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Determination of Trace Mercury in Chinese Herbal Medicine by Cold Vapour Generation-Atomic Fluorescence Spectrometry

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A highly sensitive and simple method was developed for the simultaneous determination of trace mercury in Chinese Herbal Medicine by cold vapour generation-atomic fluorescence spectrometry. The herbal medicine samples were digested by closed microwave digestion with $HNO_3 + H_2O_2$ (1:1). The digested samples were determined by cold vapour generation-atomic fluorescence spectrometry equipped with an atomizer which effectively improved the stability of the signals. The conditions for the generation of volatile mercury species were investigated in detail. The tolerance to the interference of coexisting ions was carefully examined. Under optimal conditions, the detection limits (3s) were evaluated to be 4.2 ng L^{-1} for mercury. This method was successfully applied to the determination of mercury in Chinese Herbal medicine samples (Hg in the range of 24 to 83 μg kg^{-1}). The relative standard deviations are 2.5-2.8 %. The recoveries are 94-104 %.

Key Words: Mercury, Cold vapour generation, Microwave digestion, Atomic fluorescence spectrometry, Chinese Herbal medicine.

INTRODUCTION

The toxicological effects of mercury on human bodies have received wide concern and extensive study in recent years and it can accumulate in human issues that may induce biological diseases[1-3]. The Quality Standards of Environment in Chinese says that the concentration of mercury should not exceed 0.2 μg g^{-1} in food and 0.05 μg L^{-1} in drinking water[4,5]. Therefore, determination of trace mercury in biological samples is very important.

In the past few years, a number of techniques have been applied to the determination of Hg, including flame absorption spectrometry, electrothermal atomic absorption spectrometry, inductively coupled plasma mass spectrometry,

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inductively coupled plasma-atomic emission spectrometry, atomic fluorescence spectrometry, cold vapour atomic absorption spectrometry. Each method has its advantages and disadvantages with respect to sensitivity, selectivity and rapidity[6-13].

The relatively low boiling point and high volatility of Hg brings forth the possibility of measuring mercury without additional thermal energy supplied by a flame or electrothermal heating. As a consequence, the cold vapour atomic spectrometry technique has become the preferred technique for trace mercury analysis[14-17]. The main purpose of this paper was to develop a sensitive and simple cold vapour atomic absorption spectrometry (CVAFS) method for the simultaneous determination of trace level of mercury. The experimental parameters were studied in detail. The sample preparation by closed microwave digestion was evaluated. The interference of the co-existing ions was also examined carefully. The proposed method has been successfully applied to the determination of mercury in Chinese Herbal medicine samples with good results.

EXPERIMENTAL

A model AFS-230 double-channel non-dispersive atomic fluorescence spectrometry equipped with an intermittent flow reactor (Beijing Haiguang Instrument Co., Beijing, China) was used throughout this work. Details about the instrument construction can be obtained elsewhere[18]. Mercury high performance hollow cathode lamps (HPHCLs) was used as the excitation sources for the determination of mercury. The HPHCLs were especially designed for atomic fluorescence spectrometry (AFS) measurement, which have advantages over electrodeless discharge lamps in both the stability and lifetime. A schematic diagram of the intermittent reactor is shown in Fig. 1. The working program of the intermittent reactor is described in Table-1. Polytetra-fluoroethylene (PTFE) tubing of 0.7 mm id was employed to construct the manifold. The gas-liquid separator used in this work was made of glass. After gas-liquid separation, the mercury volatile species were introduced by an argon carrier gas to the AFS detector and measured at room temperature (cold vapour). A modified atomizer was used (Fig. 2) to improve the stability of signals. The operational parameters used for the AFS instrument are given in Table-2.

A milestone MLS 1200 microwave digestion system with Teflon digestion vessels and high pressure supports (Milestone, Monroe, Connecticut, USA) was used.

All reagents were of analytical-reagent grade or higher. Ultrapure water used throughout the experiment was obtained from a Milli-Q water-purification system of Millipore (Bedford, MA, USA). The mercury standard solution (1.0 mg mL^{-1}) was obtained from the Chinese Standards

TABLE-1
WORKING PROGRAM FOR THE INTERMITTENT FLOW REACTOR

Step	Flow rate (mL min^{-1})		Time (s)	Function
1	Sample	5.3	10	Draw sample solution using sampling tube
	Reductant (2 % m/v KBH_4)	5.3		
2	Sample	0	1	Chang over of sampling tube into carrier solution
	Reductant (2 % m/v KBH_4)	0		
3	Carrier solution (0.2 mol L^{-1} HCl)	7.5	16	Propel the sample to react with reductant followed by readout
	Reductant (2 % m/v KBH_4)	7.5		
4	Carrier solution (0.2 mol L^{-1} HCl)	0	1	Return to step 1
	Reductant (2 % m/v KBH_4)	0		

TABLE-2
OPERATING PARAMETERS OF THE AFS INSTRUMENT

Parameter	Mercury
High voltage of PMT (V)	300
Peak current of lamp (mA)	30
Atomizer height (mm)	8.0
Flow rate of carrier gas (Ar, L min^{-1})	0.45
Flow rate of shield gas (Ar, L min^{-1})	1.0
Read time (s)	10
Delay time (s)	1
Sample volume (mL)	0.5
Measurement mode	Standard curve
Read mode	Peak area

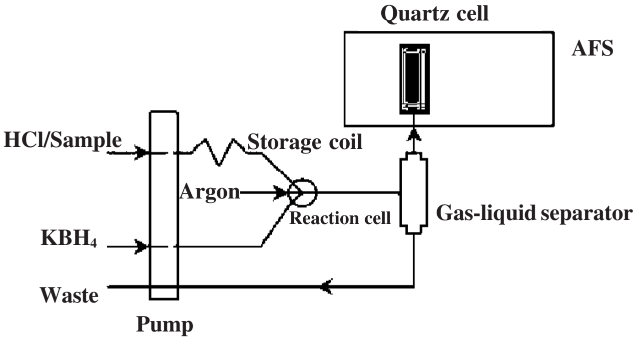

Fig. 1. Schematic diagram of the intermittent reactor: storage coillength 50 cm, id 0.7 mm PTFE tubing; gas-liquid separator made of glass with a cylindrical-shaped cavity (height 60 mm, id 15 mm); transfer tubing (from the separator to the atomizer) length 30 cm, id 0.7 mm

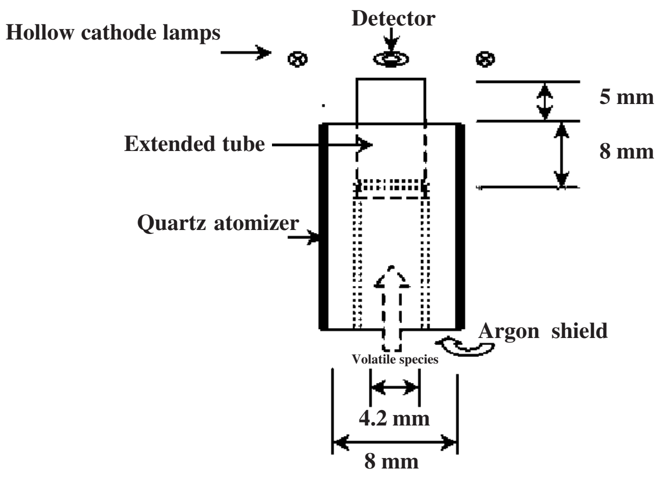

Fig. 2. Schematic diagram of the atomizer: outer quartz tube length 70 mm, id 9 mm; original inner quart tube length 62 mm, id 4.2 mm; extended tube (PTFE tube) length 13 mm, id 4 mm

Center, P.R. China and lower concentration working solutions were obtained by stepwise dilution of the stock solution just before use. The potassium tetrahydroborate(III) solutions were prepared daily by dissolving the reagent (Beijing Chemical Reagent Co., Beijing, China) in 0.5 % potassium hydroxide (Beijing Chemical Reagent Co., Beijing, China) solution. A 0.2 mol L^{-1} hydrochloric acid carrier solution was prepared from concentrated hydrochloric acid (Tianjin Zhenxing Second Chemical Co. Tianjin, China), which was also applied to acidify the samples.

Sample preparation: 0.2 g of sample was weighed accurately into the Teflon high-pressure microwave acid-digestion bomb. To which, 5.0 mL of $HNO_3 + H_2O_2$ (1:1) was added. The bombs were sealed tightly and then positioned in the carousel of the microwave oven. The system was operated at full power for 6 min. Because the oxidizing power of HNO_3 acid inhibited the formation of volatile species, the digestion solution must be heated to near dryness (about 0.5 mL) to eliminate the interference of HNO_3. But this operation must be handled gently and ensure that the digestion can not be heated to dryness. Otherwise, it may result in loss of the mercury. After cooling, 5 mL ultrapure water was added, followed by transferring to a 10 mL calibrated flask, then diluted the solution to volume with water. Reagent blanks were included in each series of digestions.

Analytical procedures: According to the working program of the intermittent flow reactor, the sample solution was drawn from sample cups to the storage coil by a peristaltic pump at a flow rate of 5.3 mL min^{-1} and then swept by 0.2 mol L^{-1} HCl carrier at a flow rate of 7.5 mL min^{-1} to a T-type reactor reacting with a stream of 7.5 mL min^{-1} 2 % KBH_4. Consequently, the volatile mercury species were introduced to the quartz atomizer by argon carrier for AFS determination.

RESULTS AND DISCUSSION

Select of digestion parameters: Microwave sample preparation is the technology of choice for trace and ultra-trace metals analysis. High temperature, closed vessel, acid digestion prepares samples in less time than older methods (minutes rather than hours), uses less acid and retains even volatile elements[19-21]. Therefore, microwave digestion was selected as sample digestion method in this method.

Four digestion reagents, HNO_3, HNO_3 + H_2O_2 (1:1), HNO_3 + $HClO_4$ (1:1) and aqua regia were compared for the digestion of Chinese Herbal medicine. The result show that HNO_3 + H_2O_2 (1:1) has a highest recoveries and less time consumption. For 0.1-0.5 g of samples, 5.0 mL of HNO_3 + H_2O_2 (1:1) used was obtained a complete sample decomposition. Thus, 5 mL of HNO_3 + H_2O_2 (1:1) was selected as digestion reagents in this work. The digesting temperature, pressure and time were also studied. The results show that the microwave system was operated at full power (1000 W power, pressure of 400 kPa, temperature of 250 ºC) for 6 min obtained a complete sample decompose. Therefore, 6 min at 1000 W was selected as digestion time.

Evaluation of AFS parameters: Investigations on the influence of lamp current indicated that the signal intensities of Hg significantly increased along with increase of the lamp current. This result is similar to that of conventional AFS analysis. Nevertheless, higher lamp current resulted in higher noise which may reduce the lifetime of the lamps. So, as a compromise, a current of 30 mA for the Hg lamp was employed in the present work.

As can be seen in Fig. 3, the fluorescence signals reach it maximum when the argon carrier flow rate was 0.4-0.5 L min^{-1}. Therefore, 0.45 L min^{-1} of carrier gas was chosen to gain better sensitivity in this work.

An argon shield gas was employed in this system to isolate the volatile species from the outside air. When the flow rate was less than 0.9 L min^{-1}, the fluorescence signal intensity of mercury decreased. There was a plateau appearing between 1.0 to 1.2 L min^{-1}. Considering the higher cost of argon, a 1.0 mL min^{-1} argon shield gas was used.

Studies on the effect of the injection volume of analyte showed that 0.5 mL of sample was optimal for mercury determination as less volume produced lower fluorescence signal and higher volumes than 0.5 mL did not improve the sensitivity.

We also tested the effect of flow rates of both sample and reductant on the fluorescence signal intensity. By increasing their flow rates, the intensity of the signal for mercury could be improved. In this work, the appropriate flow rate and time are listed in Table-1.

Optimization of chemical variables: When 2 % KBH_4 was used as the reductant, the relationship between acidity and the signals of Hg is shown in Fig. 4. The volatile Hg species can be generated over a wide range of acidity. However, the optimal acidity range of the analytes shifted to higher values when the concentration of KBH_4 in solutions increased. The influence of the HCl concentration in the carrier solution on the determination of Hg is shown in Fig. 5. The signal of Hg decreased slowly with the increase of the acidity of carrier solution. Therefore, 0.2 mol L^{-1} HCl in both the sample and carrier solution was employed in this work. The concentration of KBH_4 has an effect on the generation efficiency. The results shown in Fig. 6 illustrate the signals of Hg remained constant when the concentration of the reductant ranged from 0.5-2.5 %. As the noise increased when the concentration of KBH_4 increased, a 2 % KBH_4 in 0.5 % KOH solution was employed as the reducing agent.

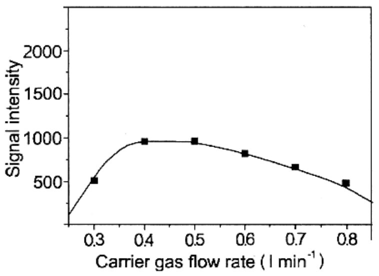

Fig. 3. Effect of the carrier gas (Ar) flow rate on the atomic fluorescence signals of Hg (1.0 μg L^{-1})

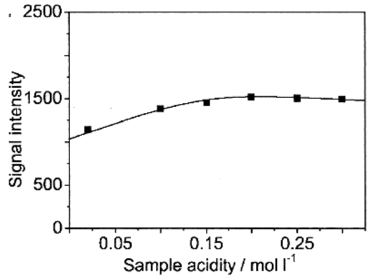

Fig. 4. Effect of HCl concentration in sample on the atomic fluorescence signals of 1.0 μg L^{-1} Hg

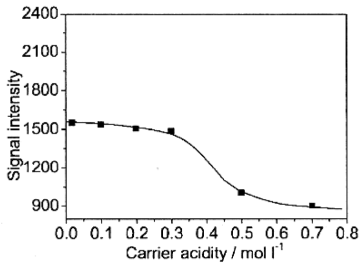

Fig. 5. Effect of HCl concentration in carrier on the atomic fluorescence signals of 1.0 μg L^{-1} Hg

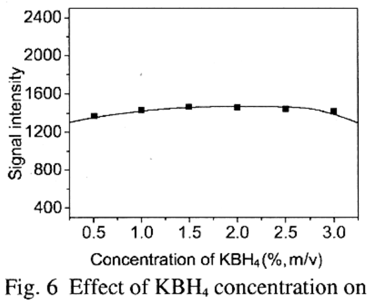

Fig. 6 Effect of KBH_4 concentration on the atomic fluorescence signals of 1.0 mg L^{-1} Hg

Interference: The selectivity of the proposed method was investigated by the determination 1.0 μg L^{-1} of Hg(II) in the presence of various ions within a relative error of ± 5 %. The results are given in Table-3. The results show that most of the foreign ions do not interfere with the determination. This method is high selectivity.

TABLE-3
TOLERANCE LIMITS FOR THE DETERMINATION
1.0 μg L^{-1} OF Hg(II) WITH A RELATIVE ERROR ± 5 %

Ion added	Tolerate (mg L^{-1})
NO_3^-, K^+, Na^+, Mg^{2+}, Cl^-, ClO_4^-, SO_4^{2-}, BO_3^{3-}	50.0
Li^+, Al^{3+}, PO_4^{3-}, NO_2^-, ClO_3^-	10.0
Ca^{2+}, Sr^{2+}, IO_3^-, BrO_3^-, B^{3+}	5.0
Mn^{2+}, Ce^{4+}, Fe^{3+}, Mo^{6+}, V^{5+}	2.0
Ti^{4+}, Bi^{3+}, Cr^{6+}, Ba^{2+}, W^{6+}, U^{4+}, Co^{2+}	1.0
Cd^{2+}, Pd^{2+}, Cr^{3+}, La^{3+}, Zn^{2+}, Zr^{4+}, Ni^{2+}	0.5
Bi^{3+}, Pb^{2+}, Pd^{2+}, Sb^{3+}, Th^{4+}, Ni^{2+}, Cu^{2+}	0.2
Se^{4+}, Te^{4+}, Au^{3+}, Ag^+, Co^{2+}, Sn^{4+}	0.1

Calibration curve and sensitivity: The calibration curve show that Beer's law is obeyed in the concentration range of 0.05-50 mg L^{-1} for Hg(II). The linear regression equation obtained was: If = 2185 C (μg L^{-1}) + 26.4, (r = 0.9993). The detect limit, based on three times the relative standard deviation of the blank 4.2 ng L^{-1}.

Sample analysis: The proposed method has been applied to the determination of Hg in Chinese Herbal Medicine samples. The accuracy of the developed method was tested with recovery experiments by spiking standard solutions into the digest solution. The analytical results and recoveries are shown in Table-4. The concentrations of Hg in Chinese Herbal Medicine were found to range from 24 to 83 μg kg^{-1}, respectively, which were in good agreement with the results of reference method (conventional

TABLE-4
DETERMINATION OF MERCURY IN THE CHINESE
HERBAL MEDICINE SAMPLE

Samples	Reference method (μg kg^{-1})	Found (μg kg^{-1})	RSD (%) (n = 5)	Recovery (%) (n = 5) (Add 0.2 μg mercury)
C. militaris	24.5	26.1	2.6	95
C. oxycephala	38.2	36.2	2.8	103
Cordyceps sinensis	46.8	44.8	2.5	104
Radix Notoginseng	82.7	84.2	2.8	94

ICP-MS). To validate the accuracy of the proposed method, tea leaves and human hair certified reference materials (GBW07601 and GBW08505) were analyzed. The results are shown in Table-5 and are in good agreement with the certified value.

TABLE-5
DETERMINATION OF MERCURY IN THE CERTIFIED STANDARD BIOLOGICAL SAMPLES

Samples	Standard value (μg g^{-1})	By this method (μg g^{-1})	RSD (%) (n = 5)
Human hair (GBW07601)	As (0.28), B (1.3), Bi (0.34), Ca (2900), Cd (0.11), Ce (1.2), Co (0.71), Cr (0.37), Cu (10.2), Fe (54), Hg (0.36), Mg (360), Mn (6.3), Mo (0.073), Ni (0.83), Pb (8.8)	0.342	2.2
Tea Leaf (GBW08505)	As (0.191), Ba (15.7), Ca (2840), Cd (0.032), Co (0.2), Cr (0.8), Cu(16.2), Fe (373), Hg (0.014), Mg (2240), Mn (766), Ni (7.61), Pb (1.06), Se (0.041), Zn (38.7),	0.0135	2.8

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