Asian Journal of Chemistry Vol. 19, No. 4 (2007), 3831-3838

Determination of Mercury and Selenium in Tobacco by Inductively Coupled Plasma Mass Spectrometry after Microwave Digestion

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A new method for the determination of mercury and selenium in tobacco by inductively coupled plasma mass spectrometry (ICP-MS) using isotope dilution calibration was studied. The samples were digested with *aqua regia* in a microwave oven. The isotope ratios used for quantification were $^{201}Hg/^{202}Hg$ and $^{77}Se/^{82}Se$. A $NaBH_4$ solution was used as reducing agent. Five certified tobacco samples were analyzed and the obtained concentrations were in good agreement with the certified values. The detection limits in the sample were 0.5 and 2 ng g^{-1}, for mercury and selenium respectively. The method is precise, accurate and rapid.

Key Words: Mercury, Selenium, ICP-MS, Microwave digestion, Isotopic dilution calibration, Tobacco.

INTRODUCTION

The quality of the analytical results mainly depends on the sample pre-treatment stages and on the detection system, particularly for determination of elements with low concentration in the samples, such as mercury and selenium. At present, there are several analytical methods for the determination of mercury and selenium including spectrophotometry, atomic absorption spectrometry, fluorescence and mass spectrometry (MS)[1-18]. Among the analytical techniques employed, inductively coupled plasma mass spectrometry (ICP-MS) is especially important due to its several advantages, such as its multi-element capacity, high sensitivity and ability to measure isotopic ratios, which allows isotope dilution calibration (ID)[11-18]. In this work, a method for the determination of mercury and selenium in tobacco, after microwave-assisted acid digestion with *aqua regia* of the samples followed by chemical vapour generation with retention of the analyte vapour in an Ir-treated graphite tube of an electrothermal vapourizer using isotope dilution calibration (ID-CVG-ETVICP-MS) is optimized and described.

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EXPERIMENTAL

All measurements were carried out with an inductively coupled plasma mass spectrometer ELAN 6000 (Perkin Elmer SCIEX, Thornhill, ON, Canada). For the ETV system, a hydride generator MHS-15 (Perkin Elmer, CO, USA) was coupled to a Perkin-Elmer HGA 600 MS electrothermal vapourizer and a Perkin Elmer AS-60 autosampler and manually operated, as described previously[18]. A 3 % (m/v) sodium borohydride solution stabilized with 1 % (m/v) sodium hydroxide was used as reducing agent in the MHS-15. The reducing agent was injected during 5 s using an argon pressure of 250 kPa. The generated vapours were transported to the ETV by an Ar flow through a 10 cm glass tube (0.1 cm i.d.) connected to a polytetra-fluorethylene (PTFE) tube (60 cm long, 0.5 cm i.d.). Pyrolytic coated graphite tubes (Perkin-Elmer, Part No. B050 8371) were used. Argon of 99.996% purity (Kunming Yangqi, Kunming, P.R.China) was used. An internal Ar flow rate of 0.1 L min^{-1} in the vapourizer, optimized previously[18], summed to the "nebulizer" gas flow rate of 1.06 L min^{-1} during vapourization, resulting in a total flow rate of 1.16 L min^{-1}, transported the aerosol to the plasma. The instrumental conditions are shown in Table-1 and the ETV temperature program, which was also optimized previously[18] is shown in Table-2. The samples for the microwave digestion were weighed using a M2P microbalance (Sartorius, Göttingen, Germany). A Model WL 5001 microwave system (1000 W, Fei Yue Analytical Instrument Factory, Shanghai, China) was employed for the digestions of the samples.

TABLE-1
ETV-ICP-MS OPERATIONAL PARAMETERS

Parameters	
RF power (W)	1000
Gas flow rate	(L min^{-1})
Principal	15
Intermediate	1.2
Carrier	1.06
Sampler and skimmer cones	Pt
Dwell time (ms)	25
Sweeps *per* reading	1
Reading *per* replicate	150
Resolution at 10% of the peak height	0.7 u.m.a
Auto lens	On
Signal measurement	Peak area

TABLE-2
ETV TEMPERATURE PROGRAM

Step	Temperature (°C)	Ramp (s)	Hold (s)	Gas flow rate (mL min^{-1})
Cooling	20	1	5	300
Pre-heating[a]	150	5	0	0
Pre-heating[b]	150	1	30	0
Cooling	20	1	8	0
Vapourization[c]	2000	1	20	100
Cleaning	2200	5	5	300
Cooling	20	1	5	300

[a]glass tube introduction into the graphite tube; [b]analyte vapour collections on the graphite tube; [c]vapourization and reading.

All the reagents were of analytical grade. The water used was deionized in a Milli-Q system (Millipore, Bedford, MA, USA). Nitric acid (High purity grade, Tianjing, P.R. China) and hydrochloric acid (High purity grade, Tianjing, P.R.China) were used. The reducing agent was prepared by dissolving $NaBH_4$ (Fluka, Buchs, Switzerland) in sodium hydroxide (High purity grade, Tianjing, P.R. China) stored in a polyethylene flask and kept under refrigeration for no longer than 2 d. A stock solution of $IrCl_3$ (Fluka, Buchs, Switzerland), 1000 mg L^{-1} Ir, was used for the treatment of the tube with the permanent modifier as described previously[20]. The enriched isotope materials were from the Cambridge Isotope Laboratories Inc. (Andover, MA, USA). The abundances of the enriched isotopes were: 96.35 % of ^{201}Hg and 93.48 % of ^{77}Se. The isotope compositions of the enriched materials were measured and the found values agreed with the informed values. Stock solutions of 30 mg L^{-1} for mercury and 200 mg L^{-1} for selenium were prepared by dissolution of an accurately weighed amount of the solid material (HgO and elemental Se) in nitric acid and diluted in 5 % v/v HNO_3. The following isotope ratios were used in the calculation of the concentrations: $^{201}Hg/^{202}Hg$ and $^{77}Se/^{82}Se$. The ratio of the signal intensities of the isotopes in the samples without the addition of the enriched isotope was compared to the natural ratio in order to check for spectral interference and mass discrimination. The correction factor for the mass discrimination was calculated by comparing the natural isotope ratio with the measured ratio for the sample without the spike. The software automatically uses the correction factor to correct the measured altered ratio.

Sample preparation: The samples analyzed are Certified Chinese Standard Material. The standard values were determined by atomic absorption spectrum (AAS), atomic fluorescence spectrum (AFS), neutron activation analysis (NAA), inductively coupled plasma-mass spectrometry (ICP-MS) and ion chromatography (IC) method.

An aliquot of *ca.* 250 mg of the sample was weighed directly in the PTFE flask of the microwave oven. Then, the solutions of the materials enriched with the isotopes ^{201}Hg and ^{77}Se were added to the flask in an adequate amount in order to obtain an altered ratio close to 1, to minimize the measurement errors. Due to the low concentrations of selenium and mercury in the samples, masses of the enriched materials at ng level were added. The masses were calculated using the ID equation for an altered ratio of 1 and converting the masses to volumes of the enriched materials solutions. The sample aliquot without spike was digested with 3.5 mL *aqua regia* plus 1.0 mL of deionized water in the microwave bombs. The sample aliquot with the spikes was also digested with 3.5 mL *aqua regia* plus a certain volume of deionized water, which was the complement to 1 mL of the volumes of the spiking solutions. In this way, all sample aliquots, without and with spikes, were digested in the same volume of liquid. 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. After the digestion was finished, all solutions were colourless, indicating an effective digestion. Following that, 1 mL of the digested sample was mixed with 1 mL of concentrated hydrochloric acid and heated to 90°C for 0.5 h, in order to guarantee the lower oxidation state for selenium, favouring its hydride generation[7]. After cooling the solution, the final volume of 10 mL was made up with deionized water, which was not strictly necessary, as isotopic dilution calibration was used, assuming that equilibration of the added isotope with the isotope in the sample have occurred during digestion. However, this final dilution to the same final volume for each sample aliquot, allowed a better control of the counting signals.

Analytical procedure: For the determination of mercury and selenium in samples, a 1 mL aliquot of the final sample solution (without or with spike), was transferred to the reaction flask of the hydride generator and the temperature program of the ETV was started. The glass tube of the hydride generator was manually introduced into the graphite tube. During the pre-heating step, the reducing agent was added to the reaction flask for 5 s and the generated vapours were transferred to the graphite tube by using argon for 30 s in the MHS-15. Before the vapourization step, the glass tube was removed and the graphite tip of the ETV arm closed the dosing hole of the graphite tube. Reading was carried out during the vapourization stage, in which the vapour is transported from the ETV to the plasma by a total argon flow rate of 1.16 L min^{-1}.

RESULTS AND DISCUSSION

The chemical vapour generation conditions and the electrothermal vapourizer temperature program were optimized previously for the determination of mercury and selenium of 3.5 mL of *aqua regia* was used. Lower

volumes do not lead to accurate results neither for mercury nor for selenium and other analytes in sediments, as slurries in an *aqua regia* plus HF medium, using external[18] or isotopic dilution calibration[19]. The isotopes ^{202}Hg (natural abundance of 29.8%) and ^{82}Se (natural abundance of 8.73%) were chosen as reference isotopes, taking into account the absence of spectral interference and their natural abundances. In this way, the concentrations were obtained by measuring simultaneously the following altered isotope ratios: ^{201}Hg/^{202}Hg and ^{77}Se/^{82}Se.

Effect of the hydrochloric acid treatment and of the *aqua regia* concentration: In order to generate the vapour, the sample should be completely digested, meaning that all organic compounds containing the analytes should be destroyed. The microwave-assisted digestion of the tobacco sample with *aqua regia* efficiently digested the tobacco samples, as clear solutions were obtained. As already mentioned, the isotope dilution is an ideal internal standardization, as the internal standard, in this case, is one isotope of the same element. In principle, if a reasonable fraction of the selenium, after the digestion, was present in its lower oxidation state, which is able to generate the hydride, the isotope calibration should compensate for the fraction of the selenium, which is on its higher oxidation state and does not generate the hydride[6]. In this way, a procedure was attempted, in which the sample solution, after digestion, was not heated with HCl, but was only taken to a final volume of 10 mL with 1 mol L^{-1} HCl. The obtained concentrations for selenium were significantly higher (from 19 to 50 %) than the certified values, indicating that the heating with HCl, which converts Se(VI) to Se(IV) is required even when isotope dilution calibration is employed, this indicates that the Se(IV) fractions in the spike and in the sample are different. However, for mercury, the results obtained, with and without heating with hydrochloric acid, are in agreement with the certified values according to the t-test for a 95 % confidence level. Thus, 1 mL of the sample solution was mixed with 1 mL of HCl and heated to 90ºC for 0.5 h after the sample digestion and before the vapour generation.

The *aqua regia* volume added to the sample mass of around 250 mg for the microwave-assisted digestion was investigated, using the GB-2102 sample. A volume of 3.5 mL of *aqua regia* was used. Lower volumes do not lead to accurate results neither for mercury nor for selenium, being the obtained concentrations much lower than the certified ones, indicating that part of the analytes are not in the appropriate forms to generate the cold vapour of mercury or the hydride of selenium. Most probably, the lower volume is not enough to mineralize completely the sample.

Figures of merit and analytical application: The limit of detection (LOD) is defined as the minimum concentration or weight of analyte that can be detected at a known confidence level. The limits of detection, in the

sample, calculated as a function of the enrichment of the isotopic spike and the linear calibration detection limits for each isotope[21] are 0.5 and 2 ng g^{-1} for mercury and selenium, respectively, indicating that the proposed procedure is able to detect very low concentrations of the analytes in tobacco samples. Santos *et al.*[5] have investigated the chemical vapour generation for mercury and selenium in biological samples as slurries in different media, prior to detection by in ICP-OES, using external calibration. For 20 mg of solid sample in 15 mL of slurry, the obtained detection limits (3 s, n = 10) for the proposed procedure were 80 and 100 ng g^{-1} for mercury and selenium, respectively, much higher (*ca.* 110 times for mercury and 33 times for selenium) than those obtained in this work, as expected due to the higher sensitivity of the ETV-ICP-MS technique. Vieira *et al.*[18] have proposed a method for the determination of arsenic, mercury, selenium and tin in sediment by slurry sampling CVG-ETV-ICP-MS, also with trapping of the vapour on a treated graphite tube, but using slurry sampling and external calibration against aqueous standards, obtaining about the same LOD (3 s, n = 10) for mercury, 0.80 ng g^{-1}, as in this work and 300 times better LOD for selenium, 0.01 ng g^{-1}.

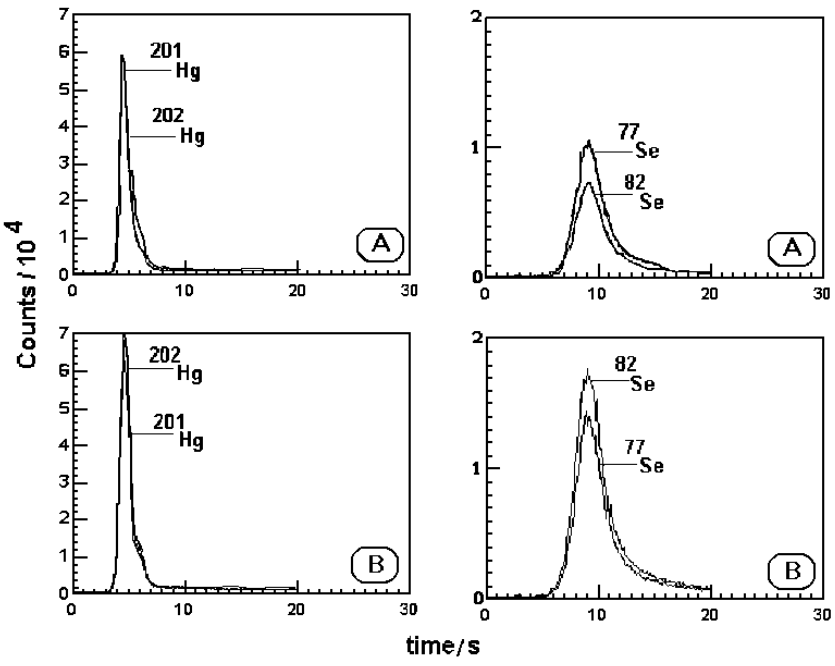

Fig. 1. Transient signals for Hg and Se in reference material GB-2102 (a) with out and (b) with the addition of the enriched isotope

In Fig. 1 some typical transient signals for mercury and selenium in reference material GB-2102, before and after addition the enriched isotopes, are shown. As shown in the Figure the condition of an altered isotopic ratio close to 1 was obtained for mercury and selenium.

The accuracies of the procedures were estimated by the analysis of fix certified reference materials. As shown Table-3, the found concentrations are in agreement with the certified values, according to the t-test for a confidence level of 95 %. The relative standard deviations (RSD) were the range from 2.8 to 3.4 % for selenium and from 2.8 to 4.1 %, for mercury, indicating an adequate precision. Probably, an efficient digestion of the sample, with complete destruction of the analytes organic compounds by microwave digestion with *aqua regia* was attained, providing the equilibration of the added isotopes with the isotopes in the sample, which is a basic requirement for the ID calibration. After equilibration, there is no need to use an exact volume of the sample solution.

TABLE-3
DETERMINED CONCENTRATIONS IN ng g^{-1} FOR MERCURY AND SELENIUM IN CERTIFIED TOBACCO SAMPLES BY CVG-ETV-ICP-MS USING ISOTOPE DILUTION CALIBRATION AFTER MICROWAVE DIGESTION WITH *aqua regia* AND TREATMENT WITH HCl TO REDUCE THE OXIDATION NUMBER OF SELENIUM (n = 5)

	Certified (ng/g)		Found (ng/g)		RSD (%) (n = 5)	
	Hg	Se	Hg	Se	Hg	Se
GB-2102	22.8	68.5	20.5	69.8	3.4	3.0
GB-2108	18.2	76.8	16.8	78.4	3.6	2.8
GB-2124	56.4	87.5	58.2	85.4	2.8	3.2
GB-2180	68.9	84.8	65.8	86.9	4.1	2.8
GB-2194	35.6	61.2	38.2	59.4	3.9	3.4

Conclusions

It was demonstrated that mercury and selenium can be determined in tobacco samples after microwave-assisted acid digestion with *aqua regia* followed by their determination by CVG-ETVICP-MS using vapour trapping in the graphite tube and isotopic dilution calibration. The treated tube with Ir was efficient for the trapping of the mercury cold vapour and the selenium hydride. The proposed procedure using ID compensate eventual analyte loss during the steps of sample preparation, including digestion and reduction of the oxidation state for selenium. The reduction of the oxidation state for selenium with hydrochloric acid prior to its hydride generation is necessary, even using isotope dilution calibration. For mercury, the reduction step with hydrochloric acid can be omitted.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (20471051) and the Key Natural Science Foundation of Yunnan Province (2005K021).

REFERENCES

1. H. Matusiewicz, R.E. Sturgeon and S.S. Berman, *J. Anal. At. Spectrom.*, **4**, 323 (1989).
2. E.J.D. Santos, A.B. Herrmann, M.A. Vieira, V.L.A. Frescura and A.J. Curtius, *Spectrochim. Acta,* **60B**, 659 (2005).
3. M.A. Vieira, A.S. Ribeiro and A.J. Curtius, *Anal. Bioanal. Chem.*, **380**, 570 (2004).
4. M.J. Cal-Prieto, M. Felipe-Sotelo, A. Carlosena, J.M. Andrade, P. Lopez-Mahia, S. Muniategui and D. Prada, *Talanta*, **56**, 1 (2002).
5. E.J.D. Santos, A.B. Herrmann, V.L.A. Frescura and A.J. Curtius, *Anal. Chim. Acta*, **548**, 166 (2005).
6. B. Welz and M. Sperling, Atomic Absorption Spectrometry, Wiley-VCH: Weinheim, edn. 3 (1999).
7. J. Petersson and A. Olin, *Talanta*, **38**, 413 (1991).
8. Q. Jin, F. Liang, H. Zhang, L. Zhao, Y. Huan and D. Song, *Trends Anal. Chem.*, **16**, 479 (1999).
9. J.A. Brisbin and J.A. Caruso, *Analyst*, **127**, 921 (2002).
10. R.S. Houk, *Anal. Chem.*, **58**, 97A (1986).
11. L.F. Dias, T.D. Saint'Pierre, S.M. Maia, M.A.M. da Silva, V.L.A. Frescura, B. Welz and A.J. Curtius, *Spectrochim. Acta*, **57B**, 2003 (2002).
12. D. Pozebon, V.L. Dressler and A.J. Curtius, *J. Anal. At. Spectrom.*, **13**, 1101 (1998).
13. T.D. Saint'Pierre, T.A. Maranhão, V.L.A. Frescura and A.J. Curtius, *Spectrochim. Acta*, **60B**, 605 (2005).
14. F. Vanhaecke, S. Boonen, L. Moens and R. Dams, *J. Anal. At. Spectrom.*, **12**, 125 (1997).
15. M. Tibi and K.G. Heumann, *J. Anal. At. Spectrom.*, **18**, 1076 (2003).
16. C. Chang and S. Jiang, *J. Anal. At. Spectrom.*, **12**, 75 (1997).
17. S.M. Maia, D. Pozebon and A.J. Curtius, *J. Anal. At. Spectrom.*, **18**, 330 (2003).
18. M.A. Vieira, T.D. Saint'Pierre, B. Welz and A.J. Curtius, *J. Anal. At. Spectrom.*, **19**, 297 (2004).
19. M.A. Vieira, A.S. Ribeiro, L.F. Dias and A.J. Curtius, *Spectrochim. Acta*, **60B**, 643 (2005).
20. M.A. Vieira, A.J. Curtius and B. Welz, *Spectrochim. Acta*, **57B**, 2057 (2002).
21. L.L. Yu, J.D. Fassett and W.F. Guthrie, *Anal. Chem.*, **74**, 3887 (2002).

(*Received*: 4 July 2006; *Accepted*: 7 March 2007) AJC-5488