Determination of Hg (II) by Hybrid Micellar Liquid Chromatography
การวิเคราะห์โลหะไอออนปรอทด้วยเทคนิคโครมาโทกราฟีแบบไฮบริดไมเซลล์

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ABSTRACT

Hybrid micellar liquid chromatography (HMLC) with UV-visible detection was developed for the determination of Hg^{2+} after its complexation with ammonium pyrrolidinedithiocarbamate (APDC). Optimum chromatographic conditions were a C_{18} μBondapak column and the mobile phase containing 50 mmol L^{-1} sodium dodecyl sulfate (SDS), 10 mmol L-1 phosphate buffer (pH 3.5), 38 mmol L^{-1} tetrabutylammoniumbromide (TBABr) and 68% (v/v) acetonitrile, and UV detection at 275 nm. Under the optimum condition, Hg-APDC complex was detected at 6 min. Linear range was ranging from 1 to 800 μg L^{-1} and the detection limit of 0.32 μg L^{-1}. The proposed method was applied to the analysis of Hg^{2+} in whole blood samples.

บทคัดย่อ

การวิเคราะห์โลหะไอออน Hg^{2+} โดยใช้เทคนิคโครมาโทกราฟีแบบไฮบริดไมเซลล์หลังการเตรียมอนุพันธ์กับแอมโมเนียมไพโรลิดินไดไทโอคาร์บาเมต (APDC) สภาวะที่เหมาะสมในการวิเคราะห์ปรอท คือ คอลัมน์ C18 μBondapak เฟสเคลื่อนที่ประกอบด้วย โซเดียมโดเดคซิลซัลเฟตเข้มข้น 50 มิลลิโมลต่อลิตร เตตระบิวทิลแอมโมเนียมโบรไมด์เข้มข้น 38 มิลลิโมลต่อลิตร สารละลายบัฟเฟอร์ฟอสเฟตเข้มข้น 10 มิลลิโมลต่อลิตร pH 3.5 และ 68 เปอร์เซ็นต์ อะซิโตไนไตรล์ ความยาวคลื่นที่ใช้ในการตรวจวัด คือ 275 นาโนเมตร ภายใต้สภาวะที่เหมาะสมสามารถตรวจวัดปรอท-APDC ได้ที่เวลา 6 นาที ช่วงความเป็นเส้นตรงของโลหะปรอทอยู่ในช่วง 1-800 ไมโครกรัมต่อลิตร ขีดจำกัดของการตรวจวัด คือ 0.32 ไมโครกรัมต่อลิตร ได้ประยุกต์ใช้วิธีที่นำเสนอนี้ในการหาปริมาณปรอทในตัวอย่างเลือด

Key Words: Ammonium pyrrolidinedithiocarbamate, Mercury, Hybrid micellar liquid chromatography
คำสำคัญ : แอมโมเนียมไพโรลิดินไดไทโอคาร์บาเมต ปรอท โครมาโทกราฟีแบบกึ่งไมเซลล์

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Introduction

Mercury compounds are well known to be highly toxic, organomercury compounds are neurotoxic and Hg^{2+}compounds are nephrotoxic (Gundacker et al, 2006). Mercury is present in trace amount in air (vapour, Hg^0), water (inorganic Hg) and food (methylmercury in seafood and freshwater fish). The toxicity of mercury depends on the chemical state of the element. This metal is one of the main pollutants, especially in water: its maximum allowable concentration in drinking water is 1 $\mu g\ L^{-1}$ (Théraulaz and Thomas, 1994).

Mercury can be inhaled as vapor or pass through the skin and into the blood stream. Compounds of mercury can enter the body through the lungs, skin or by ingestion. Repeated exposure to mercury has adverse health effects whose symptoms are well documented. Individuals at high risk of exposure or who are suspected of mercury intoxication are typically monitored through analysis of blood and urine samples. The mercury blood test will detect all types of mercury but because mercury remains in the bloodstream for only a few days the test should be performed soon after exposure.

Several techniques, including inductively coupled plasma mass spectrometry (ICP-MS), cold-vapor atomic absorption spectrometry (CVAAS) and hydride generation atomic absorption spectrometry (HGAAS) have been widely used for the determination of mercury in different samples because of their sensitivity, high precision and accuracy, and also wide availability of instruments. However, the mentioned techniques require significant investment and functioning cost (Batáriová et al, 2006). Colorimetric methods for the determination of mercury were proposed but all require complexation of mercury and time-consuming. In addition, qualitative and quantitative determination of mercury by UV-visible spectrometry can be hindered by spectra overlapping of ligand and Hg complex (Théraulaz and Thomas, 1994).

However, complexation of metal ions with ligands is still useful because it can be used in combination with other techniques for the determination of metal ions. Ammonium pyrrolidine dithiocarbamate (APDC) is a bidentate univalent anionic ligand, having two donor sulfur atoms (Ueno et al, 1992). APDC is the most widely used ligand to determine the metal ions, such as Cd^{2+}(Lee et al, 2001), Pb^{2+} (Daorattanachai et al, 2005) and Hg^{2+}(Dong et al, 2004). Usually, metal-APDC complexes are insoluble in aqueous solution, thus they can be measured by UV-visible spectrophotometry and flame atomic absorption spectrometry, after extraction with nonpolar organic solvent. Solvent extraction techniques are time-consuming, tedious and usually involve harmful solvents.

Recently, hybrid micellar liquid chromatography (HMLC) has been introduced as an alternative to RP-HPLC (Berthod et al, 1986). Mobile phase of HMLC is the one used in RP-HPLC with the addition of surfactant above critical micelle concentration (CMC). HMLC has proved to be a useful technique in the determination of non-polar analyte in RP-HPLC. However, the separation of insoluble metal-APDC complexes using hybrid micellar mobile phase has not been reported before.

This study is aimed to develop liquid chromatographic method for the determination of Hg (II). The method consists of pre-column derivatization of Hg (II) ion with APDC and then analysis by HMLC. The proposed method will be applied to determine Hg (II) ion in whole blood samples. The results of the proposed method will be compared with those obtained by HGAAS.

Experimental

1. Chemicals and reagents

All reagents were of analytical grade. The atomic absorption standard solution (1000 mg L^{-1}) of Hg (II) was obtained from Carlo Erba (France). Ammonium pyrrolidinedithiocarbamate (APDC) and tetrabutylammonium bromide (TBABr) were purchased from Fluka (Australia). Sodium dodecyl sulphate (SDS) was purchased from BDH (England). Acetonitrile was of HPLC grade from Lab-Scan (Thailand). Aqueous solutions were prepared with deionized water obtained from RiO_s^{TM} Type I simplicity 185 (Millipore Waters, USA) throughout the experiment. The standard solution of Hg (II) was prepared on daily basis by diluting appropriate aliquots of 1000 mg L-1 stock Hg (II) solution with water. Nitric acid (65%) was purchased from Merck. APDC solutions were prepared by dissolving the appropriate amounts of APDC in water.

2. Instruments

The chromatographic set-up consisted of a Waters 6000A Dual Pump, a Rheodyne injector with 20 µL sample loop and a Waters 484 Tunable Absorbance Detector (Waters, USA) equipped with the Millennium32 Software data acquisition system. Separation was carried out using a µBondapak C_{18} column (300x3.9 mm i.d., 10 µm particle sizes).

The HGAAS set-up consisted of AAnalyst 100 (Perkin Elmer, USA) equipped with flow injection system hydride generator (FIAS 100 mercury/hydride chemifold, Perkin Elmer, USA) and Electrodeless Discharge lamp (Perkin Elmer, USA).

The absorption spectrum of Hg complex was obtained on an Agilent 8453 UV-visible spectrophotometer (Agilent, USA).

Blood samples were wet ashed using a microwave digestion system (MAR-5, CEM Corporation, USA).

3. Procedures

3.1 Sample preparation

Blood samples of approximately 4 mL from healthy volunteers were collected into Monovettes containing heparin as an anticoagulant for trace element analysis (BD vacutainer, USA) and thoroughly shaken to avoid coagulation and to achieve homogeneity. Samples were transferred cooled to the laboratory and stored at 4 °C until further treatment. Thawed blood samples were homogenized in an ultrasonic bath. Samples (1.00 mL) were wet ashed with 10 mL 65% HNO_3 using microwave digestion. The digestion solution and 3x4 mL rinsed by solutions were transferred to a 50 mL volumetric flask and made up to 50 mL with water and then were transferred to polyethylene (PE) tube and stored at 4 °C until determination.

3.2 Pre-column derivatization reaction

In the case of standard solutions, APDC chelate of Hg (II) was formed by mixing appropriate amounts of metal ion stock solutions and APDC (0.4 mL, $5x10^{-3}$ mol L^{-1}) with the metal:APDC molar ratio of 1:3. The solution was

then adjusted to pH 3.5 with 20 mmol L^{-1} phosphate buffer pH 3.5 containing 50% acetonitrile and 50 mmol L-1 SDS. The Hg-APDC complex solution was filtered through 0.45 μm nylon membranes (Millipore) before being injected into the HPLC system.

In the case of blood samples, the digested sample (100.00 μL) was derivatized by complexing with APDC using the same procedure as standard Hg (II). The obtained complex was injected into HPLC and the obtained peak area was used for quantification of Hg (II).

3.3 Recovery

Recovery was studied via the matrix-match calibration curve (including blood blank). Five different concentrations of standard Hg (II) including 0.016, 0.024, 0.032, 0.040, 0.048 μg L^{-1} were spiked into the blood samples before being digested and analyzed by both the proposed method and HGAAS.

3.4 Chromatographic determination of Hg (II) by HMLC

The optimum mobile phase was a mixture of 38 mmol L^{-1} TBABr, 50 mmol L^{-1} SDS and 68% acetonitrile (v/v) in 10 mmol L^{-1} phosphate buffers (pH 3.5). The flow rate was 1.0 $mLmin^{-1}$ and detection wavelength was 275 nm. In order to ensure that there was no surfactant ion retained on the column between runs, the column was washed with MeOH/H_2O (50/50) for 30 min column-volumes and was then equilibrated with the mobile phase being used for 1 hr before injection.

3.5 Determination of mercury in whole blood by HGAAS

A flow-injection HGAAS was used for the determination of total mercury in whole blood. The sample solution from section 2.3.1 and matrix-matched calibration standards were loaded onto a autosampler mounted on top of a flow injection mercury system (FIMS). The sample solution (500 μL) was injected into a 10% (v/v) HCl carrier stream. This FI stream was merged with a reductant stream consisting of 0.2% (w/v) of sodium tetrahydroborate in 0.05% (w/v) NaOH and was then carried into the quartz cell (100°C) by means of an argon stream. The measurement parameters were as follows: wavelength, 253.6 nm; lamp current, 60 mA.

Results and Discussion

1. Optimization of HPLC conditions

Compositions of mobile phase were optimized including pH and concentrations of buffer solution, acetonitrile, SDS and TBABr.

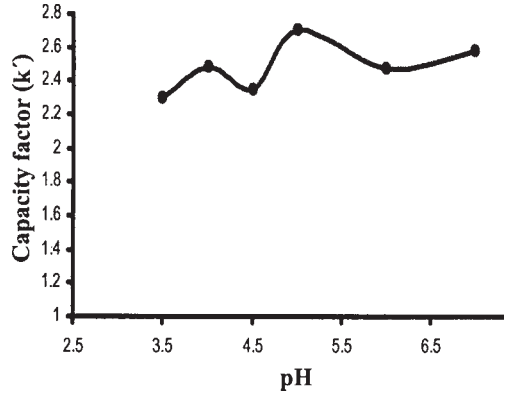

Figure 1 Effect of pH of mobile phase, HPLC conditions: 68% acetonitrile, 30 mM SDS.

To examine the optimum pH, phosphoric acid-phosphate buffers in the pH range of 3.5-7.0 were studied. Figure 1 demonstrates the effect of pH on retention behavior of Hg-APDC, the retention decreased with the increasing of pH. However, there was a report on effect of pH that changing of pH caused small variation in retention

times (Zivanovic et al, 2004). According to the result, it is obvious that the sensitivity of Hg-PDC complex mainly depend on the pH value. The optimum pH value in the mobile phase was 3.5 (phosphate buffer).

Concentrations of phosphate buffer pH 3.5 were studied in the range of 5.0 - 20.0 mmol L^{-1}. Since the role of buffer is to control the pH of the system, as long as it was able to provide enough buffer capacity varying in concentration should not severely influence the retention behavior of the complex. It was found that the concentration of buffer has insignificant effect on retention time but the peak areas were obviously affected, as shown in Figure 2. (Vílchez et al, 2004).

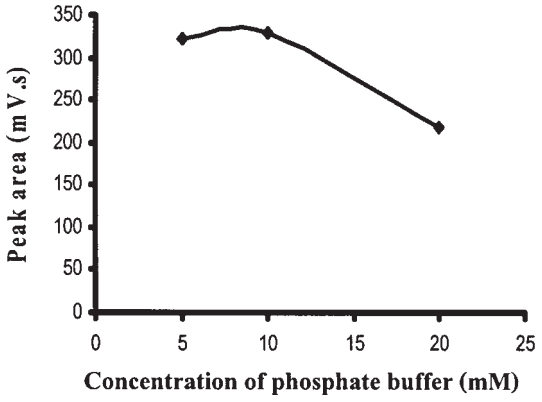

Figure 2 Influence of buffer concentration in mobile phase, HPLC conditions: 68% acetonitrile, 40 mM SDS, 38 mM TBABr, phosphate buffer pH 3.5

In hybrid micellar liquid chromatography (HMLC), the addition of sodium dodecylsulfate (SDS) is aimed to increase aqueous solubility, to form the hydrophilic layer above the surface of the silica and reduce the penetration depth of the analyte into the bonded phases. The ability of surfactant to self-assemble at its critical micellar concentration and form micelles was used to increase the elution strength of mobile phase and decrease the concentration of organic solvent needed (Zivanovic et al, 2004). In the present study, SDS concentration was varied in the range 10-50 mmol L^{-1}.

Peak area of the Hg-APDC was increased when concentration of SDS was increased. Using eluents containing high concentration of SDS resulted in the decreasing of retention times and the theoretical plates (Loginova et al, 2006). The best compromise in terms of run time, efficiency and pressure of HPLC system was found to be 50 mmol L-1 in SDS concentration. Fig. 3 shows a plot of reciprocal of capacity factor ($1/k'$) versus SDS concentration. The results show that the retention behavior of Hg-APDC is fitted well with the HMLC (Gil-Agust et al, 2006). These results indicated that Hg-APDC complex form inclusion complexes both with the micelles and the surfactant adsorbed on the stationary phase (Vílchez et al, 2004).

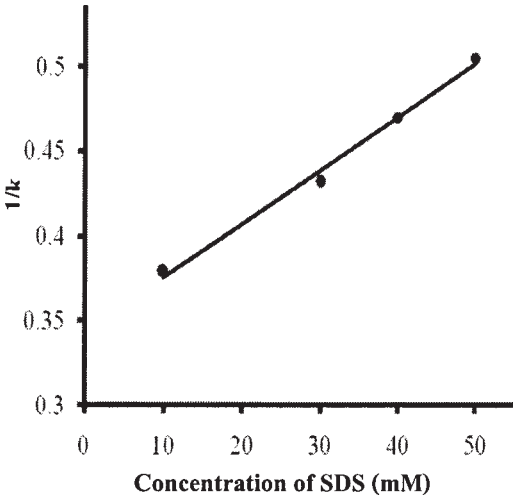

Figure 3 Effect of SDS concentration in mobile phase, HPLC conditions: 68% acetonitrile, 38 mM TBABr, 10 mM phosphate buffer pH 3.5.

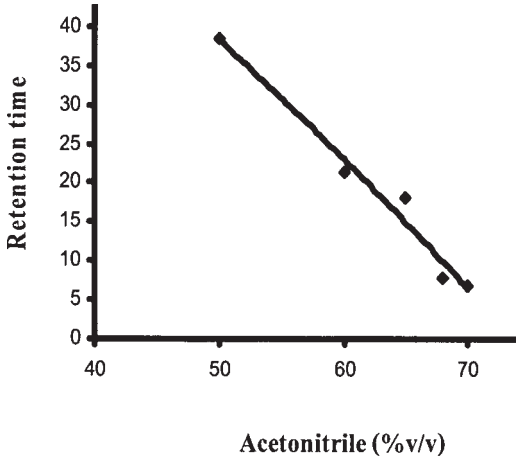

Figure 4 Effect of acetonitrile in mobile phase, HPLC condition: 40 mM SDS, 38 mM TBABr, 10 mM phosphate buffer pH 3.5

The effect of percentage acetonitrile is shown in Figure 4. Increasing of acetonitrile resulted in the decreasing of retention which is the behavior of reversed phase liquid chromatography.

In HMLC, the use of micellar mobile phase contained tail-reducing agents including ammonium acetate, TBABr and triethylamine was reported (V?lchez et al, 2004). In the present study, the micelles of Hg-APDC and SDS are expected to be anionic micelles, TBABr was chosen as the tail-reducing agent in HMLC mobile phase. The concentration of TBABr ranging from 5-40 mmol L^{-1} was investigated. The retention time and peak shape of Hg-APDC were optimized at 38 mmol L^{-1} TBABr.

The optimum mobile phase obtained was a mixture of 38 mmol L^{-1} TBABr, 50 mmol L^{-1} SDS and 68% acetonitrile (v/v) in 10 mmol L^{-1} phosphate buffers (pH 3.5) which Hg-APDC was detected at 6 min.

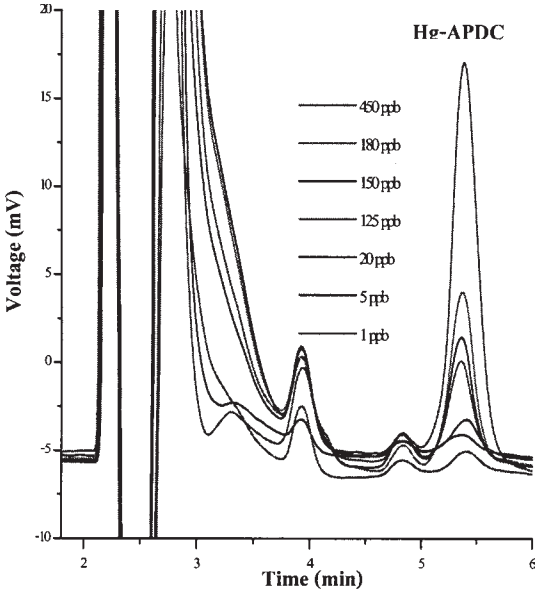

Figure 5 Chromatograms of Hg-APDC at various concentrations of Hg (II)

2. Analytical Performance

The calibration curve is linear at the concentration range of 0.001 to 0.800 mg L^{-1}. Figure 5 depicts the chromatograms obtained for calibration curve study. The correlation coefficient obtained was 0.9924. The limit of detection (LOD) and the limit of quantitation (LOQ) were deduced as the amount of Hg (II) which gives a signal to noise (S/N) ratio of 3 and 10, respectively. LOD and LOQ were 0.32 μg L-1 and 0.64 μg L-1, respectively. The repeatability was studied using 0.020 and 0.800 mg L-1 Hg (II) by performing five independent determinations. The repeatability was deduced from five replicates in one day (n=5). The relative standard deviation (RSD) of retention time was lower than 0.09% and RSD of peak area was lower than 3.24%. The reproducibility was obtained from the experiments in 3 days (n=3x5). The RSD of retention time was lower than 1.53% and RSD of peak area was lower than 11.71%.

3. Recovery and the determination of Hg (II) in blood samples

Table 1 summarizes the recovery of Hg (II) using HMLC and HGAAS. The recoveries from HMLC were good at various concentration levels ($R^2 > 0.99$) and are in good agreement with the results obtained from HGAAS.

Among 8 samples studied, only one sample found Hg (II) at 0.99 ($\mu g\ L^{-1}$). However, the content found is below the maximum permissible mercury concentration in whole blood (usually 2.3 $\mu g\ L^{-1}$) used for many countries (Batáriová et al, 2006).

Table 1 Recovery of Hg (II) from whole blood sample

	%Recovery	
	(HMLC)	(HGAAS)
Blood1	70.78-82.24	85.20-93.00
Blood2	98.12-103.34	86.60-91.10
Blood3	96.01-108.68	96.47-101.04
Blood4	87.56-107.28	85.33-108.04
Blood5	103.05-103.50	101.6-104.95
R^2	0.9942- 0.9984	0.9929-0.9984

Conclusion

The analytical method based on complexation of Hg (II) with APDC before the determination by HMLC was developed. The method provides both qualitative (via retention time) and quantitative data (via peak area). Using HMLC, the interference from excess APDC was removed. The proposed method can be applied to determine Hg (II) in whole blood samples.

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