon tubing for the peristaltic pump, and PTFE tubing [1/8 in (3 mm) o.d.] for the remainder, with the exception of the glass gas–liquid separator, which had been previously silanized with $(CH_3)_2Cl_2Si$. Data were collected from the atomic absorption spectroscopy (AAS) and analysed with the aid of Shimadzu EZChrom software run on a PC, or with an HP 3390A integrator. Measurement of pH was carried out with an Accumet Model 15 pH meter (Fisher Scientific) after the sample and acid had been mixed, but before the $NaBH_4$ was added.

In order to achieve lower detection limits, an HG/purge-and-trap system coupled on-line to an inductively coupled plasma mass spectrometer (ICP MS) (VG Plasmaquad PQ2 Turbo, VG Elemental) as an element-specific detector was used (Method B). The apparatus for Method B is shown in Fig. 1 and consisted of unsilanized glass except where PTFE tubing (1/8 in o.d.) was used between the hydride generator and the water trap. The trap that was filled with non-polar chromatographic material served as a simple, robust chromatographic column for separation. The operational conditions for this apparatus are given in Table 1. The data from the ICP MS were collected with the instrument software in the time-resolved analysis (TRA) mode. Data were processed and analysed using chromatographic software developed by Koelbl *et al.*[10]

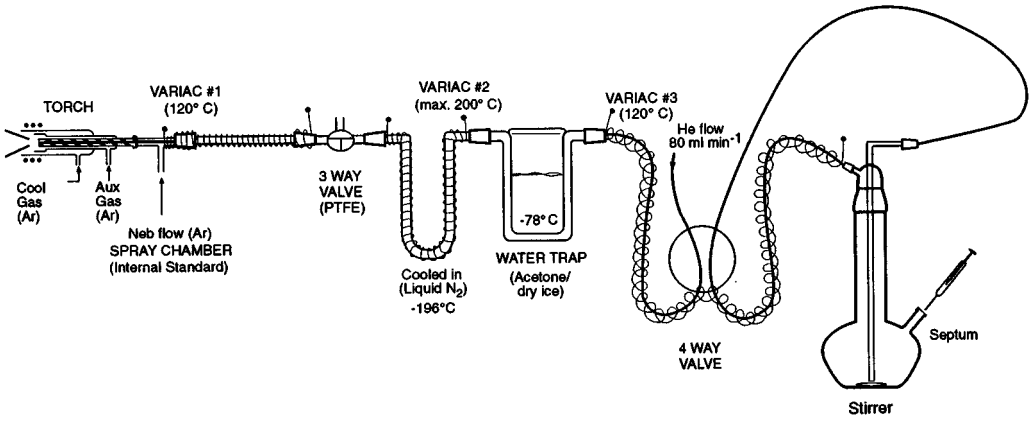

Figure 1 HG–GC–ICP MS apparatus for Method B.

Table 1 Operational parameters for GC–ICP–MS

Gas chromatograph	
Column temperature	−196 to +150 °C
Cryogenic supply	Liquid nitrogen
Carrier gas	Helium
GC–ICP interface	T-piece of quartz tube, 6 mm o.d.
Flow rate	133 ml min^{-1}
Column	22 cm × 6 mm o.d. packed with 10% SP-2100 on Chromosorb 45/60 mesh
Transfer line	PTFE tubing, 100 cm length, 0.3 mm i.d. at 120 °C
Inductively coupled plasma	
Forward power	1350 W
Reflected power	<7 W
Cooling gas flow rate	13 l min^{-1}
Auxiliary gas flow rate	0.6 l min^{-1}
Nebulizer type	DeGalan
Nebulizer gas flow rate	0.950 l min^{-1}
Spray chamber	Scott, water-cooled, 4–6 °C
Mass spectrometer	
Sampler, skimmer orifice	Nickel, 1.0 mm, 0.7 mm
Expansion chamber	<2 mbar
Intermediate chamber	$<1\times10^{-4}$ mbar
Analyser chamber	1×10^{-6} mbar

Reagents

Me_3SbCl_2 was synthesized as described elsewhere.[11] Stock solutions were made by dissolving Me_3SbCl_2 in deionized water to 100 mg l^{-1} as Sb. Standard working solutions were made by diluting the stock solution with deionized water as necessary. $NaBH_4$ (reagent grade, Aldrich) was dissolved in deionized water (fresh daily) to give a concentration of 2% w/v for Method A and 6% w/v for Method B. Hydrochloric acid (reagentgrade, Fisher Scientific) was redistilled for use in Method B and diluted with deionized water to the appropriate concentration. Glacial acetic acid, citric acid, sodium hydroxide (for pH adjustment), maleic acid and concentrated sulphuric acid were all reagentgrade and obtained from common distributors.

Sample preparation

A water sample was taken from the Greater Vancouver Regional District (GVRD) municipal waste deposit, located in Burns Bog, Delta. The water was sampled from standing rainwater through which landfill gas containing trimethylstibine[12] had percolated.

The second sample was taken from a biological experiment in which mycelia of the pink oyster mushroom *Pleurotus flabellatus* (Western Biologicals, Aldergrove, BC, Canada) were grown, with shaking, in 400 ml potato dextrose broth (Difco) in a 1 l Erlenmeyer flask. Me_3SbCl_2 solution was added to the broth to give a concentration of 1 mg l^{-1} in antimony. After a 14-day growing period, the mycelia, as spherical pellets, were harvested by centrifugation and rinsing with distilled water. They were then homogenized with an Ultraturrax T25 homogenizer (Jak and Kunkel) to give a solution of lysed fungal cells, and analysed by HG–GC–AAS. A control experiment was carried out in which the fungus was grown in the same manner, only without the addition of antimony to the potato dextrose broth.

Method of analysis

In method A, a peristaltic pump was used to deliver the Me_3SbCl_2 or sample solution (usually 0.1–0.2 ml of 1 mg l^{-1} Me_3SbCl_2 solution and 0.1–3 ml of sample solution) to mix with the acid or buffer at its appropriate concentration and then to mix with a solution of $NaBH_4$ (2%, w/v) in a reaction coil. The gases evolved were separated with a gas–liquid separator and then swept by a flow of helium into a PTFE U-tube, where they were trapped at −196 °C. Continuous hydride generation and trapping were carried out for 3 min. The peristaltic pump was then stopped (making the system semicontinuous) and the U-tube was heated to 70 °C, allowing the gases to be swept onto a Poropak PS column, which was then heated from 70 to 150 °C at a rate of 30 °C min^{-1}, whereby the gases were separated. They were detected by AAS. The details of this method are described elsewhere.[8]

In Method B, 0.1 ml of a 10 μg l^{-1} solution of Me_3SbCl_2 were mixed with the appropriate amount of 1 M HCl and 10 ml of deionized water in the glass flask represented in Fig. 1. A 0.8 ml portion of $NaBH_4$ solution (6%, w/v) was added with a syringe and the reaction mixture was purged with stirring for 6 min. After being passed through a dry ice/acetone trap to remove water, the gas mixture from the purge was cryogenically focused and trapped with liquid nitrogen on the column packed with 10% SP2100 on Chromosorb (Supelco). The column

contents were heated with a nichrome wire connected to a Variac from −196 to 150 °C in 3 min. The stibines were separated and detected at m/z 121 and 123. More details of this method can be found in Table 1 and Fig. 1.

RESULTS AND DISCUSSION

Dodd *et al.*[6] observed that peaks corresponding to SbH_3, $MeSbH_2$ and Me_2SbH, as well as the expected Me_3Sb, appeared when the hydride generation apparatus had not been preconditioned with the reagents used for analysis (2% $NaBH_4$ and 4 M acetic acid). In an attempt to replicate this observation, experiments were carried out using the same method, but with an AA spectrometer instead of a mass spectrometer as the detector (Method A). Me_3SbCl_2 was analysed after a preconditioning step which consisted of rinsing all tubing with water, as well as after rinsing with 2% $NaBH_4$ and 4 M acetic acid. In all replicates (five for each), the results were the same when Me_3SbCl_2 was reduced and analysed: Me_3Sb and a minor amount of Me_2SbH (corresponding to <2% demethylation) were detected. The large amount of demethylation that was observed by Dodd *et al.* could not be replicated.

When HCl was used as the acid in the reaction (in place of 4 M acetic acid), increased demethylation was observed. Different concentrations of HCl led to the appearance of SbH_3, $MeSbH_2$ and Me_2SbH in differing amounts, suggesting a pH dependence. Hence, demethylation at different acidities was studied, and the results obtained using Method A are illustrated in Fig. 2. It was assumed that the response of each species is the same (which was observed to be the case for Me_3Sb and SbH_3), allowing the amount of each stibine to be shown as a fraction of the total concentration of antimony measured for each analysis. A minimum of three replicates were carried out at each pH, but reproducibility of the

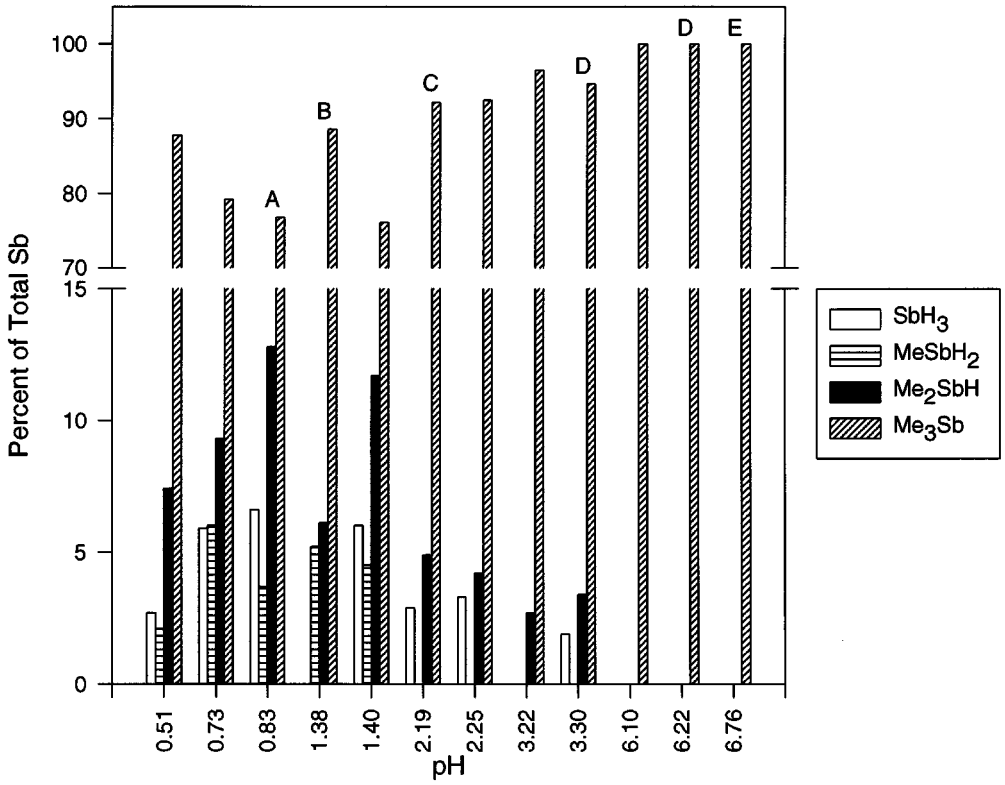

Figure 2 Percentages of stibines at varying pH values using Method A (HG–GC–AAS). Amounts of stibines may not add up to 100% because of imprecision in blank correction. Except where indicated, unbuffered HCl was used to adjust the pH. A, sulphuric acid; B, maleic acid; C, citric acid; D, citrate buffer; E, water.

normalized amounts was poor, with relative standard deviations averaging 22% (0.1–50% for amounts approaching the detection limit). The acid used in most of the experiments was unbuffered HCl, but at some pH values different buffers and acids were tested to determine if the demethylation was specific to HCl. These buffers and acids are specified in Fig. 2. Small amounts of Me_2SbH appear at pH 3.22 and pH 3.30, but at the next pH (6.10) no demethylation is seen for the concentration of antimony being studied.

Although a significant dependence of demethylation on the acid used was not observed for the acid concentrations studied, it must be noted that certain acid systems give less demethylation than would be expected considering the pH. For example, reactions carried out using 4 M acetic acid resulted in less demethylation than experiments carried out at similar pH (2.2) using other acids. However, when 0.6 M acetic acid was tested, the amount of demethylation was not significantly different from other acid systems at similar pH. More studies must be carried out to elucidate the mechanism of the demethylation phenomenon and hence to explain this observed behaviour.

Two analysis methods were used in this study to verify that the demethylation phenomenon is independent of the materials and the method used for the derivatization. Dodd *et al*.[6] suggested that surface effects may be involved and hence the two apparatuses were compared here, consisting of different materials and derivatization procedures (PTFE and silanized glass, and semicontinuous derivatization in method A; unsilanized glass and batch-type derivatization in method B).

When experiments similar to those described above were carried out using Method B, the results were comparable, as shown in Table 2. In this set of experiments the pH was not measured, so that the pH dependence is expressed with respect to unbuffered HCl concentrations. Here, the maximum demethylation appears to be taking place at the lowest pH, or highest acid concentration, i.e. at 0.1 M HCl.

An important difference between the results for Methods A and B is that the demethylation is general appears to be occurring to a lesser extent when using Method A (maximum 24% compared with 68% for B). This may be due to the shorter time that stibines are exposed to the reaction mixture in Method A. The possibility that the different surfaces used in the apparatus for Method B led to increased demethylation cannot be discounted since this effect was not studied for Method B. However, no differences were observed in the amount of demethylation for silanized or unsilanized glass surfaces, or for a glass or PTFE reaction coil in Method A, which was also observed by Dodd *et al*.,[6] so this possibility is considered to be unlikely.

Table 2 Percentages of stibines[a] at varying concentrations of hydrochloric acid (Method B, HG–GC–ICP MS)

HCl concentration (M)	Calculated pH	SbH_3	$MeSbH_2$	Me_2SbH	Me_3Sb
0.1	1	35	4	29	32
0.03	1.5	10	10	40	40
0.01	2	1.2.	3.6	48	48
0.001	3	1.0	0	9.1	90
0.0001	4	1.0	0	11	88
0	7	1	0	2	97

[a] Amounts may not add up to 100% because of imprecision in blank correction and calibration.

Another observation of importance during analysis with Method B was that demethylation was always observed. Even at neutral pH, up to 3% demethylation was seen. The ability to detect stibines in addition to Me_3Sb at such low levels is due to the superior sensitivity of Method B (detection limit 0.1 ng; range used 0.1–10 ng) compared with Method A (detection limit 4 ng; range used 10–1000 ng). When high levels of Me_3SbCl_2 (500–1000 ng) were analysed with Method A, demethylation was observed at neutral pH as well, due to the levels of SbH_3, $MeSbH_2$ and Me_2SbH being sufficiently high for detection. The detection limits are estimated to be between 1 and 5 ng for all the stibines, although standards are not available for methyl- and dimethyl-antimony species.

The demethylation pattern is not dependent on the concentration of antimony being analysed for either method. Small ranges of concentration (two orders of magnitude) were tested, however, so the possibility that the demethylation pattern changes at higher concentrations cannot be discounted.

The ability of Method B to detect very low concentrations of antimony led to a serious cross-contamination problem after Me_3SbCl_2 standard solutions were analysed at the nanogram level, or after samples with nanogram levels of trimethylantimony species were ana-

lysed. Me_3Sb appears to contaminate the system regardless of reaction conditions (acidic or neutral), and this can lead to erroneous results. The detection limits of Method B are limited by the high blank signals, which occur especially for Me_3Sb. Thus, the detection limit is no lower than approximately 0.1 ng, because of the poor reproducibility of the blank level (approx. ±20%).

The purity of the synthesized Me_3SbCl_2 standard was confirmed by NMR and microanalysis. A saturated D_2O solution of the compound was analysed to give one peak in ^1H NMR at δ 1.80 and one peak in ^{13}C NMR at δ 11.71. The results found from the elemental analysis were: C, 15.24; H, 3.91%; calculated for $C_3H_9SbCl_2$: C, 15.15; H, 3.81%. This rules out any possibility that 'demethylation' is due to impurities in the standard. The melting point was 218–220 °C (uncorrected) and has not been published previously to our knowledge.

To illustrate the importance of establishing the presence of demethylation during the analysis of trimethylantimony species, two samples of unknown elemental composition were analysed by using different reaction conditions during hydride generation. The first sample, consisting of water from a Vancouver municipal waste deposit, was analysed by Method B. Figure 3(a) shows the water sample analysed at low pH (unbuffered) and Fig. 3(b) shows the same water analysed at neutral pH (water only, unbuffered). The effect of pH on the analysis is clearly shown by the dramatic difference in the proportions of methylstibines in the two graphs. Inorganic, methyl-, dimethyl- and trimethyl-antimony species appear to be present in the water sample according to Fig. 3(b), which is deemed to be more reliable than the result shown in Figure 3(a). Assuming again that the response of each species is the same, the sum of the areas of all four peaks can represent the total antimony available for hydride generation. At pH 7, this total Sb was found to be 85% of the total Sb measured at pH 2. This difference may be due to

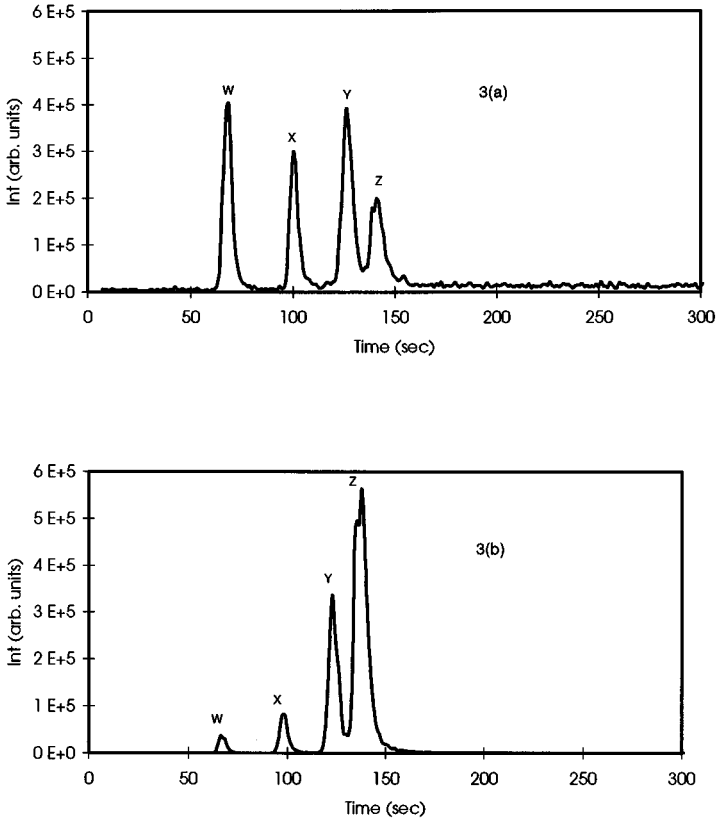

Figure 3 (a) Chromatogram of standing landfill water analysed at pH 2 (unbuffered), Method B. (b) Chromatogram of standing landfill water analysed at neutral pH (water only, unbuffered), Method B. W, SbH_3; X, $MeSbH_2$; Y, Me_2SbH; Z, Me_3Sb.

the higher percentage of SbH_3 determined at pH 2, which may indicate the presence of inorganic Sb(V), a species that is not derivatized to SbH_3 at neutral pH. The demethylation of standard Me_3SbCl_2 at different pH values may be used to interpret antimony speciation by comparing the amounts of the antimony species in standards and samples. If the demethylation at each pH is the same for a sample as for the standard, then the corrected amounts of stibines for a sample would be the same at pH 2 and 7. After correcting for the expected demethylation of trimethylantimony species at each pH (2 and 7), the distribution of the species in the rainwater-puddle water sample show the occurrence of dimethyl- and methyl-antimony species in addition to inorganic and trimethylantimony species. However, the amounts of stibines at each pH do not match each other meaning that it was not only pH that influenced the rearrangement of the methylated species. Other possible influences that were not studied for this sample were the possible demethylation and rearrangement of the other methylated species, and the effect of sample matrix.

The second sample, analysed by Method A, shows the effect that the sample matrix may have on the hydride generation of methylstibines. The term 'sample matrix' refers to any physical, chemical or biochemical components other than the analytes (antimony species) that make up the sample. Figure 4(a) shows a chromatogram of an aqueous extract of the fungus *Pleurotus flabellatus* (10 mg ml^{-1} of protein) that had been grown in liquid culture and amended with Me_3SbCl_2. The stibines were generated with the aid of

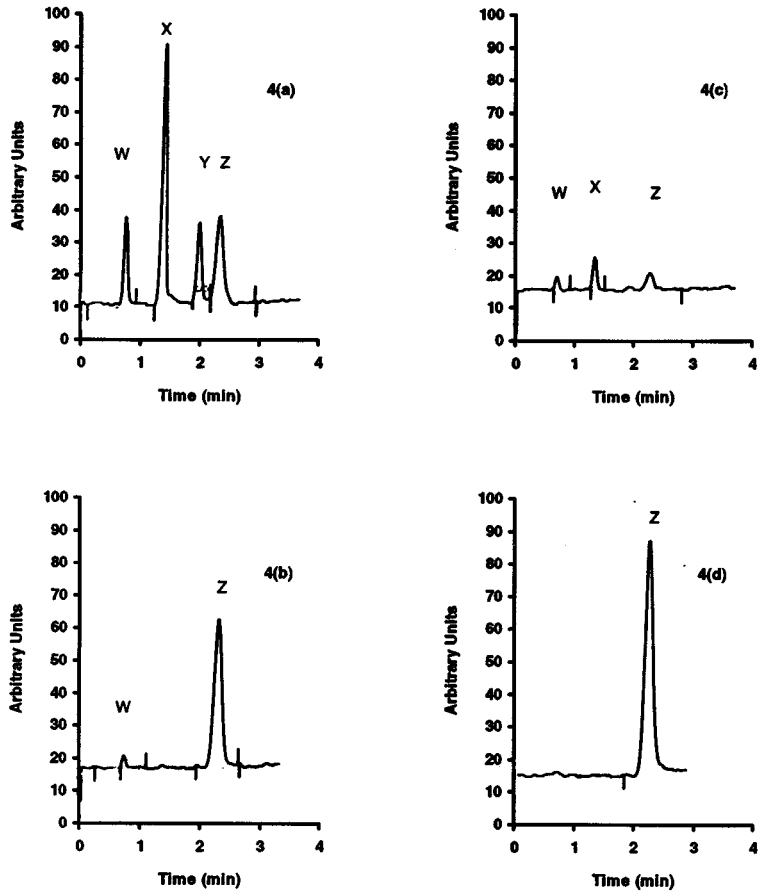

Figure 4 (a) Chromatogram of an aqueous fungus extract analysed at neutral pH (water only, unbuffered), Method A. (b) Chromatogram of the same aqueous fungus extract analysed at pH 6.2, citrate buffer, method A. (c) Chromatogram of antimony-free aqueous fungus extract spiked with 200 ng Me_3SbCl_2, analysed as in (a). (a) Chromatogram of 200 ng Me_3SbCl_2 in water analysed as in (a). W, SbH_3; X, $MeSbH_2$; Y, Me_2SbH; Z, Me_3Sb.

aqueous borohydride with no acid or buffer, conditions under which the water sample described above showed an increase in trimethylantimony species (pH 5.6). Figure 4(b) shows the same fungus extract analysed using a 0.05 M citrate buffer at pH 6.2. Most of the demethylation appearing in Fig. 4(a) was eliminated by using a buffer. To determine whether the demethylation observed in Fig. 4(a) was caused by the sample matrix, a control sample from the mushroom culture that had not been amended with antimony, and hence with the identical matrix but found previously to contain no antimony above the detection limit, was analysed after the addition of 200 ng of Me_3SbCl_2. The sample was analysed in the same manner as the sample in Fig. 4(a) and the result is shown in Fig. 4(c). The same demethylation pattern can be seen, indicating that this specific sample matrix causes the demethylation. The efficiency of the hydride generation reaction is decreased by the sample matrix, as shown by Fig. 4(d), which is the chromatogram that resulted when 200 ng of Me_3SbCl_2 dissolved in water was analysed in the same manner as the samples in Figs 4(a) and (c). The amount of total hydrides in Fig. 4(d) is much greater than that in Fig. 4(c). Figures 4(a–d) show the importance of matrix effects in this fungal extract on the hydride generation of Me_3Sb.

The authors recommend, therefore, that when analysing a sample for methylated antimony compounds by the method of hydride generation, the reaction conditions be carefully tested with standard compounds such as Me_3SbCl_2. The issue of 'rearrangement' associated with methyl- or dimethyl-antimony compounds has not been addressed, since no standard compounds are available, but similar problems may exist for these species, making the synthesis and study of these compounds imperative. As well, the behaviour of methylated antimony species in the III oxidation state is completely unknown, although their presence in the environment is not unlikely. Earlier hydride generation studies that report the presence of methylated antimony compounds should be regarded with caution, especially any quantitative aspects, since the presence and amounts of these compounds may be an artifact of the method. The reason for and mechanism of demethylation are currently being investigated.

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