Simultaneous Determination of Trace Cadmium and Arsenic in Biological Samples by Hydride Generation-Double Channel Atomic Fluorescence Spectrometry

Yun-kai Lü,[†,‡] Han-Wen Sun,[‡] Chun-Gang Yuan,[‡] and Xiu-Ping Yan*,[†]

College of Chemical and Environmental Sciences, Hebei University, Baoding 071002, China, and Central Laboratory, Research Center for Analytical Sciences, College of Chemistry, Nankai University, 94 Weijin Road, Tianjin 300071, China

Hydride generation atomic fluorescence spectrometry (HG-AFS) has been used for determination of hydride-forming elements because of its high sensitivity, simplicity, and low costs, but most of such work has been concentrated on single element analysis, and reports dealing with multielement determination by HG-nondispersive (ND)AFS are rare. In this work, a sensitive HG-NDAFS method was developed for simultaneous determination of trace cadmium and arsenic in biological materials. The conditions for the generation of volatile cadmium and arsenic species from the reaction with KBH_4 in aqueous solution were investigated using a double-channel AFS integrated with an intermittent flow reactor. Like thiourea and Co(II), ascorbic acid was found to significantly enhance the generation efficiency of volatile Cd and As species. The interferences of coexisting ions were evaluated. Under optimal conditions, the detection limits for Cd and As were determined to be 10 and 150 ng L^{-1}, respectively. The precision for 11 replicate determinations at the 1 μg L^{-1} Cd level and the 10 μg L^{-1} As level were 3.5 and 2.7% (RSD), respectively. The recoveries of spike analytes in the biological samples studied ranged from 94 to 109%. The proposed method was successfully applied to the simultaneous determination of Cd and As in a variety of biological samples.

Determination of toxic nonessential trace elements cadmium and arsenic in biological materials is an important screening procedure in the studies of environmental pollution and occupational exposure. Several techniques have been employed for such a purpose, including electrothermal atomic absorption spectrometry (ETAAS),[1,2] hydride generation atomic absorption spectrometry (HGAAS),[3,4] inductively coupled plasma-atomic emission spectrometry (ICPAES),[5,6] inductively coupled plasma mass spectrometry (ICPMS),[7] and hydride generation atomic fluorescence spectrometry (HGAFS).[8,9]

Recently, several systems for the generation of volatile cadmium species have been developed. Cacho et al.[10] described the formation of a volatile species of cadmium by treating Cd(II)-diethyldithiocarbomate (Cd-DDTC) complex in an acidic organic medium of *N,N*-dimethylformamide (DMF) with $NaBH_4$. D'Ulivo and Chen[11] analyzed cadmium in aqueous samples on the basis of its derivatization using $NaBH_4$ as the ethylating agent. Sanz-Medel and co-workers reported the determination of Cd by ICPAES[5] and AAS[12,13] on the basis of the generation of volatile cadmium species from a vesicular medium. Guo and co-workers[14] found that the presence of thiourea and Co(II) greatly improved the generation of cadmium volatile species.

AFS has been used for determination of hydride-forming elements because of its high sensitivity, wide linear dynamic range, speed of analysis, ease of use, and low costs.[8,9,14,15] A literature survey revealed that most of such work concentrated on single element analysis, whereas papers dealing with multi-element determination with HG-nondispersive (ND)AFS are rare.

In this work, a sensitive HG-double channel NDAFS method was developed for simultaneous determination of trace Cd and As in biological materials. In addition to thiourea and Co(II), ascorbic acid was found to significantly enhance the vapor generation efficiency of Cd and As. The factors that may affect the generation, delivery, and atomization of volatile cadmium and arsenic species were studied in detail. The developed method was successfully applied to the simultaneous determination of trace cadmium and arsenic in biological samples.

* Corresponding author. Fax: (86) 22-23503034. E-mail: xpyan@nankai.edu.cn.
† Nankai University.
‡ Hebei University.

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Table 1. Working Program for the Intermittent Flow Reactor

step	flow rate, mL min^{-1}		time, s	function
1	sample reductant (5% m/v KBH$_4$)	7.2 7.2	10	insertion of sampling tube into sample solution
2	sample reductant (5% m/v KBH$_4$)	0 0	1	change over of sampling tube into carrier solution
3	carrier solution (0.5 mol L^{-1} HCl) reductant (5% m/v KBH$_4$)	7.2 7.2	16	readout
4	carrier solution (0.5 mol L^{-1} HCl) reductant (5% m/v KBH$_4$)	0 0	1	return to step 1

Table 2. Operating Parameters of the AFS Instrument

parameter	Cd	As
high voltage of PMT, V	300	300
atomizer temperature, °C	300	300
atomizer height, mm	7.0	7.0
peak current of lamp, mA	80	80
flow rate of carrier gas(Ar), mL min^{-1}	400	400
flow rate of shield gas(Ar), mL min^{-1}	800	800
read mode	peak area	peak area

EXPERIMENTAL SECTION

Instrumentation. A model AFS-230 double-channel nondispersive atomic fluorescence spectrometer fitted with a programmable intermittent reactor (Beijing Haiguang Instrument Co., Beijing, China) was employed throughout this work. Details about the construction of the instrument can be found elsewhere.[14,15] The Cd and As hollow cathode lamps (HCLs) with a duty factor of 24 and a period of 200 μs for the modulated pulse that were specially designed for AFS were used as the radiation sources. The distance between the cathode and the front window of the lamp was much shorter than that of typical HCLs used for AAS. This type of HCLs gave improved stability and a longer lifetime, as compared with previous electrodeless discharge lamps. The lamps were operated in a double-modulated mode to discriminate between the fluorescence signals of the two elements so that high lamp currents could be applied for generating a high intensity of radiation. A "solar blind" photomultiplier was used as the detector in the nondispersive detection system.

A schematic diagram of the intermittent reactor is described in ref 14. Briefly, the configuration of the device is similar to a continuous flow reactor, but the operation of the pump can be programmed in several steps for each measurement. The duration and rotation rate of the pump can be programmed for each step. The working program of the intermittent reactor is described in Table 1.

An electrically heated quartz furnace was used as the atomizer into which the volatile species and the hydrogen evolved from the reactor were swept by an argon flow. The gas mixture was self-ignited at the outlet of the furnace, and a hydrogen−argon−air-entrained flame was maintained without the addition of any auxiliary hydrogen. The operating parameters used for the AFS instrument are given in Table 2.

Reagents. All reagents used were of highest available purity, and of at least analytical grade. Doubly deionized water (DDW) was used throughout. The arsenic(III) standard stock solution,

1.000 g L^{-1}, was prepared by dissolving 1.320 g of As$_2$O$_3$ (Merck) in 25 mL of 20% m/v KOH (The Second Chemical Co., Beijing), neutralizing with 20% v/v H$_2$SO$_4$ (The Second Chemical Co., Beijing), and diluting to 1000 mL with 1% v/v H$_2$SO$_4$. The cadmium stock solution, 1.000 g L^{-1}, was prepared by dissolving 1.1423 g of CdO (Merck) in 20 mL of 5 mol L^{-1} HCl, and diluting to 1000 mL with DDW. Working standard solutions of Cd and As(III) were prepared by stepwise dilution of the stock solutions just before use.

The KBH$_4$ solutions were prepared by dissolving the reagent (Tianjin Institute of Chemical Reagents, Tianjin) in 0.5% m/v potassium hydroxide solution. Ascorbic acid and thiourea solutions were prepared by dissolving ascorbic acid (The Second Chemical Co., Beijing) and thiourea (The Second Chemical Co., Beijing) in DDW. The solution of 100 mg L^{-1} Co was prepared by dissolving a suitable amount of CoCl$_2$·6H$_2$O (The Second Chemical Co., Beijing) in DDW.

Sample Pretreatment. Approximately 0.2000−1.0000 g of the biological sample, depending on the concentrations of the analytes in the sample, was accurately weighed and placed in a PTFE vessel to which 13 mL of concd HNO$_3$ and 2 mL of concd HClO$_4$ were added. Acid digestion of the biological sample was carried out in sealed PTFE vessels using a model Qwave-3000 microwave digestion system (Questron, U.S.A.). All instrumental parameters for the digestion were chosen according to the recommendation of the manufacturer. The clear digest was transferred to a PTFE crucible and gently heated to near dryness. After cooling, 5 mL of 1 mol L^{-1} HCl was added, followed by gentle heating until a clear solution was obtained. The solution was transferred to a 25-mL calibrated flask and neutralized with potassium hydroxide solution. Concentrated HCl, thiourea, ascorbic acid, and Co(II) solutions were added to make their final concentrations of 0.3 mol L^{-1}, 1% m/v, 0.5% m/v, and 1 mg L^{-1}, respectively.

RESULTS AND DISCUSSION

Optimization of AFS Instrument Parameters. Studies on the influence of lamp current show that an increase in the lamp current significantly improves the signal intensities of Cd and As. However, higher lamp currents would produce higher signal noise, and reduce the lifetime of the lamps. A current of 80 mA was, therefore, used as a compromise.

Results for the influence of carrier gas flow rate on the signal intensities of Cd and As are shown in Figure 1. There was a maximum of the signal intensities as the flow rate of carrier gas increased (at 400 mL min^{-1} for Cd, 200−400 mL min^{-1} for As). At lower flow rates, the signal intensities increased with the flow

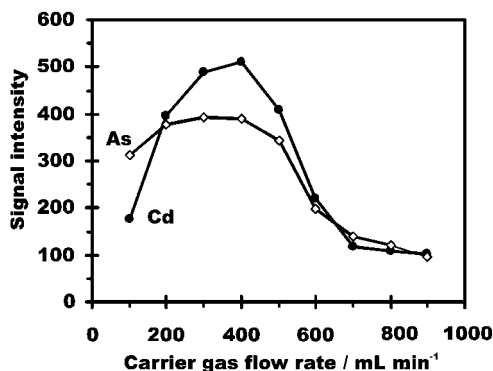**Figure 1.** Effect of the carrier gas (Ar) flow rate on the atomic fluorescence signals of Cd (1 μg L^{-1}) and As (10 μg L^{-1}) in the presence of 1% m/v thiourea, 0.5% m/v ascorbic acid, and 1 mg L^{-1} Co(II). All other conditions as in Tables 1 and 2.
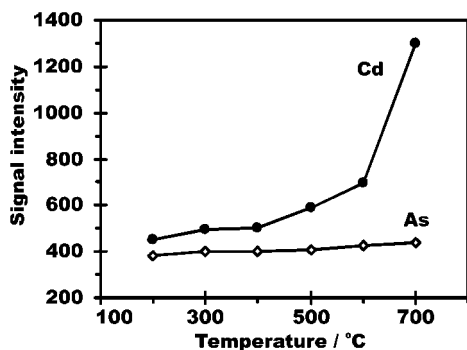

Figure 2. Effect of the atomizer temperature on the atomic fluorescence signals of Cd (1 μg L^{-1}) and As (10 μg L^{-1}) in the presence of 1% m/v thiourea, 0.5% m/v ascorbic acid, and 1 mg L^{-1} Co(II). All other conditions as in Figure 1, Tables 1 and 2.

Figure 3. Effect of KBH$_4$ concentration on the atomic fluorescence signals of Cd (1 μg L^{-1}) and As (10 μg L^{-1}) in the presence of 1% m/v thiourea, 0.5% m/v ascorbic acid, and 1 mg L^{-1} Co(II). All other conditions as in Figure 1, Tables 1 and 2.

Figure 4. Effect of HCl concentration on the atomic fluorescence signals of Cd (1 μg L^{-1}) and As (10 μg L^{-1}) in the presence of 1% m/v thiourea, 0.5% m/v ascorbic acid, and 1 mg L^{-1} Co(II). All other conditions as in Figure 1, Tables 1 and 2.

rate of the carrier gas. However, at higher flow rates, the dilution of the evolved volatile species and short residue of the analyte species in the atomizer would be dominant, leading to the decrease of the signal densities. Therefore, an argon flow rate of 400 mL min^{-1} was employed.

In this work, an argon shield gas flow was employed to prevent extraneous air from entering the flame, avoiding any reactions of gaseous atoms of Cd and As with air. Studies on the effect of the flow rate of the shield gas revealed that a shield gas flow rate of 800 mL min^{-1} was optimal for giving higher signal intensities and a better signal-to-noise ratio.

Studies on the effect of the atomizer temperature showed that an increase in the temperature of the quartz atomizer significantly enhanced the signal intensity of Cd but gave little change of the As signal intensity (see Figure 2); however, the noise of the signal also increased with an increase in the temperature of the quartz atomizer. This is probably due to the increase of furnace radiation at higher temperatures. Consequently, the operating temperature of the electrically heated quartz furnace should be as low as possible.[15] In this study, an atomizer temperature of 300 °C was chosen, which gave an optimal signal-to-noise ratio.

Observation height is the distance from the quartz furnace outlet to the point where the atomic fluorescence signal is measured. A close examination of the effect of the observation height on the determination of Cd and As is essential to achieve better analytical performance of the present system. For cadmium,
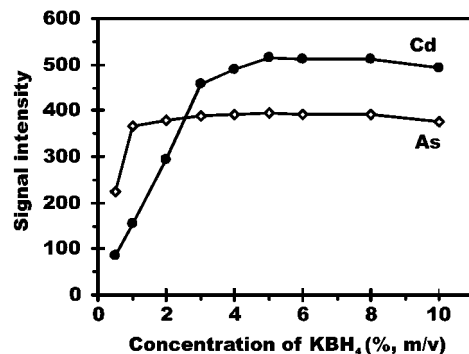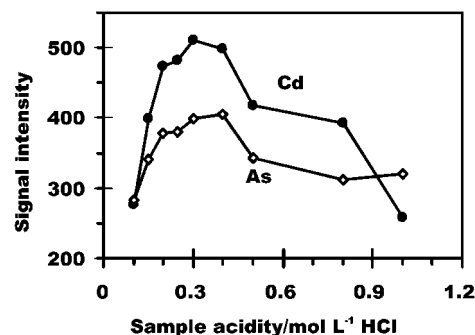
the signal intensity increased with an increase in the observation height from 5 to 7 mm, then leveled off between 7 and 14 mm; however, the fluorescence signal intensity of arsenic became remarkably reduced as the observation height increased. For further experiments, an observation height of 7 mm was used.

Optimization of Chemical Variables. In the present method, KBH$_4$ is used not only as the reductant, but also as the hydrogen supply to sustain the argon−hydrogen flame. The concentration of KBH$_4$ had a large impact on the hydride generation process and the argon−hydrogen flame. Figure 3 illustrates the effect of the concentration of KBH$_4$ on the signal intensities of Cd and As. Obviously, the optimal concentration of KBH$_4$ ranged from 4 to 10% m/v for Cd, and from 1.5 to 10% m/v for As. A 5% m/v of KBH$_4$ solution was employed for simultaneous determination of Cd and As.

The influence of the HCl concentration in the sample on the determination of Cd and As is shown in Figure 4. The optimal sample acidity was found to be in the range of 0.2−0.4 mol L^{-1} HCl for determination of Cd and As. Studies of the influence of the HCl concentration in the carrier solution on the fluorescence signals of Cd and As show that a carrier solution of 0.5 mol L^{-1} HCl gave relatively high signals of Cd and As. Therefore, a HCl concentration of 0.3 mol L^{-1} was chosen as the optimal sample acidity, and a 0.5 mol L^{-1} HCl solution was employed as the carrier.

Sensitivity Enhancement. Guo et al.[14] reported that thiourea gave the most promising result for the generation of volatile Cd
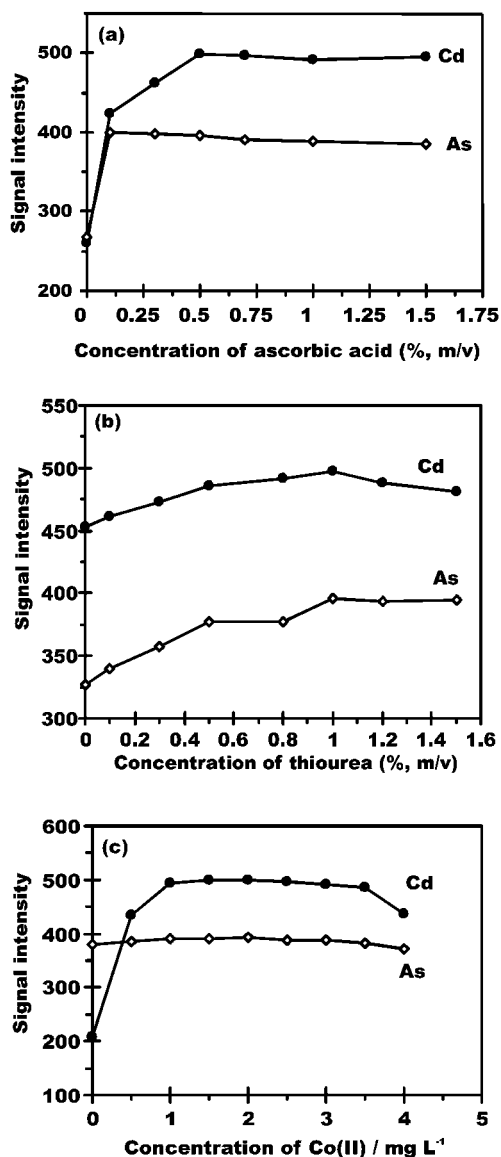
Figure 5. Effect of the concentrations of (a) ascorbic acid, (b) thiourea, and (c) Co(II) on the atomic fluorescence signals of Cd (1 μg L^{-1}) and As (10 μg L^{-1}). All other conditions as in Figure 1, Tables 1 and 2.

species among the organic reagents tested, mainly nitrogen- and sulfur-containing reagents and that the presence of thiourea together with Co(II) further enhanced the generation efficiency of the volatile species.

In this work, the influences of several organic reagents together with Co(II) on the atomic fluorescence intensities for Cd and As were investigated. It was found that the presence of ascorbic acid greatly enhanced the signal intensities of cadmium and arsenic (See Figure 5a). Similarly to previous results,[14] the enhancement of Cd sensitivity was observed in the presence of thiourea and Co(II) (Figures 5b, c). Thiourea also enhanced the signal intensity of As (Figures 5c). The signal intensity of As, however, was not affected by Co(II) concentrations. A combination of 1% m/v of thiourea, 0.5% m/v ascorbic acid, and 1 mg L^{-1} Co(II) was found to be optimal, giving the maximal signal intensities of Cd and As.

Table 3. Effects of Coexisting Ions on Determination of Cd (1 μg L^{-1}) and As (10 μg L^{-1})

coexisting ion	concn (mg L^{-1})	[M]/[As]	[M]/[Cd]	signal change (%) As	signal change (%) Cd
Fe(III)	100	10 000	100 000	−1	+112
	10	1000	10 000	+1	+6
Ni(II)	10	1000	10 000	−7	−48
	1	100	1000	−3	+5
Pb(II)	10	1000	10 000	0	−67
	0.1	10	100	0	−9
Te(IV)	10	1000	10 000	0	−11
	1	100	1000	0	+5
Zn(II)	10	1000	10 000	−4	+6
	1	100	1000	+4	+8
Bi(III)	1	100	1000	+7	−56
	0.1	10	100	+4	−7
Hg(II)	1	100	1000	−1	+7
	0.1	10	100	0	+1
Sn(II)	1	100	1000	0	+13
	0.1	10	100	0	+7
Se(IV)	1	100	1000	+1	−10
	0.1	10	100	−1	+2
Cu(II)	0.5	50	500	+98	+78
	0.1	10	100	+2	+5

Evaluation of Interferences. The effects of coexisting ions on the generation of the volatile species are shown in Table 3. For the determination of As, no significant interferences from 10 mg L^{-1} of Ni(II), Pb(II), Te(IV), and Zn(II); 100 mg L^{-1} of Fe(III); 0.1 mg L^{-1} of Cu(II); and 1 mg L^{-1} of Bi(III), Hg(II), Se(IV), and Sn(II) were observed. The presence of 10 mg L^{-1} of Fe(III); 0.1 mg L^{-1} of Pb(II); 10 mg L^{-1} of Te(IV) and Zn(II); 0.1 mg L^{-1} of Cu(II) and Bi(III); and 1 mg L^{-1} of Hg(II), Ni(II), Se(IV) and Sn(II) had no significant influence on the determination of Cd. As can be seen later, the above tolerance levels of coexisting ions permit the use of simple aqueous standard calibration for the determination of Cd and As in the biological samples of interest.

Analytical Performance. The analytical characteristic data of the proposed method are given in Table 4. It should be noted that the calibration functions for simultaneous determination of cadmium and arsenic were in good agreement with those for individual determination of the two elements, indicating that the proposed method for simultaneous determination of cadmium and arsenic is reliable. The calibration graphs were found to be linear up to at least 5 and 100 μg L^{-1} for Cd and As, respectively, which corresponds to a linear range of 3 orders of magnitude.

To demonstrate the accuracy of the present method, we applied it to the simultaneous determination of trace Cd and As in the following certified biological reference materials (National Center for Standard Materials, Beijing): GBW08551 (pork liver), GBW08501 (peach leaf), GBW07601 (human hair), and GBW08571 (mussel). The results for the simultaneous determination of Cd and As in these certified reference materials (CRMs) using simple aqueous standard calibration technique are shown in Table 5. It can be seen that the concentrations of Cd and As in the CRMs obtained by the present method were in good agreement with the certified values.

The developed method was also successfully applied to the simultaneous determination of Cd and As in a number of rat kidney and cock liver samples. The analytical results and recoveries are shown in Table 6. The recoveries for Cd and As

Table 4. Detection Limits, Precision, and Calibration Functions

measurement method	element	calibration function (I_f, signal intensity; C, μg L^{-1})	correlation coeff	RSD %	DL μg L^{-1}
double channel	Cd	$I_f = 23.89 + 476.7\ C_{Cd}$	0.9995	3.5	0.010
	As	$I_f = 39.85 + 35.12\ C_{As}$	0.9991	2.7	0.150
single channel	Cd	$I_f = 32.59 + 475.6\ C_{Cd}$	0.9993	4.6	0.011
	As	$I_f = 14.73 + 36.43\ C_{As}$	0.9996	1.5	0.130

Table 5. Analytical Results[a] for Determnation of Cadmium and Arsenic in the Certified Reference Materials (CRMs)

CRMs	cert concn (μg g^{-1}) Cd	As	found concn (μg g^{-1}) Cd	As
pork liver (GBW 08551)	0.067 ± 0.002	0.044 ± 0.004	0.066 ± 0.003	0.043 ± 0.012
peach leaf (GBW 08501)	0.018 ± 0.004	0.34 ± 0.03	0.018 ± 0.006	0.32 ± 0.07
human hair (GBW 07601)	0.11 ± 0.02	0.28 ± 0.04	0.105 ± 0.012	0.33 ± 0.05
mussel (GBW 08571)	6.1 ± 0.6	4.5 ± 0.3	$5.93 + 0.06$	4.47 ± 0.12

[a] Mean $\pm$ s, $n = 6$.

Table 6. Analytical Results[a] for Simultaneous Determination of Trace Cadmium and Arsenic in Biological Samples

samples	concn, μg g^{-1} Cd	As	recovery, %[b] Cd	As
rat kidney 1	0.113 ± 0.008	0.191 ± 0.007	109	95.5
rat kidney 2	0.021 ± 0.001	0.396 ± 0.011	106	97.0
rat kidney 3	0.045 ± 0.001	0.628 ± 0.006	94.5	105
rat kidney 4	0.023 ± 0.001	0.487 ± 0.007	93.5	103
rat kidney 5	0.101 ± 0.003	0.327 ± 0.004	100	97.7
rat kidney 6	0.240 ± 0.014	1.29 ± 0.01	104	95.9
rat kidney 7	0.088 ± 0.001	1.31 ± 0.01	97.1	106
cock liver 1	0.025 ± 0.002	0.880 ± 0.003	96.0	94.0
cock liver 2	0.027 ± 0.001	0.980 ± 0.005	103	107
cock liver 3	0.018 ± 0.002	1.15 ± 0.01	97.0	103

[a] Mean $\pm$ s, $n = 6$. [b] Values obtained by spiking the standard analyte solutions into the digests so that the spiked concentrations of the analytes were similar to those in the original digests.

from these rat kidney and cock liver samples ranged from 94 to 109%, permitting the use of simple aqueous standard solution for calibration. The concentrations of Cd and As in the rat kidney samples were found to be in the range of 0.02−0.24 and 0.2−1.3 μg g^{-1}, respectively, and the concentrations of As and Cd in the cock liver samples ranged from 0.018 to 0.027 and from 0.9 to 1.1 μg g^{-1}, respectively.

CONCLUSIONS

This work demonstrated the feasibility for simultaneous determination of trace cadmium and arsenic in biological samples by HG-double-channel NDAFS. The presence of thiourea, ascorbic acid and Co(II) significantly enhanced the sensitivities of Cd and As. The developed method is simple and sensitive and is promising for routine analysis of trace Cd and As in biological materials.