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Gas chromatographic determination of organomercury following aqueous derivatization with sodium tetraethylborate and sodium tetraphenylborate
Comparative study of gas chromatography coupled with atomic fluorescence spectrometry, atomic emission spectrometry and mass spectrometry

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Abstract

Several hyphenated analytical techniques, including gas chromatography (GC) coupled with atomic fluorescence spectrometry (AFS), microwave-induced plasma atomic emission spectrometry (AES), and mass spectrometry (MS), have been evaluated for methylmercury and ethylmercury analysis following aqueous derivatization with both sodium tetraethylborate and sodium tetraphenylborate. Both GC–AFS and GC–AES were shown to be excellent techniques with detection limits in the range of sub-picogram levels (0.02–0.04 pg as Hg). Both techniques have wide linear ranges, although setting of the AFS sensitivity has to be selected manually based on the concentration of mercury in the sample. Phenylation seems to be more favorable in this study because of its capability of distinguishing between ethylmercury and inorganic mercury, and low cost compared to ethylation. Although sensitivity of GC–MS is poor with detection limits ranging from 30 to 50 pg as Hg, it is an essential technique for confirmation of the derivatization products.

Keywords: Detection, GC; Derivatization, GC; Organomercury compounds; Sodium tetraethylborate; Sodium tetraphenylborate

1. Introduction

Mercury is one of the most prevalent and toxic contaminants in the environment. Different mercury species differ greatly in their physico–chemical properties, such as solubility, rates of bioaccumulation by organisms and others. It is in its methyl form

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that mercury is most hazardous [1–3]. The growing awareness of the strong dependence of the toxicity of mercury on its chemical forms has led to an increasing interest in the quantitative determination of specific mercury species [4]. In its various analytical manifestations, chromatography is a powerful tool for separation and subsequent measurement of a vast variety of chemical species, so it is not surprising that chromatographic techniques coupled to highly sensitive and element-specific detectors have been widely exploited for the analysis of organomercury species. The use of the traditional technique, gas chromatography with electron-capture detection (GC–ECD) for organomercury analysis, is limited due to the non-specificity of ECD, and halogen bearing compounds, co-extracted with organomercury, interfere with the determination. Therefore, this method is being replaced by more selective techniques. The most commonly used hyphenated techniques are a combination of GC or high-performance liquid chromatography (HPLC) with element-specific detection methods, such as atomic absorption spectrometry (AAS) [5,6], atomic fluorescence spectrometry (AFS) [7–9], microwave-induced plasma atomic emission spectrometry (MIP-AES) [10,11], and inductively coupled plasma mass spectrometry (ICP-MS) [4,12]. Some of these techniques (ICP-MS, MIP-AES) are capable of analyzing several elements simultaneously. GC–AFS is becoming an emerging technique for mercury analysis because of its high sensitivity and selectivity [9]. The recent availability of a commercial GC–AFS instrument makes this the technique of choice for organomercury analysis. Although GC–MS is not normally used in the determination of mercury, it is still very useful for structural confirmation.

Organomercury compounds are generally present in the sample matrix as ionic species. For GC analysis, these compounds need to be extracted from the sample and to be converted to volatile species. One of the commonly used derivatization methods is aqueous ethylation with sodium tetraethylborate ($NaBEt_4$) [7,13]. Although this derivatization reaction provides a fast and easy procedure for methylmercury (MeHg) determination, it suffers a major drawback. It does not distinguish between ethylmercury (EtHg) and inorganic mercury (Hg^{2+}). EtHg has not generally been found in marine and fresh animals [14], but its occurrence has been reported in soil and sediment, both from polluted and natural environments [15,16]. It is therefore preferable to use some other aqueous derivatization reagent when both MeHg and EtHg information is needed. In this regard, sodium tetraphenylborate ($NaBPh_4$) seems to be an excellent alternative [11,17].

In the present study, we compare the performance of three techniques, namely GC–AFS, GC–MIP-AES, and GC–MS, for the determination of MeHg and EtHg following aqueous derivatization using $NaBEt_4$ and $NaBPh_4$.

2. Experimental

2.1. Materials

All mercury standards were purchased from Ultra Scientific (N. Kingstown, RI, USA). Standard stock solutions of methylmercury chloride (MeHgCl) and ethylmercury chloride (EtHgCl) were prepared by dissolving appropriate amounts of the standards in methanol. These solutions were stored in dark brown glass bottles at room temperature (20°C).

$NaBEt_4$ (98%) and $NaBPh_4$ (98%) were purchased from Strem (Newburyport, MA, USA). Fresh 1% (w/v) solutions of $NaBEt_4$ and $NaBPh_4$ were prepared daily in deionized water. A buffer solution of pH 4.5 was prepared by mixing appropriate amounts of sodium acetate (0.2 *M*) and acetic acid (0.2 *M*). Other reagents used were of analytical grade or better.

2.2. Instrumentation

2.2.1. GC–AFS

Mercury analysis was performed using the P.S. Analytical (PSA) mercury speciation system Model PSA 10.723. This is an integrated gas chromatography–mercury atomic fluorescence instrument which is comprised of an Ai Cambridge (UK) Model GC 94 gas chromatograph equipped with a CTC A200S autosampler, an optic injector module, coupled to the PSA Merlin detector via a pyrolysis oven held at 800°C. A fused-silica analytical column with dimensions of 15 m×0.53 mm I.D. (Megabore), coated with a 1.5 μm film thickness of DB-1 (J&W

Scientific) was used. The column temperature was held at 80°C for 1 min, programmed at 30°C/min to 175°C, which was held for 3 min, then programmed at 20°C/min to a final temperature of 250°C, and then held for 3 min. A split/splitless injector was used in the splitless mode and maintained at 250°C. Injection volume was 5 μl. The carrier gas and make-up gas flow-rates were 4.0 ml/min and 60 ml/min of argon, respectively. For the PSA Merlin detection system, the sheath gas flow was 150 ml/min of argon. Other parameter settings were the same as those reported previously [9]. Data were acquired by a real-time chromatographic control and data acquisition system (E-Lab, Version 4.10R, OMS Tech, USA).

2.2.2. GC–AES

The GC–AES instrument used was a HP 6890 series gas chromatograph interfaced to a HP G2350A atomic emission detector. Operation conditions are listed in Table 1.

2.2.3. GC–MS

The GC–MS system used consisted of a HP 5890 Series II gas chromatograph interfaced to a HP 5971A mass-selective detector. A DB-1 MS capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness) was utilized. The temperature of the injection port was set at 280°C and the splitless mode was employed. The oven temperature was held at 80°C for 1 min, programmed at 8°C/min to 240°C, and then held for 5 min. Injection volume was 1 μl. The MS detector was operated at scan mode to be able to gain full spectra of the analytes of interest.

2.3. Procedures

Standard solutions of MeHg and EtHg were prepared by reacting methyl- and ethylmercury chlorides with $NaBEt_4$ or $NaBPh_4$ in a buffer solution. Generally, 1.20 ml of acetate buffer (pH 4.5), 0.2 ml of 1% $NaBEt_4$ or $NaBPh_4$ solution, 50 μl of 100 pg/μl mercury standard solution, and 1 ml of hexane were added to a 7-ml glass reaction vial. The vial was shaken for 20 min with a Gyrator shaker, then centrifuged for 5 min before the organic phase was transferred into a GS glass vial for analysis. The mercury concentration in the final extract was estimated to be approximately 5 pg/μl as Hg. The real yields, however, for these two derivatization reactions were not investigated. In order to meet the detection limit of the individual detection technique, different concentrations of mercury standard were prepared by changing the quantity of mercury chlorides used. Before the figures of merit were evaluated, the analytical operation conditions for each technique were optimized.

3. Results and discussion

3.1. GC–AFS

Optimization of the parameters used for GC–AFS

Table 1
Operation conditions for GC–AES

GC parameters	
Injection port	Splitless
Injection port temperature	220°C
Column	HP-1, 25 m×0.32 mm I.D., 0.17 μm film
Oven temperature program	40°C (3 min), 30°C/min to 270°C, held 3 min
Carrier gas (He) flow	2.5 ml/min
Injection volume	1 μl
AES parameters	
Wavelength	253.65 nm
Transfer line temperature	300°C
Cavity temperature	260°C
Make up gas (He) flow	60 ml/min
Reagents gas pressure	H_2: 20 p.s.i., O_2: 20 p.s.i.

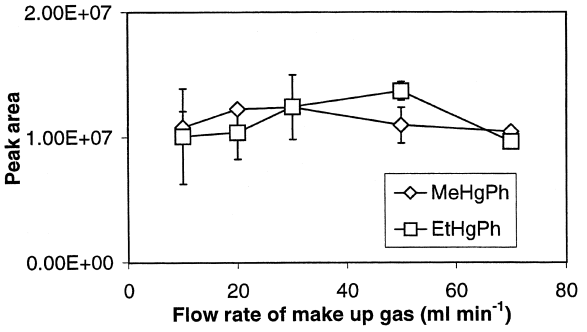

Fig. 1. Effect of argon make up gas flow on the sensitivity of GC–AFS for the determination of MeHgPh and EtHgPh.

has been investigated previously for methyl- and ethylmercury bromide analysis [8,9]. The flow-rates of sheath gas and make up gas were found to have critical effects on the performance of the AFS detector and the effects often change with individual detectors. In this work both gas flows were evaluated for the analysis of ethylation and phenylation derivatives. Fig. 1 shows the effect of argon make up gas flow on the AFS sensitivity for the determination of phenylation product. Relative constant response was observed for a gas flow-rate in the range of 10–70 ml/min. However, as shown in Fig. 2, the argon sheath gas flow has to be maintained at more than 40 ml/min. As expected, the analysis of ethylation products showed the similar trend. Fig. 3a and b show typical chromatograms obtained for the analysis of ethylation and phenylation derivatives under optimum gas flow conditions.

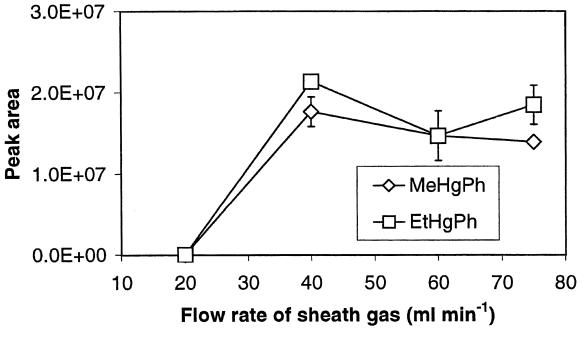

Fig. 2. Effect of argon sheath gas flow on the sensitivity of GC–AFS for the determination of MeHgPh and EtHgPh.

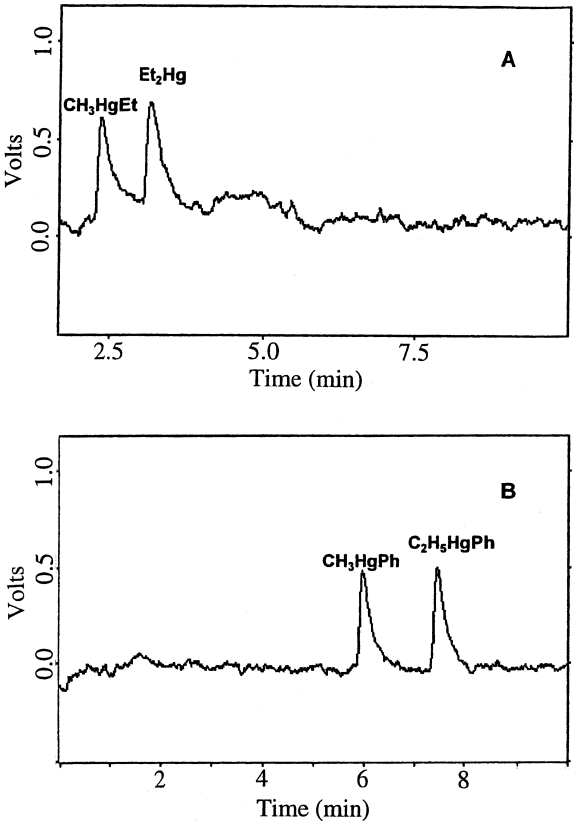

Fig. 3. Typical GC–AFS chromatograms of MeHg and EtHg standards (5 pg as Hg) obtained using ethylation (A) and phenylation (B).

In order to evaluate the reproducibility of this technique, eight sample replicates, in which 100 μl of 50 pg/μl standard solution of MeHg and EtHg were used, were prepared according to the procedures described above. The concentration of mercury in the final extract was 5 pg/μl as Hg. The relative standard deviations (RSDs) were found to be less than 6% for both ethylation and phenylation. The manual amplification control on the AFS detector allows it to be used over five orders of magnitude [12]. Linear calibrations have been only tested between 0 and 50 pg on the most sensitive setting. Calibration curves had correlation coefficients of better than 0.9900 for both MeHg and EtHg. This value is good considering that the samples went through the whole sample preparation procedures, including derivatization and extraction. The linearity

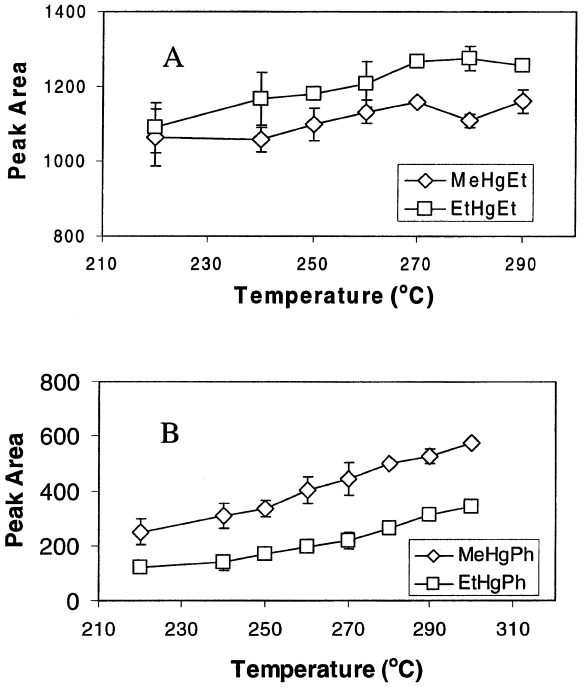

Fig. 4. Effect of transfer line temperature on the analysis of ethylation (A) and phenylation (B) derivatives using GC–AES.

of the AFS response at higher concentration was not tested in this work. The limit of detection, calculated as three-times the standard deviation of the baseline noise, was 0.04 pg as Hg for both MeHg and EtHg using phenylation and for MeHg using ethylation.

3.2. GC–AES

Important parameters, such as temperatures of the transfer line and the cavity, hydrogen (as reagent gas) pressure, and helium make up gas flow-rate, were optimized before figures of merit were evaluated. The transfer line temperature profiles are shown in Fig. 4a and b for ethylation and phenylation derivatives, respectively. The AFS responses to ethylation products were slightly increased with temperature from 220 to 270°C. Further increase in temperature did not change the signal significantly. Sharper increases in the AFS signal with temperature were observed for phenylation derivatives in the range of 220 to 300°C. Such results were expected since the boiling points of phenylation derivatives are higher than that of ethylation, requiring higher transfer line temperature. Compared to the temperature of transfer line, changes in cavity temperature showed less effect on the AFS signal. A slight decrease in AFS response was observed with increase of the cavity temperature in the range between 220 and 320°C.

The analysis of mercury requires a mixture of oxygen and hydrogen as the reagent gas. The function of oxygen is to prevent carbon deposits forming

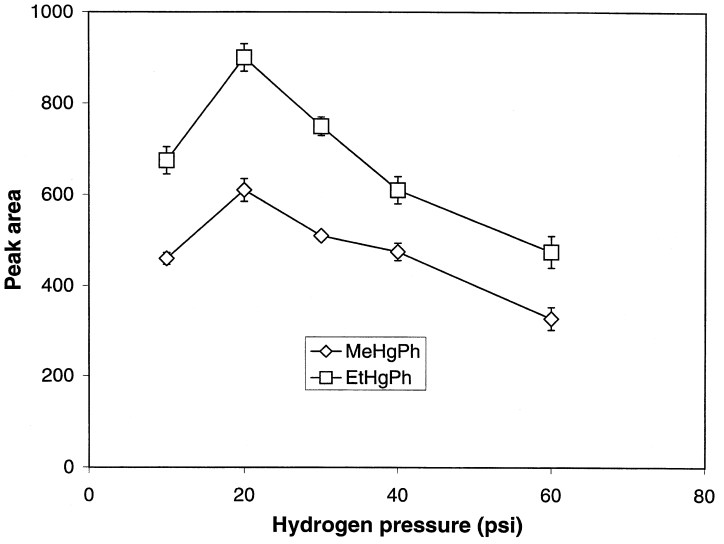

Fig. 5. Effect of hydrogen pressure on the sensitivity of GC–AES for the determination of MeHgPh and EtHgPh.

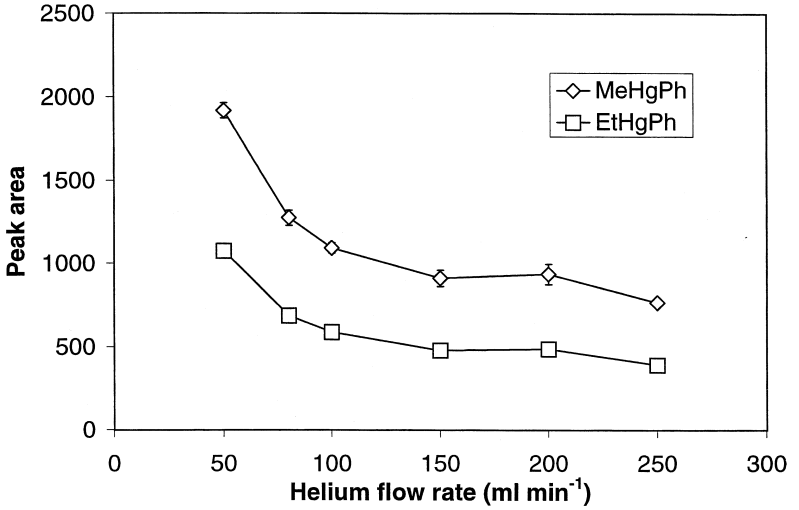

Fig. 6. Effect of helium make up gas flow on the sensitivity of GC–AES for the determination of MeHgPh and EtHgPh.

on the discharge tube during the sample excitation process [18]. The pressure of oxygen used in this work was 20 p.s.i. (1 MPa=145 p.s.i.) as recommended by the manufacturer. The effect of hydrogen pressure on the sensitivity for mercury analysis was investigated by repeated injections of 1 μl of 200 pg/μl standard at different hydrogen pressures (10 to 60 p.s.i.). The results for the determination of phenylation derivatives are shown in Fig. 5. It was observed that an increase in hydrogen pressure resulted in an increased sensitivity in mercury detection, until a maximum was reached at 20 p.s.i., after which the sensitivity fell rapidly. The introduction of a large amount of hydrogen may lead to an increased dilution of the analyte in the plasma, resulting in decline of the AFS signal.

The effects of make up gas flow was investigated in the range of 50 to 250 ml/min by repeated injections of 1 μl of 200 pg/μl standard at different helium gas flow-rates. Results (Fig. 6) show that the sensitivity decreased rapidly with increase of flow-rate of helium make up gas, indicating a reduction of residence time of the emitting species in the plasma. The optimum make up gas flow-rate of 50 ml/min was selected for the subsequent work.

Figures of merits were investigated under optimized conditions. As with the GC–AFS method, the reproducibility was tested by using eight sample

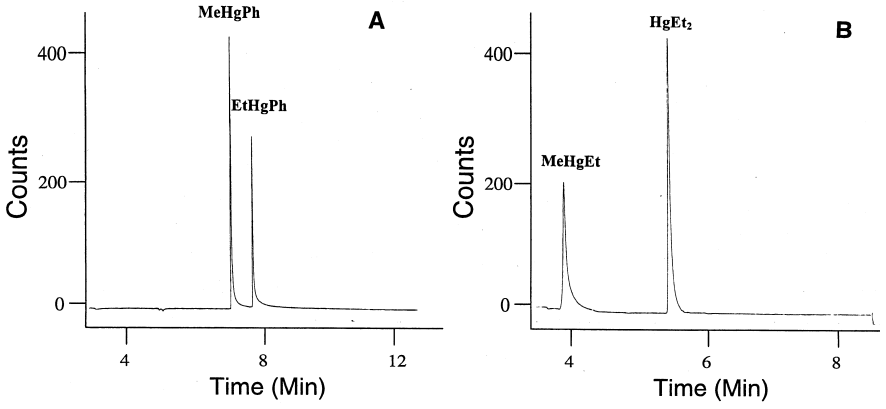

Fig. 7. GC–AES chromatograms of MeHg and EtHg standards obtained using phenylation (A) and ethylation (B).

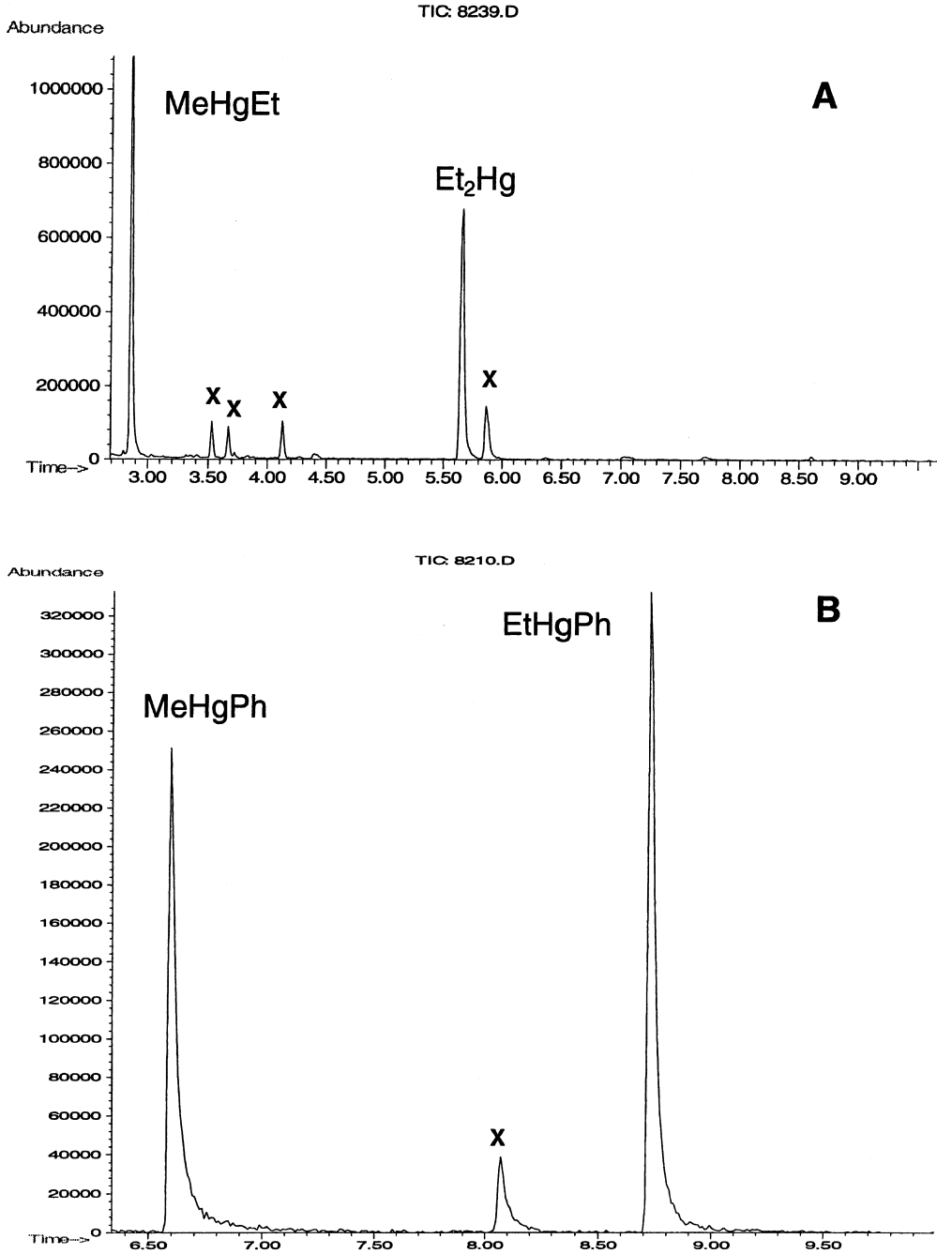

Fig. 8. GC–MS chromatograms of MeHg and EtHg standards obtained using ethylation (A, 80 μg as Hg) and phenylation (B, 40 μg as Hg). X=Non-mercury containing impurities. Time scales in min.

Table 2
Comparisons of the figures of merit for the analysis of mercury using GC–AFS, GC–AES and GC–MS

	GC–AFS			GC–AES			GC–MS		
	Phenylation		Ethylation, MeHg	Phenylation		Ethylation, MeHg	Phenylation		Ethylation, MeHg
	MeHg	EtHg		MeHg	EtHg		MeHg	EtHg	
Linear range	0–50 pg	0–50 pg	0–50 pg	0–500 pg	0–500 pg	0–500 pg	0–80 μg	0–80 μg	0–80 μg
Detection limit	0.04 pg	0.04 pg	0.04 pg	0.02 pg	0.03 pg	0.04 pg	48.4 pg	34.1 pg	31.2 pg
RSD (%)	3.7	3.2	5.4	1.0	1.4	2.1	14.7	14.9	10.6

replicates of 5 pg/μl of ethylation and phenylation standards synthesized following the procedures described above. The RSDs were found to be 1.0 and 1.4% for MeHg and EtHg by phenylation, and 1.6% for MeHg by ethylation, respectively. The linearity for the AES response to mercury was obtained at least in the range of 0 to 500 pg with correlation coefficients of better than 0.9900 for both types of derivatives. The limits of detection, calculated based on the three-times standard deviation of the baseline noise, were 0.02 and 0.03 pg for MeHg and EtHg using phenylation and 0.04 pg for MeHg using ethylation (Fig. 7).

3.3. GC–MS

GC–MS has been used in the previous studies to confirm the derivatization products obtained using ethylation [19] and phenylation [17] reactions. The experimental conditions were adapted mainly from those studies and used in this work. GC–MS chromatograms are shown in Fig. 8. For the analysis of ethylation derivatives, the solvent used was methylene chloride instead of hexane because of the rapid elution of MeHg from the column (less than 3 min). The solvent delay time was set at 2.65 min and initial oven temperature was set at 40°C.

The linear range extended at least up to 80 μg as Hg with correlation coefficients of better than 0.9900 for phenylation and 0.9700 for ethylation. Eight sample replicates were also run to test reproducibility. The RSDs obtained were 14.7 and 14.9% for MeHg and EtHg, respectively, using phenylation, and 10.6% for MeHg using ethylation,. The limits of detection were found to be 48.4 and 34.1 pg as Hg for MeHg and EtHg for phenylation and 31.2 pg as Hg for EtHg using ethylation.

4. Conclusions

Table 2 summarizes the comparative results for the evaluation of the three analytical techniques using both phenylation and ethylation reactions. The data show that both GC–AFS and GC–AES are suitable techniques for organomercury speciation. Both techniques are extremely sensitive and selective, indicated by their sub picogram levels of detection limit and very clean chromatograms. A wide linear range, over at least three-orders of magnitude, is easily obtained using GC–AES, while pre-selected sensitivity setting can be done manually based on the concentration range of mercury compounds. Both techniques are very reproducible with RSDs of less than 5% ($n=8$) in all cases, even including the derivatization and extraction steps. The main advantage of GC–AES is its capability for multi-elemental analysis, whereas GC–AFS has the advantage of simple operation and comparatively low cost. Compared with $NaBEt_4$ the advantages of $NaBPh_4$ as an aqueous derivatization reagent for organomercury speciation are its low cost and capability of differentiating ethylmercury and inorganic mercury. Although GC–MS is perhaps the most important technique for the purpose of identification and confirmation, it is unlikely to provide the required sensitivity to be used for the determination of mercury in most environmental samples.

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