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Determination of inorganic Hg(II) and organic mercury compounds by ion-pair high-performance liquid chromatography

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Abstract

The ion-pair chromatographic behavior of inorganic mercury(II) and organomercurials was investigated with tetra-*n*-alkylammonium bromides as ion-pair reagents and sodium halides in a methanol–water mixture as mobile phases. UV and three-electrode direct current argon plasma specific element detection were employed. The effects of the type and the concentration of sodium halide and ion-pair reagent, and the level of methanol on chromatographic behavior of mercury compounds were evaluated.

Both inorganic mercury(II) and benzylmercury species showed much greater UV response than other mercurials, while all analytes showed more consistent mercury-specific responses although there was some bias seen toward a greater response for the smaller organometallics.

1. Introduction

It is well known that inorganic mercury is introduced to the environment by various natural and pollutant processes and can be converted into more toxic alkyl mercury species by biological activity [1]. Also alkyl and aryl mercury compounds are frequently used in industrial manufacturing and in agriculture for mold and pest control [1]. The toxicity of mercury in environmental and biological systems has been evaluated differently according to the valence and the exact chemical form of the mercury.

High-performance liquid chromatography (HPLC) utilizing sensitive and selective detection is an efficient and rapid tool for the speciation and quantitation of mercury compounds. In 1974, normal-phase HPLC was first employed by Funasaka et al. [2] for the separation of organomercurials on Corasil I with *n*-hexane as eluent.

Reversed-phase HPLC is suited to separations of non-polar and moderately polar species while more polar and ionic species have been separated by utilizing secondary equilibria such as ion suppression, ion pair and ion exchange. A mode of reversed-phase HPLC termed on-column complexation or charge neutralization chromatography has proved particularly amenable to the determination of trace metal ions. The charged species injected are complexed on-column by a complexing agent added to the eluent and sepa-

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ration of inorganic mercury(II) and ionic organomercurials has been effected by the on-column formation of their neutral 2-mercaptoethanol complexes. This technique has been widely utilized for mercury speciation in drugs, fish, water and biological samples [3–9].

Several alternative detectors including electrochemical detection (ED) [4–7], inductively coupled argon plasma (ICP) [10] and graphite furnace atomic absorption (GFAA) [11] have been used for HPLC of mercury compounds with good sensitivity, linearity and/or selectivity.

ED may be sensitive and can monitor several different organometallics simultaneously, but is not highly selective and any interferent reducible species must be removed from solvent systems. The argon ICP provides continuous monitoring, low detection limits and simultaneous multielement monitoring but restrictions on the choice of mobile phase, peak broadening from nebulizer systems and high investment costs are drawbacks. ICP-mass spectrometry has been applied effectively for some organometallic compounds, its performance for arsenic compounds having been compared with hydrogen–argon flame atomic absorption spectrometry [12]. GFAA has been used for organometallics but discontinuous sampling and monitoring give decreased chromatographic resolution. The direct current argon plasma (DCP) is simple to interface and provides a convenient element-selective detector [13–15]

It has been shown [16–18] that quaternary ammonium halide salts are effective extractants for mercury compounds, both inorganic mercury(II) and organomercurials forming extractable anionic complexes in the presence of halide ions. The mechanism of extraction was expressed by Tajima et al. [19] who first applied the scheme shown in the following equations to analyze Hg(II) halides in the presence of counter ions such as tetra-*n*-butylammonium halides:

$$Hg^{2+} + 4X^- = HgX_4^{2-}$$

$$2R_4N^+ + HgX_4^{2-} = (R_4N)_2HgX_4$$

$$[2R_4NX + HgX_2 = (R_4N)_2HgX_4]$$

or

$$R'HgX + X^- = R'HgX_2^-$$

$$R_4N^+ + R'HgX_2^- = (R_4N)R'HgX_2$$

$$[R_4NX + R'HgX = (R_4N)R'HgX_2]$$

Gast and Kraak [20] also reported that in the separation of organomercurials by normal-phase HPLC, a significantly larger capacity ratio, good reproducibility and no decomposition of diphenylmercury were found upon addition of quaternary ammonium halide salts to the eluent.

2. Experimental

2.1. Instrumentation

An IBM Instruments (Danbury, CT, USA) Model LC/9533 ternary gradient liquid chromatograph equipped with Model LC/9522 ultraviolet detector set at 254 nm was used. Sample was introduced by a Rheodyne (Cotati, CA, USA) Model 7125 injector with a 10-μl loop. A Spectraspan IV (ARL, Sunland, CA, USA) DCP emission spectrometer was used with a Houston Instruments (Austin, TX, USA) Omniscribe chart recorder. A 5-μm Octadecyl column (250 × 4.6 mm I.D.) was used (IBM Instruments). A single-channel Model "Rabbit" peristaltic pump (Rainin, Woburn, MA, USA) and was used to pump standard solutions into the DCP.

2.2. Reagents and standards

Tetrabutylammonium bromide (TBABr) (Aldrich, Milwaukee, WI, USA), tetraethylammonium bromide (TEABr) (Fisher Scientific, Fairlawn, NJ, USA) and tetramethylammonium bromide (TMABr) (Eastman Kodak, Rochester, NY, USA) were used as received. HPLC-grade water was made from distilled water further purified using a NANOpure II system (Barnstead, Boston, MA, USA). Methylmercury chlo-

ride and ethylmercury chloride were obtained from Strem Chemical (Newburyport, MA, USA) and benzylmercury chloride was obtained from M.D. Rausch (Department of Chemistry, University of Massachusetts, Amherst, MA, USA); all were purified twice by recrystallization from methanol. Phenylmercury chloride (Alfa Inorganics, Beverly, MA, USA) was purified first by recrystallization from methanol and repurified by sublimation at 100°C and 0.5 Torr (1 Torr = 133.322 Pa). Mercuric chloride was obtained from Fisher Scientific and used as received. Each standard solution was prepared in HPLC-grade methanol, except the mercuric chloride, which was prepared by dissolution in a minimum amount of water and diluted with methanol. All the solutions were prepared weekly and stored in the dark at 2°C when not in use.

2.3. Sample preparation

A river water sample was obtained from the Connecticut River, sampled in Sunderland, MA, USA and spiked with appropriate amounts of mercury compounds. It was then filtered through a 0.45-μm membrane filter (Millipore, Bedford, MA, USA) prior to analysis.

2.4. Procedure

The mobile phases were prepared by dissolving the appropriate amount of tetra-*n*-alkylammonium bromide salts and sodium halide salts in a mixture of methanol and water. In order to achieve the maximum selectivity and sensitivity of DCP response, the position of the plasma and the wavelength setting needed to be optimized. First, the $HgCl_2$ aqueous solution was pumped directly into the DCP nebulizer through PTFE tubing by a peristaltic pump. Then the wavelength setting and orientation of the electrode assembly were adjusted appropriately. The interfacing of HPLC to DCP was accomplished by directly connecting PTFE tubing (50 cm × 0.25 mm I.D.) to the DCP nebulizer from the output of the UV detector.

3. Results and discussion

3.1. Separation of inorganic mercury(II) and organomercury compounds

Fig. 1B shows an isocratic separation of $HgCl_2$, CH_3HgCl, C_2H_5HgCl, C_6H_5HgCl and $C_6H_5CH_2HgCl$ on a C_{18} column with 0.01 *M* TBABr as the ion-pair reagent in methanol–water (60:40, v/v) used as the mobile phase. The amounts of mercury compounds injected were 62.5 ng for Hg(II) and benzyl, and 1250 ng for methyl, ethyl and phenyl species. The mercury compounds were well resolved and eluted with good peak shape, reproducibility and chromatographic efficiency. The elution order of organomercury compounds was methyl < ethyl < phenyl < benzyl, following the expected trend for homologues in reversed-phase HPLC for increasing elution volume as the number of methylene groups or the molecular mass increases.

3.2. Chromatographic behavior of mercury compounds under different mobile phase conditions

A number of experimental factors were investigated for effects on the chromatographic behavior.

Influence of the type and the concentration of sodium halide

Inorganic and organic mercury species can form stable charged bromide complexes, $HgBr_4^{2-}$ and $RHgBr_2^-$ respectively, with the bromide ion from the 0.01 *M* ion-pair reagent, TBABr. When sodium chloride was added to the eluent, the retention times decreased sharply. Fig. 1A shows the chromatogram obtained when 0.15 *M* NaCl was added to the eluent. This trend may be accounted for by the formation of the more soluble chloride complexes $HgCl_4^{2-}$ (log β_4 = 15.3) and $HgCl_2^-$, even though they have lower stability constants than analogous bromide complex ions (log β_4 = 22.23 for $HgBr_4^{2-}$) [21]. In addition to decreasing retention time, the addition of sodium chloride gave more symmetrical

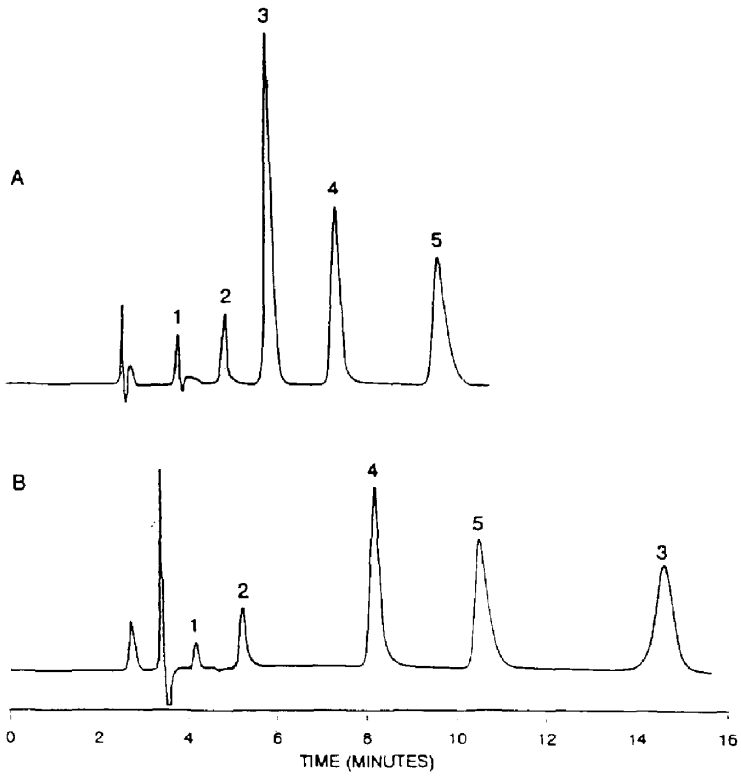

Fig. 1. Separation of inorganic mercury(II) and organomercurials on IBM C_{18} column, 5 μm, 250 × 4.6 mm I.D. Mobile phase: (A) 0.01 *M* TBABr and 0.15 *M* NaCl in methanol–water (60:40, v/v), (B) 0.01 *M* TBABr in methanol–water (60:40, v/v). Flow-rate: 1 ml/min. Detection: UV 254 nm. Peaks: 1 = CH_3Hg^+; 2 = $C_2H_5Hg^+$; 3 = Hg^{2+}; 4 = $C_6H_5Hg^+$; 5 = $C_6H_5CH_2Hg^+$.

and sharper peak shapes; thus in all further experiments sodium halide was always present in the eluent.

Fig. 2 shows graphically the effect of the concentration of chloride ion on the capacity factors of mercury compounds. Sodium chloride concentration was varied from 0.0 to 0.2 *M*. The drop in the capacity factor of inorganic mercury(II) with increase of chloride concentration can be attributed to the formation of more $HgCl_4^{2-}$, resulting in greatly increasing solubility. In contrast, the organomercury species only formed singly negative charged complexes and the capacity factors showed little change with increase of Cl concentration. A comparison of the influence of chloride and bromide concentrations on the capacity factors of the mercury compounds showed slightly larger values with Br^- than with Cl^-, consistent with the solubility order of the halides.

Influence of the level of organic modifier

The effect of the organic modifier concentration on the capacity factors of mercury compounds was investigated for methanol, the data being shown in Fig. 3. The logarithm of capacity factors decreases linearly with increase in the content of methanol in the eluent in the range of 50–70% as is commonly found for reversed-phase systems. However, the individual slopes are different, which indicates a change in selectivity when varying the methanol percentage. For organomercury compounds, the decrease in log k' almost parallels the increase in methanol content, which indicates no significant selectivity changes among them. In contrast to organomer-

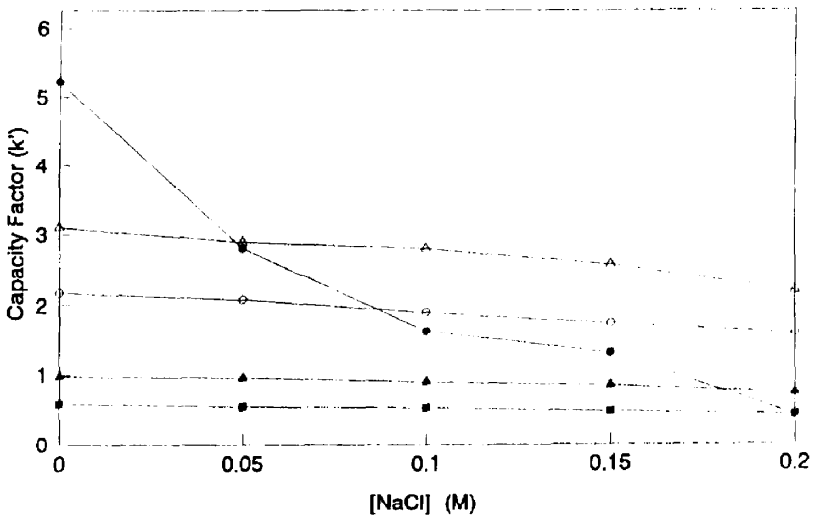

Fig. 2. Effect of sodium chloride concentration on the capacity factor, k', of mercury compounds. Chromatographic conditions as in Fig. 1A. ■ = CH_3Hg^+; ▲ = $C_2H_5Hg^+$; ● = Hg^{2+}; ○ = $C_6H_5Hg^+$; △ = $C_6H_5CH_2Hg^+$.

cury species, however, the capacity factor of inorganic mercury(II) drops markedly with increase in methanol content.

Influence of ion-pair reagent concentration

Fig. 4 shows the relationship between the capacity factor and the concentration of TBABr over the range 0.001 to 0.0316 *M*. As is typical in ion-pair chromatography, the capacity factors increase with the increase in ion-pair reagent concentration. The much larger slope for inorganic mercuric species corresponds to a higher stability constant for HgX_4^{2-}, which indicates that it requires a greater amount of ion-pair

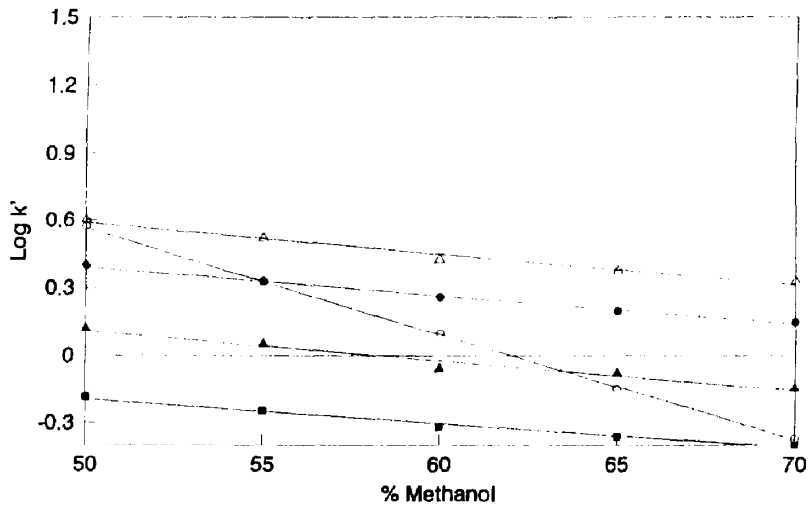

Fig. 3. Effect of methanol concentration on the capacity factor, k', of mercury compounds. Chromatographic conditions as in Fig. 1A. ■ = CH_3Hg^+; ▲ = $C_2H_5Hg^+$; ● = $C_6H_5Hg^+$; ○ = Hg^{2+}; △ = $C_6H_5CH_2Hg^+$.

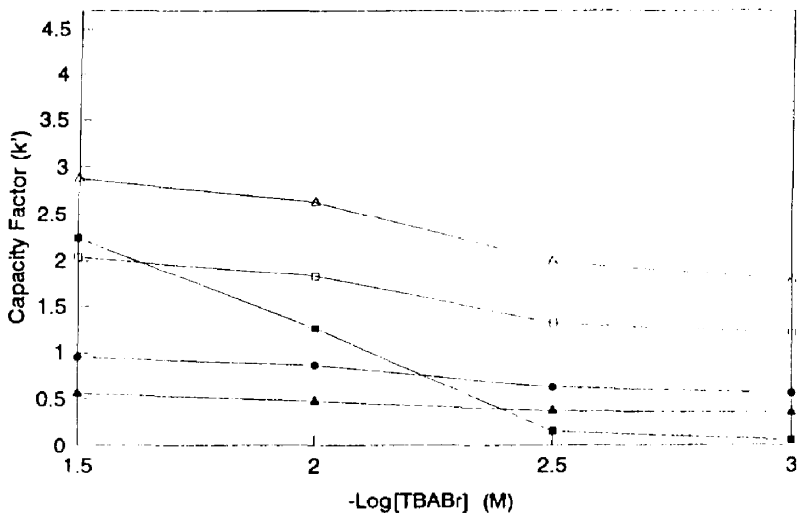

Fig. 4. Effect of ion-pair concentration on the capacity factor, k', of mercury compounds. Chromatographic conditions as in Fig. 1A. ■ = Hg^{2+}; ▲ = CH_3Hg^+; ● = $C_2H_5Hg^+$; ○ = $C_6H_5Hg^+$; △ = $C_6H_5CH_2Hg^+$.

reagent for complete ion-pair formation. At lower TBABr concentrations, the inorganic mercury(II) species eluted close to the void volume, while at higher TBABr concentration, it was strongly retained. This indicates that the retention of Hg^{2+} is greatly dependent on the concentration of ion-pair reagent.

In contrast to the inorganic mercury(II), organomercury species show lower capacity factor slopes. In addition, the decrease in k' closely parallels the decrease in TBABr concentration suggesting similar structures for the ion pairs and similar stability constants of $RHgX_2^-$ species despite their differences in k'. This also indicates that the capacity factors for organomercury species are primarily dependent on their own chemical properties, rather than on the concentration of ion-pair reagent. For both Hg^{2+} and RHg^+ species, the peak shapes were found to be broader and less reproducible at higher TBABr concentrations; 0.01 *M* of TBABr was found optimal for HPLC.

Influence of the types of ion-pairing reagent

The effect of the different ion-pair reagents, TMABr, TEABr and TBABr, on the capacity factors of inorganic mercury(II) and organomercury species was examined The capacity factors of the organomercury species showed very little change with decreasing molecular size of ion-pair reagents, but the capacity factors for Hg^{2+} increased notably with increase in molecular size of the ion-pair reagents. This suggests that the more ionic complex ion species, HgX_4^{2-}, requires a larger counter-ion than the less ionic organomercury complex ions, $RHgX_2^-$ to pair effectively.

3.3. Calibration curves and detection limits with UV detection

Although alkylmercury compounds show a low molar absorption in the UV region [22], Hg^{2+} forms HgX_4^{2-} species which have a charge absorption band in the UV range [23]. Table 1 shows analytical data for UV detection for inorganic and organomercury species. Both Hg^{2+} and $C_6H_5CH_2Hg^+$ are detected with much greater sensitivity than the CH_3Hg^+, $C_2H_5Hg^+$ and $C_6H_5Hg^+$ species.

The detection limit of each mercury species was determined by measuring the minimum amount which had to be injected to provide a peak signal approximately twice the noise while the detector was at the most sensitive setting, AUFS 0.001. The detection limits were in the

Table 1
Analytical data for inorganic and organic mercury species by ion-pair HPLC with detection at 254 nm

Cation	Linear range (ng)	Slope	Correlation coefficient
CH_3Hg^+	8.0–200	0.0025	0.9989
$C_2H_5Hg^+$	3.8–200	0.0035	0.9987
Hg^{2+}	0.8–400	0.1260	0.9998
$C_6H_5Hg^+$	1.3–300	0.0060	0.9940
$C_6H_5CH_2Hg^+$	0.2–400	0.1300	0.9998

range of 0.2–8.0 ng which represents the range between the most sensitive benzylmercury and the least sensitive methylmercury species.

3.4. Mercury determination in a spiked river water sample

Fig. 5 shows the UV detection chromatogram of a river water sample "spiked" with Hg^{2+} (5 μg/ml) and CH_3Hg^+ (80 μg/ml) to illustrate the relative sensitivities of inorganic and organic mercury species. Recoveries were found to be $80.02 \pm 1.62\%$ and $96.71 \pm 2.32\%$ for Hg^{2+} and CH_3Hg^+, respectively, measured against aqueous standards. The lower percentage recovery

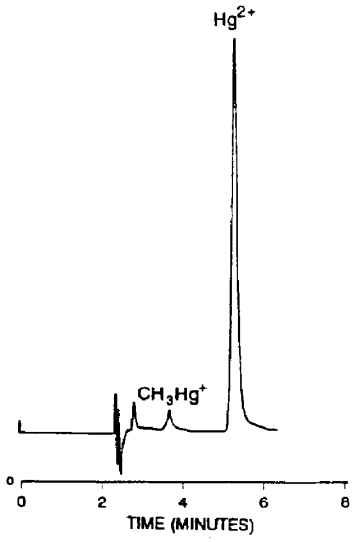

Fig. 5. Chromatogram of spiked water sample with inorganic mercury (5 μg/ml) and methylmercury (80 μg/ml). Column and conditions as in Fig. 1A.

for Hg^{2+} may be attributed to the matrix effects of the water sample whereby the ion reacted with any reactive substrate resulting in a low amount of "free" mercuric ion. In contrast the less ionic methylmercury species appeared without apparent loss in the system.

Although ion-pair chromatography with UV detection provides an improvement in the determination of Hg^{2+} through the formation of a highly UV absorbing HgX_4^{2-} species, the determination of Hg^{2+} may sometimes remain problematic in an environment where other relatively stable mercury(II) complexes may predominate.

3.5. DCP as a detection method

Fig. 6 shows a chromatogram of mercury compounds with both DCP and UV detection. The DCP plasma was found to be stable and no serious baseline noise was found when there was a high content of organic solvent (methanol) and the organic ion-pair reagent in the eluent. No substantial increase in retention times was observed upon incorporation of the 50 cm long interface tube. The UV trace shows response for each mercury species. However, in the DCP trace, inorganic mercury(II) and the benzylmercury species are missing from the chromatogram, while the methylmercury, ethylmercury and phenylmercury species show response. As noted previously, Hg^{2+} and $C_6H_5CH_2Hg^+$ show much higher UV sensitivity than the other three organomercury species. In order to bring the UV response to a comparable level for each mercury species, the amount of each injected was modified appropriately, only a very small amount (62.5 ng each) of Hg^{2+} and $C_6H_5CH_2Hg^+$ being injected on the column.

Fig. 7 shows another chromatogram also with UV and DCP detection, with equal amounts (4 μg) of each mercury species injected. The DCP trace shows similar response for each species, while in the UV trace, the response is off scale for the Hg^{2+} and $C_6H_5CH_2Hg^+$ species. The notable difference between the DCP and the UV responses occurs because the former is energetic

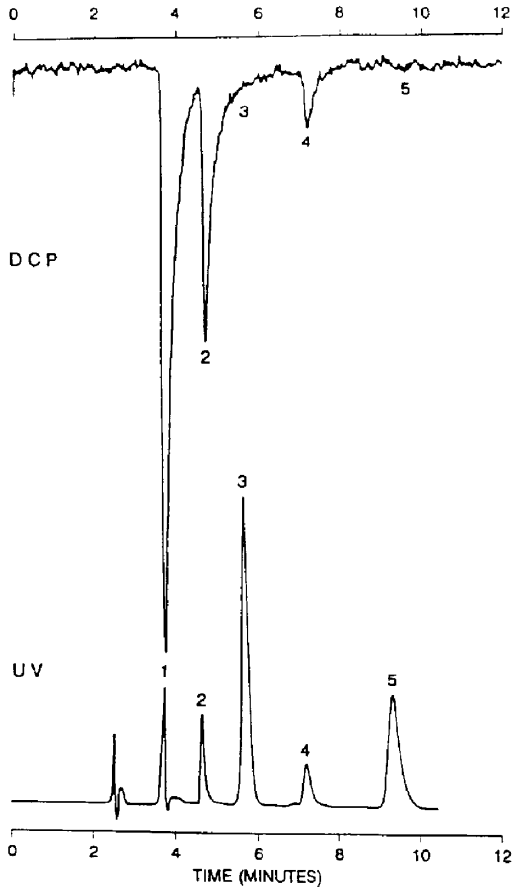

Fig. 6. Dual-detection chromatogram of mercury compounds. Column and conditions as in Fig. 1A. Lower chromatogram, UV detection at 254 nm; upper chromatogram, DCP emission detection at 253.6 nm. Peaks: 1 = CH_3Hg^+; 2 = $C_2H_5Hg^+$; 3 = Hg^{2+}; 4 = $C_6H_5Hg^+$; 5 = $C_6H_5CH_2Hg^+$.

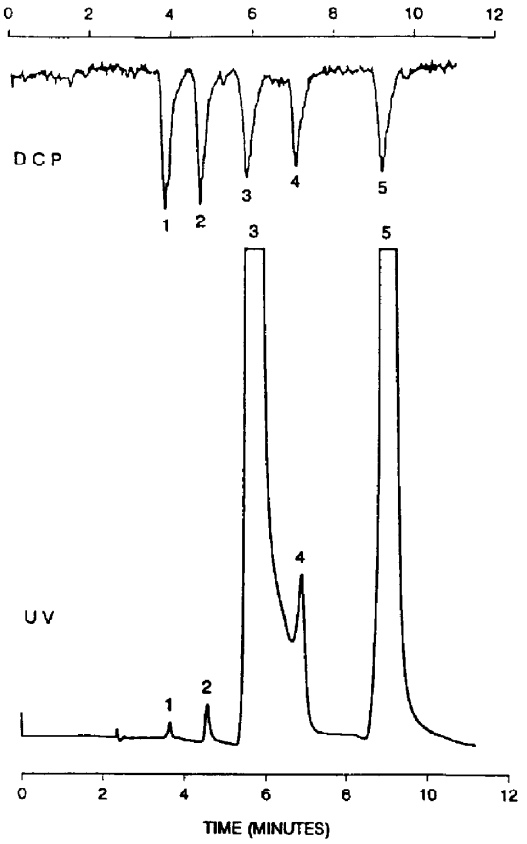

Fig. 7. Dual-detection chromatogram of mercury compounds (4 μg for each species). Column and conditions as in Fig. 1A. Lower chromatogram, UV detection at 254 nm; upper chromatogram, DCP emission detection at 253.6 nm. Peaks as in Fig. 6.

enough to completely atomize each molecule, thus its response is relatively independent of the matrix and chemical effects seen in the UV trace.

Fig. 8 shows the DCP calibration curves for the mercury compounds, the sensitivities decreasing in the order $CH_3Hg^+ > C_2H_5Hg^+ > C_6H_5Hg^+ > Hg^{2+} > C_6H_5CH_2Hg^+$. The sequence is the same order as volatility decrease, but is the reverse order of molecular size and UV responses. As is commonly found in atomic emission spectroscopy, the more volatile species will have the greater emission intensity. Also, the larger size of molecule may penetrate less efficiently into the DCP than the smaller one, resulting in decreased atomization efficiency and emission intensity.

The relatively high detection limits for mercury compounds, 175 ng (methyl)–255 ng (benzyl) may be attributed to the high content of carbon due to organic solvent and organic ion-pair reagent in the eluent which contributed to the noisy background. Another factor could conceivably be the formation of larger ion-pair species, which find it harder to penetrate into the DCP, resulting in low atomization efficiency.

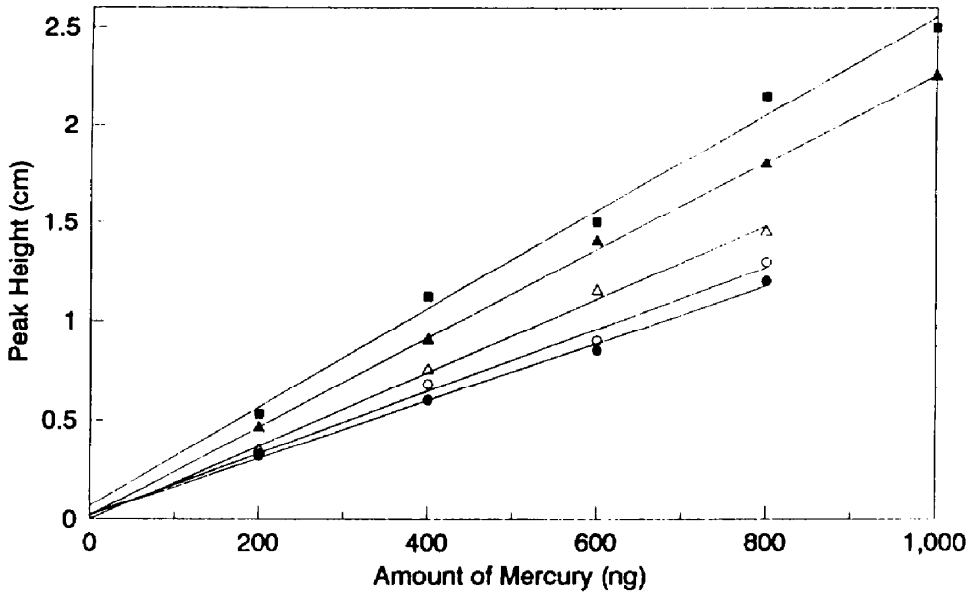

Fig. 8. Calibration curves for mercury compounds with mercury-specific detection at 253.6 nm. Chromatographic conditions as in Fig. 1A. ■ = CH_3Hg^+; ▲ = $C_2H_5Hg^+$; ● = $C_6H_5CH_2Hg^+$; ○ = Hg^{2+}; △ = $C_6H_5Hg^+$.

4. Conclusions

Ion-pair HPLC, with TBABr as an ion-pairing reagent, was shown to be effective for the separation of both inorganic and organic mercury compounds. The addition of sodium chloride to the mobile phase gives better peak shapes and lower retention times, the latter probably being due to increased competition for the pairing ion, as occurs for ion exchange. Increased complexation, by addition of more chloride, should increase retention due to the greater negative charge on the complex; however this is overridden by the decrease in retention caused by the increased ionic strength and perhaps solubility.

The halide in TBABr forms a high-UV-absorbing charged complex, with Hg(II) species enabling direct sensitive UV detection of the mercuric species with no need for conversion into organomercurial species by a complicated or time-consuming derivatization method.

In contrast to the UV detection, DCP showed better responses to the smaller molecules such as methyl or ethyl mercury species, this being of value to compensate for their low response to UV detection.

Acknowledgements

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