Determination of Beryllium and Selenium in Human Urine and of Selenium in Human Serum by Graphite-Furnace Atomic Absorption Spectrophotometry

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For human urine beryllium (Be), each sample (500 μ l) was diluted (1+1) with Nash reagent (containing 0.2% (v/v) acetylacetone and 2.0 M ammonium acetate buffer at pH 6.0) and then a 20- μ l volume of Triton X-100 (0.4%, v/v) aqueous solution was added. An aliquot (10 μ l) of the diluted urine mixture was introduced into a graphite cuvette and was atomized according to a temperature program. The method detection limit (MDL, 3σ) for Be was 0.37 μ g/l in the undiluted urine sample and the calibration graph was linear up to 65.0 μ g/l. Calibration graphs were prepared by the standard addition method. Accuracies of 98.6–102% were obtained when testing standard reference material (SRM 2670) freeze dried human urine samples. Precision (relative standard deviation, RSD) for urine Be was $\leq 2.3\%$ (within-run, $n = 5$) and was $\leq 3.0\%$ (between-run, $n = 3$). For human urine and serum selenium (Se), samples (100 μ l) were diluted with HNO₃ (0.2%, v/v) to make a (1+1) dilution for urine analysis or a (1+4) dilution for serum analysis. An additional aliquot (10 μ l) of Triton X-100 (0.1%, v/v) was added to each 200 μ l of (1+1) diluted urine (or 20 μ l of the Triton X-100 was added to each 500 μ l of (1+4) diluted serum) sample. After the diluted sample mixture (10 μ l) was introduced into a graphite cuvette, the corresponding chemical modifier (10 μ l, containing Ni²⁺ + Pd + NH₄NO₃ in HNO₃ (0.2%, v/v)) was added to it and the mixture was atomized. The MDL (3σ) for Se in urine and in serum was 4.4 and 21.4 μ g/l in undiluted sample, respectively, and the calibration graphs were linear up to 150 and 400 μ g/l. Accuracies of urine Se were 98.9–99.4% by testing SRM 2670 (NIST) urine standards with RSD (between-run, $n = 3$) within 2.9%; and that of serum Se was 97.2% when testing a certified second-generation human serum (No. 29, #664) with RSD (between-run, $n = 3$) of 1.4%. The proposed method can be applied easily, directly, and accurately to the measurement of Be and Se in real samples (including six urine Se and four serum Se from patients of Blackfoot Disease in Taiwan).

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Beryllium (Be) and its metal alloys (such as Cu, Al, Mg, Ni) have been widely used for electrical equipment, electronic instrumentation, structural components for aircraft, missiles, satellites, and nuclear reactors.^{1–3} Be and its compounds are very toxic and may cause lung disease (berylliosis),^{1–3} inhibit alkaline phosphatase, hepatic adenosine triphosphatase,⁴ and DNA synthesis.⁵ The levels of Be in human urine for normal healthy persons are very low (0.04–0.76 μ g/l),⁶ but a concentration greater than 20 μ g/l is considered to be toxic.⁷

Graphite-furnace atomic absorption spectrometry (GFAAS),^{8,9} or electrothermal atomic absorption spectrometry (ETAAS),⁶ with a suitable chemical modifier (such as, lutetium,⁶ ammonium 12-molybdophosphate,⁸ palladium,⁹ magnesium nitrate and Triton X-100¹⁰) has been commonly used for the measurement of Be in urine samples. In another method, a urine sample (25 ml) was digested with acids, followed by fluorometric detection.¹¹

The first part of this paper describes how the concentration (0.37–65.0 μ g/l) of Be in undiluted urine samples can be directly determined by GFAAS after the urine sample was diluted (1+1) with a Nash reagent containing acetylacetone (0.2%, v/v) in ammonium acetate buffer of pH 6.0. The accuracy was tested with standard reference materials (SRM 2670 freeze-dried human urine).

Selenium (Se) is an essential trace element in living organisms^{2,12} because Se acts as a cofactor in the glutathione peroxidase of cell membrane and is important in cellular detoxification of peroxides.¹³ From the intake of daily food and drinking water, Se or its salts accumulates in kidneys,¹² liver,^{12,13} plasma,¹⁴ blood,¹⁴ and serum,^{13–19} or is excreted in the urine.^{15,19–28} The normal levels of urine Se are in the range of 10–100 μ g/l,⁷ but a concentration greater than 400 μ g/l is considered toxic.⁷ Several normal levels of serum Se are reported as follows: 80–270 μ g/l from the Clinical Guide Laboratory Test;⁷ 29–139 μ g/l from a review¹⁷ by collecting the results of healthy subjects from Germany, France, Italy, Ireland, Switzerland, Hungary, Greece, USA, and Spain; 5–160 μ g/l from the Glasgow Royal Infirmary Trace Element Unit.²⁹ Smaller concentrations of Se may cause diseases, such as anemia,³⁰ heart disease,^{13–17,28} or cancer;^{13,28} whereas large concentrations of Se cause changes in diastolic blood pressure,³⁰ higher uric acid,³⁰ or gastrointestinal troubles.¹⁷

Several techniques used for the determination of Se in human urine and serum are inductively coupled plasma-mass spectrometry (ICP-MS) after diluting samples;^{15,28} HPLC-ICP-MS for urine Se speciation^{23–25} and hydride-generation atomic absorption spectrometry (HGAAS) after digesting samples with acids;^{13,20} flow-injection HGAAS;^{14,21} HPLC-HGAAS;²² fluorometric detection;¹⁹ constant current stripping analysis;²⁶ and GFAAS (or ETAAS);^{16–18} or GFAAS after microwave

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Table 1 Suitable temperature programs for (a) beryllium and (b) selenium in (1+1) diluted urine sample and (c) selenium in (1+4) diluted serum sample by GFAAS

Sample	(a) Be in urine		(b) Se in urine		(c) Se in serum	
Step	Temperature/°C	Duration/s	Temperature/°C	Duration/s	Temperature/°C	Duration/s
Drying	80 – 120	30	80 – 120	50	80 – 120	60
Ashing	600 – 1200	45	120 – 400	90	120 – 400	60
			400 – 1000	60	1000 – 1200	10
			1000 – 1200	10	1200 – 1200	60
			1300 – 1300	15		
Atomization ^a	2800 – 2800	4	2800 – 2800	4	2800 – 2800	4
Cleaning	2800 – 2800	5	2800 – 2800	5	2800 – 2800	5

a. Flow was stopped during atomization step.

digestion.²⁷

The second part of this paper describes how the contents of Se in urine (4.4 – 150 µg/l) and serum (21 – 400 µg/l) samples can be directly measured by GFAAS after (1+1) and (1+4) dilution with HNO₃ (0.2%, v/v), respectively, and using appropriate amounts of Ni²⁺ + Pd + NH₄NO₃ as the chemical modifier. The accuracies were tested with certified reference materials (SRM 2670 freeze-dried human urine and a second-generation biological human serum). The proposed method can be applied to the analysis of real samples (including six urine and four serum samples from the patients in Blackfoot Disease Treatment Center in Taiwan).

Experimental

Apparatus

A Hitachi Z-8000 graphite-furnace atomic absorption spectrophotometer, equipped with a Zeeman background corrector, was used for the atomic absorption measurement of Be at 234.9 nm (or of Se at 196.0 nm) with a slit width of 1.3 nm. Hollow cathode lamps of Be and Se (Hitachi Co., Japan) were operated at 10 mA and 12.5 mA, respectively. Uncoated graphite tube cuvettes (No. 180-7400, Hitachi Co., Japan) were purchased.

Reagents and solutions

All chemicals used were of analytical grade or better. Nitric acid (double distilled, Seastar Co., Canada) was used to prepare 0.2% HNO₃ (v/v) with pure water (Barnstead NANOpure system). Stock standard solutions of 1000 mg/l for Be (Be(NO₃)₂·4H₂O in 0.5 M HNO₃) and for Se (SeO₂ in 0.5 M HNO₃) were purchased from Merck (Germany). Working standard solutions of Be and Se were prepared daily by diluting 10.0 mg/l of the corresponding standard solutions with 0.2% HNO₃ (v/v). Sodium chloride was superpure grade (Merck) and Triton X-100 was from Sigma (USA). A Nash reagent containing 150 g ammonium acetate (Merck), 3.0 ml glacial acetic acid (99.99%, Aldrich), and 2.0 ml acetylacetone (Merck) per liter of aqueous solution (*i.e.*, 2.0 M ammonium acetate buffer at pH 6.0 together with 0.2% acetylacetone (v/v)) was prepared weekly. Palladium nitrate [Pd(NO₃)₂·xH₂O], nickel nitrate hexahydrate (99.999%), and ammonium nitrate (99.99%) were from Aldrich and were used to prepare chemical modifiers for Se.

Standard reference samples of freeze-dried human urine (SRM 2670, NIST, USA) contain a normal level ≤0.5 µg/l of Be and 30 ± 8 µg/l of Se; and an elevated level of 33 µg/l of Be and 460 ± 30 µg/l of Se after reconstitution in 20.0 ml of pure water.

The following two certified freeze-dried human serums were purchased. Seronorm Trace Elements Batch No. 116 (Nycomed Pharma As, Norway) contains (100 ± 6) µg/l of Se after reconstitution in 3.00 ml of pure water. A second-generation biological reference material (Department of Internal Medicine, University Hospital, Ghent, Belgium)³¹ contains 1.05 ± 0.05 µg/g of Se (dry weight) after reconstitution in 1.00 ml of pure water.

Samples of human urine and serum

For Be test, two urine samples (#1, of about 1.2 l and #2, of about 1.5 l) were obtained by collecting urine for 24 h from two normal persons in cleaned polyethylene bottles and were refrigerated at –20°C while not in use. For Se test, one urine sample (#3, of about 1.8 l) was obtained by collecting urine for 24 h from a normal person. Another six urine samples (#4 to #9) collected at one time (10 ml for urine Se measurements and another 10 ml for creatinine analysis, containing 36, 78, 88, 66, 48, and 38 mg/100 ml of creatinine, respectively) and four serum samples (#1 to #4) were obtained from patients in the Blackfoot Disease Treatment Center (Provincial Hsing-Yin Hospital, Pei-Men, Tainan County, Taiwan) through the help of Dr. Ming-Yuh Wang.

Analytical procedure for Be in urine

Since the concentrations of Be in the urine of two normal persons [about 0.042 µg/l, obtained from standard addition method (0 – 40 µl of 200 µg/l of Be)] were below the method detection limit (0.37 µg/l in undiluted urine), one of them (urine sample #1) was used as the matrix of urine and calibration graphs were prepared by the following standard additions method. Five hundred microliters of urine sample #1 were pipetted into vials (10 ml) and various amounts of standard solutions of Be (0 – 100 µl of 200 µg/l or 80 µl of 400 µg/l in HNO₃ (0.2%, v/v)) and Nash reagent (400 – 500 µl) were added to make a (1+1) dilution. In order to decrease the sample viscosity and to dispense the sample more evenly in the graphite cuvette, an additional aliquot of 20 µl Triton X-100 (0.4%, v/v) was added to each 1000 µl of (1+1) diluted sample. A NaCl (0.9%, w/v) solution⁷ was correspondingly diluted and was used as a simulated blank for urine. A portion (10 µl) of the (1+1) diluted urine sample mixture, or the blank solution, was introduced into a graphite cuvette and atomized according to a temperature program (Table 1(a)). Peak heights in absorbance were used for quantitative analysis. Real urine samples were diluted (1+1) with Nash reagent before adding Triton X-100 and then analyzing by the procedure described above.

Fig. 1 Effect of ashing and atomization temperature on absorbance with (open symbols) and without (solid symbols) chemical modifier for 40 pg Be in 10 μ l of (1+1) diluted urine ($\circ$); 0.62 ng Se in 10 μ l of (1+1) diluted urine (Δ); and 0.96 ng Se in 10 μ l of (1+4) diluted serum ($\square$), respectively.

Analytical procedure for Se in urine and in serum

Calibration graphs were prepared by pipetting 100 μ l of SRM 2670 normal level human urine (or Seronorm Batch No. 116 human serum) reconstituted solution into vials (10 ml). Various amounts of HNO_3 (0.2%, v/v) and a standard solution of Se (100 μ g/l in HNO_3 (0.2%, v/v)) were added to make a (1+1) dilution for urine, or a (1+4) dilution for serum sample, together with standard additions. Real samples of human urine (#3 to #9) were diluted (1+1), or human serum (#1 to #4) were diluted (1+4), with HNO_3 (0.2%, v/v), respectively. An NaCl (0.9%, w/v) solution⁷ was correspondingly diluted and was used as a simulated blank for urine and serum. An additional aliquot of 10 μ l of Triton X-100 (0.1%, v/v) was added to each 200 μ l of (1+1) diluted urine sample, or 20 μ l of Triton X-100 (0.1%, v/v) was added to each 500 μ l of (1+4) diluted serum sample. After the diluted sample mixture, or the blank solution (10 μ l), was introduced into a graphite cuvette, the corresponding chemical modifier [10 μ l, containing Ni^{2+} (150 μ g) + NH_4NO_3 (100 μ g) + Pd (5.5 μ g) in HNO_3 (0.2%, v/v) for urine and Ni^{2+} (25 μ g) + NH_4NO_3 (100 μ g) + Pd (3.7 μ g) in HNO_3 (0.2%, v/v) for serum] was added to it and the mixture was atomized according to a temperature program (Table 1(b) or 1(c)).

Results and Discussion

For Be in urine

The effect of ramping ashing temperatures (from 600 – 600 to 600 – 1500°C for 45 s) and atomization temperatures (2400 – 2800°C for 4 s) on absorbance with and without the chemical modifier was tested with 40 pg Be in 10 μ l of (1+1) diluted urine sample. The results (Fig. 1) indicate that the sensitivity of absorbance for Be in urine is enhanced by a factor of 1.3 in the presence of the chemical modifier. When a ramping ashing temperature is higher than 600 – 1200°C for 45 s, the absorbance begins to decrease; and when atomization temperature increases from 2400 to 2800°C for 4 s, the absorbance increases. Hence, suitable ramping ashing (600 – 1200°C) and atomization (2800°C) temperatures were obtained as tabulated in Table 1(a).

The effect of the pH (3.0 – 7.0) of Nash reagent (containing 2.0 M ammonium acetate buffer and 0.2%(v/v) acetylacetone) on the absorbance of Be was tested with 40 pg Be in 10 μ l of

(1+1) diluted urine sample. The result indicates that the absorbances are large when the pH is in the range of 4.0 – 6.5. Since the formation of beryllium acetylacetonate³² is favored in the pH range of 5.5 – 6.0,³² the acetate buffer pH of 6.0 was selected. Similarly, amounts of acetylacetone (0 – 60 μ l) and ammonium acetate buffer (0.20 – 2.0 mmol of pH 6.0) in the Nash reagent were varied; the results indicate that the absorbances are relatively large when Nash reagent contains 10 – 20 μ l of acetylacetone and 0.80 – 2.0 mmol ammonium acetate buffer. Hence, 20 μ l of acetylacetone and 1.0 mmol of ammonium acetate buffer in the Nash reagent (*i.e.*, 2.0 M ammonium acetate buffer and 0.20%(v/v) acetylacetone) were used in this study.

Following the proposed method, the MDL for Be was determined as the concentration corresponding to three times the standard deviation of twelve replicates using 10 μ l of the (1+1) diluted urine mixture at the lowest concentration (1.0 μ g/l) of the calibration graph. The MDL (3σ) of human urine Be from the mean of seven determinations was found to be 0.18 ± 0.01 μ g/l in the (1+1) diluted sample. This corresponds to 0.37 μ g/l (or 3.7 pg) in the undiluted urine sample. The limit of detection (LOD, 3σ) values of Be reported and chemical modifiers used are compared as shown in Table 2. The MDL value of Be obtained in this work is higher than 0.019 pg,⁶ but is comparable to others^{8,10,11} reported elsewhere. A typical calibration graph for Be from standard addition method is $y = 9.27 \times 10^{-3}x + 2.11 \times 10^{-3}$ and is linear up to 32.0 μ g/l in (1+1) dilution (or 0.37 – 65.0 μ g/l in undiluted urine samples) with a correlation coefficient of 0.9995.

The accuracies of the proposed method were checked by testing with 4.95, 8.25, and 16.5 μ g/l of Be in (1+1) dilution prepared from the certified normal level and elevated level of SRM 2670 reconstituted urine samples. The results of 98.6 – 102% are listed in Table 3(a). The precision (RSD) for urine Be in within-run measurements ($n = 5$) is $\leq 2.3\%$ and in between-run ($n = 3$) measurements is $\leq 3.0\%$.

Concentrations of Be in real urine samples (#1 and #2) from two normal persons were measured by the proposed method and were found to be N.D. (<0.18 μ g/L of Be in (1+1) dilution). After spiking 4.00 and 8.00 ng of Merck Be in 500 μ l of urine samples, respectively, the average recoveries from triplicates were 98.3 – 103% with RSD within 2.5%. The proposed method uses acetylacetone to react with Be(II) in the presence of ammonium acetate buffer of pH 6.0 to form a chelate of beryllium acetylacetonate,^{32,33} which enhances the sensitivity for Be. The proposed method can be applied easily and accurately to the measurement of Be (0.37 – 65.0 μ g/l) in urine samples. The linear range covers the toxic level of >20 μ g/l of Be in the urine.⁷

For Se in urine

The effect of final ashing temperatures (1100 – 1500°C for 15 s) and atomization temperatures (2400 – 2800°C for 4 s) on absorbance with and without the chemical modifier was tested with 0.62 ng of Se in 10 μ l of (1+1) diluted urine sample. The results (Fig. 1) indicate that without the chemical modifier, Se is lost completely during pyrolysis. In the presence of the chemical modifier during ashing, absorbance increases from 1100 to 1300°C for 15 s and decreases after 1400°C. During atomization, the absorbance increases as temperature increases from 2400 to 2800°C for 4 s. Hence, suitable ashing (1300°C) and atomization (2800°C) temperatures were obtained as tabulated in Table 1(b).

The effect of chemical modifiers [10 μ l, containing various amounts of Ni^{2+} (0 – 175 μ g), Pd (0 – 7.4 μ g), and NH_4NO_3 (0 –

Table 2 Comparative values of limit of detection (L.O.D.) and characteristic mass (m_o) for urine Be, urine Se, and serum Se using different chemical modifiers

Element	Chemical modifier	L.O.D. (3σ) ^a /μg l ⁻¹	m_o ^b /pg	Condition	Ref. No.
urine Be	acac (0.2%)/NH ₄ OAc buffer (2 M)	0.37 (3.7 pg)	1.9	(1+1) dilution; 10 μl injected	this work
urine Be	Lu (6 μg)	(0.019 pg)	0.45	without dilution; 20 μl injected	6
urine Be	ascorbic acid/(NH ₄) ₃ PMo ₁₂ O ₄₀	(1.0 pg)	0.44	(1+1) dilution; 20 μl injected	8
urine Be	Mg(NO ₃) ₂ /HNO ₃ /Triton X-100	0.20	1.7	(1+3) dilution; 20 μl injected	10
urine Be	Aniline- <i>N</i> -salicylidene	0.5		25 ml urine digested	11
urine Se	Ni ²⁺ (150 μg)/ Pd (5.5 μg)/ NH ₄ NO ₃ (100 μg)	4.4 (44 pg)	104	(1+1) dilution; 10 μl injected	this work
urine Se		0.5 (975 pg)		6.5 ml urine digested; 1.95 ml used	26
urine Se(IV)	acetic acid (5%)	1.8		(1+1) dilution; using Int. Std. ^c	28
urine Se	Ni ²⁺ (20 μg)	1.2		5 ml urine digested, diluted to 25 ml, 20 μl injected	27
urine Se	ethanol (5%)/HNO ₃ /Triton X-100/Y	2.7		(1+10) dilution; using ⁷⁸ Se Int. Std.	15
	Ni ²⁺ (75 μg)/HNO ₃ /Triton X-100	14.5		(1+4) dilution; 20 μl injected	15
urine Se	NaBH ₄ (0.5%)/NaOH (0.16%)	3		HPLC, digestion, 100 μl injected	22
urine Se	NaBH ₄ (3%)/NaOH (1%)	4.73		standard addition method	20
urine Se	NH ₄ OAc buffer/methanol	5 (26–32 pg)		(1+1) dilution; 50 μl injected	23
urine Se	Ir	6		1 ml urine digested, diluted to 50 ml, 500 μl injected to FI	21
urine Se(IV)	HNO ₃ (1%)	(40 pg)		(1+4) dilution; 200 μl injected	24
urine Se(IV)		(50 pg)		(1+1.5) dilution; 100 μl injected	25
serum Se	Ni ²⁺ (25 μg)/ Pd (3.7 μg)/ NH ₄ NO ₃ (100 μg)	21.4 (214 pg)	364	(1+4) dilution; 10 μl injected	this work
serum Se	ethanol (5%)/HNO ₃ /Triton X-100/Y	2.7		(1+10) dilution; using ⁷⁸ Se Int. Std.	15
serum Se	Ni ²⁺ (75 μg)/HNO ₃ /Triton X-100	14.5 (290 pg)		(1+4) dilution; 20 μl injected	15
serum Se	Pd (5 μg)/Mg(NO ₃) ₂ (15 μg)	14.5 (290 pg)		(1+4) dilution; 20 μl injected	16
serum Se	NaBH ₄ /NaOH	31 (6200 pg)		0.5 ml serum digested, (1+19) dilution, 200 μl injected	13
serum Se	Ir	12 (180 pg)		(1+4) dilution; 15 μl injected	18
	Pd (2.5 μg)	13 (195 pg)			
	Pd/Mg (NO ₃) ₂	13 (195 pg)			
	Ni ²⁺ (2.5–5.0 μg)	14 (210 pg)			
	Ir/Mg(NO ₃) ₂	16 (240 pg)			
	Cu ²⁺	16.5 (248 pg)			

a. Concentration in the undiluted sample. b. The mass (pg)/0.0044 absorbance unit. c. Using ⁷⁶Se, ⁷⁷Se, ⁷⁸Se, and ⁸²Se internal standards.

200 μg)] on absorbance is shown in Fig. 2 by testing with 0.62 ng Se using the temperature program listed in Table 1(b). When 10 μl of chemical modifier contains 5.5 μg of Pd and 100 μg of NH₄NO₃, the absorbance increases rapidly as Ni²⁺ increases from 0 to 150 μg and becomes stable after 150 μg. Ni²⁺ may form the stable complex (NiSe)³⁴ with Se and eliminate the interference from phosphorous containing molecules (PO and P₂) in urine and serum.³⁵ Therefore, the sensitivity³⁶ for Se in the urine sample is enhanced. When the chemical modifier contains 150 μg Ni²⁺ and 100 μg NH₄NO₃, the absorbance slightly increases as Pd increases from 0 to 5.5 μg and then decreases after 5.5 μg. Pd may form stable complexes (SePd^{37,38} and/or SePd₂^{34,38}) with Se and eliminate the phosphorous-molecular interference in urine and serum.³⁵ Therefore, the sensitivity for Se is enhanced. When the chemical modifier contains 150 μg Ni²⁺ and 5.5 μg Pd, the absorbance slightly increases as NH₄NO₃ increases from 0 to 100 μg and then decreases after 100 μg. NH₄NO₃ may volatilize NaCl³⁶ in the urine and serum samples⁷ and enhance the sensitivity for Se. Hence, a chemical modifier (10 μl) containing Ni²⁺ (150 μg) + Pd (5.5 μg) + NH₄NO₃ (100 μg) in HNO₃ (0.2%, v/v) was used for human urine samples in this work. The results of LOD for urine and serum Se obtained in this work (Table 2) are comparable to those using Ni²⁺,^{15,18,27} Pd,¹⁸ Pd/Mg(NO₃)₂,^{16,18} Cu,¹⁸ Ir,^{18,21} Ir/Mg(NO₃)₂¹⁸ for urine and/or serum Se.

Following the proposed method, the MDL was determined as

the concentration corresponding to three times the standard deviation of twelve replicates using 10 μl of the (1+1) diluted urine at 14.3 μg/l of Se prepared from the normal level of SRM 2670 urine sample. The MDL (3σ) of human urine Se from the mean of four determinations was found to be (2.1 ± 0.2) μg/l in the (1+1) diluted sample. This corresponds to 4.4 μg/l (or 44 pg) of Se in the undiluted urine sample. The LOD values of urine Se reported and chemical modifiers used are compared as shown in Table 2. The MDL value obtained in this work is comparable to those (using ICP-MS,^{15,28} stripping analysis,²⁶ HGAAS,²⁰ HPLC-HGAAS,²² HPLC-HG-ICP-MS,²³ FI-HG-ETAAS,²¹ or HPLC-ICP-MS^{24,25}) reported elsewhere. A typical calibration graph for urine Se from standard addition method is $y = 5.90 \times 10^{-4}x + 4.32 \times 10^{-4}$ and is linear up to 71.4 μg/l in (1+1) dilution (or 150 μg/l of Se in undiluted urine sample) with a correlation coefficient of 0.9990.

The accuracy of the proposed method was checked by testing 34.8 and 55.2 μg/l of Se in (1+1) diluted samples prepared from the elevated and normal levels of SRM 2670 urine standards with HNO₃ (0.2%, v/v). The results of (98.9–99.4)% were obtained from the mean of three determinations with RSD (between-run) within 2.9%, as tabulated in Table 2(b). Real urine samples (#3 to #9) were measured by using the proposed method and were found to contain 24.6 ± 0.8, 23.7 ± 0.2, 55.9 ± 2.3, 16.0 ± 0.4, 33.8 ± 0.6, 18.1 ± 0.6, and 13.2 ± 0.6 μg/l of Se, respectively, in undiluted samples from triplicates. The RSD

Table 3 Accuracy tests for (a) Be and (b) Se in urine and (c) Se in serum

(a) Conc. of Be(II) in urine/ $\mu\text{g l}^{-1}$		
Prepared ^a	Found ^b	Accuracy ^b , %
4.95	4.88 ± 0.03	98.6 ± 0.6
8.25	8.42 ± 0.08	102 ± 1
16.5	16.7 ± 0.5	101 ± 3
(b) Conc. of Se in urine/ $\mu\text{g l}^{-1}$		
Prepared ^a	Found ^b	Accuracy ^b , %
34.8	34.6 ± 1.0	99.4 ± 2.9
55.2	54.6 ± 0.6	98.9 ± 1.1
(c) Conc. of Se in serum/ $\mu\text{g l}^{-1}$		
Prepared ^c	Found ^b	Accuracy ^b , %
28.5	27.7 ± 0.4	97.2 ± 1.4

a. At (1+1) dilution prepared from certified normal level and elevated level of SRM 2670 reconstituted urine samples.

b. Mean of three determinations with standard deviation.

c. At (1+4) dilution prepared from the second-generation human serum (No. 29, #664, Dept. of Internal Medicine, University Hospital, Ghent, Belgium).

(within-run, $n = 3$) was within 4.5%. The concentrations of Se measured in urine samples from one normal person and six patients in Blackfoot Disease Treatment Center were in the normal range (10–100 $\mu\text{g/l}$).⁷ The urinary Se concentrations obtained from six Blackfoot Disease patients are comparable to those (2.2–57.1 $\mu\text{g/l}$) reported by Horng *et al.*²⁷ After spiking Merck Se standard solutions (14.3–28.6 $\mu\text{g/l}$) to three urine samples (#3, #4, and #5) in (1+1) dilution, the average recoveries from triplicates were 98.9–102% with RSD within 4.6%. It is concluded that the proposed method provides an easy, accurate, and direct technique for the measurement of Se in (1+1) diluted urine samples using Ni^{2+} (150 μg) + Pd (5.5 μg) + NH_4NO_3 (100 μg) as a chemical modifier.

For Se in serum

The effect of final ashing temperatures (1100–1600°C for 60 s) and atomization temperatures (2400–2800°C for 4 s) on absorbance with and without the chemical modifier was tested with 0.96 ng of Se in 10 μl of (1+4) diluted serum sample (prepared from Seronorm Trace Elements Batch No. 116). The results (Fig. 1) indicate that without the chemical modifier, Se is lost completely during pyrolysis. In the presence of the chemical modifier, absorbance increases from 1100–1200°C for 60 s and decreases after 1300°C during ashing. During atomization, the absorbance increases as temperature increases from 2400 to 2800°C for 4 s. Hence, suitable ashing (1200°C) and atomization (2800°C) temperatures were obtained as tabulated in Table 1(c).

Similarly, the effect of chemical modifiers (10 μl , containing various amounts of Ni^{2+} (0–150 μg), Pd (0–14.8 μg), and NH_4NO_3 (0–300 μg)) on absorbance was compared by testing with 0.96 ng of serum Se using the temperature program (Table 1(c)). Similar to Fig. 2, a chemical modifier containing Ni^{2+} (25 μg) + Pd (3.7 μg) + NH_4NO_3 (100 μg) was selected for human serum in this work.

The MDL of serum Se was determined similarly to that described in urine Se by using 10 μl of (1+4) diluted serum

Fig. 2 Effect of amount of each constituent in 10 μl chemical modifier on atomization absorbance for 0.62 ng Se in (1+1) diluted urine. [●, varying Pd while keeping Ni^{2+} (150 μg) and NH_4NO_3 (100 μg); Δ, varying NH_4NO_3 while keeping Ni^{2+} (150 μg) and Pd (5.5 μg); □, varying Ni^{2+} while keeping Pd (5.5 μg) and NH_4NO_3 (100 μg)].

sample at 19.2 $\mu\text{g/l}$ of Se prepared from Seronorm Batch No. 116 human serum. The MDL (3σ) value for serum Se was found to be (4.11 ± 0.27) $\mu\text{g/l}$ in (1+4) dilution from the mean of three determinations. This corresponds to 21.4 $\mu\text{g/l}$ (or 214 pg) of Se in the undiluted serum. The LOD values of serum Se reported and chemical modifiers used are compared as shown in Table 2. The MDL value obtained in this work is higher than 2.7 $\mu\text{g/l}$ by ICP-MS,¹⁵ but is comparable to others (HGAAS,¹³ and ETAAS using Pd,¹⁸ Ni^{2+} ,¹⁸ Pd/Mg(NO_3)₂,^{16,18} Ir,¹⁸ Ir/Mg(NO_3)₂, or Cu^{2+} ,¹⁸ as a chemical modifier) reported elsewhere.

Following the proposed method, a typical calibration graph for serum Se is $y = 5.22 \times 10^{-4}x - 8.30 \times 10^{-4}$ and is linear up to 76.9 $\mu\text{g/l}$ in (1+4) dilution (or 400 $\mu\text{g/l}$ in undiluted serum sample) with a correlation coefficient of 0.9995. The accuracy of the proposed method was checked by testing 28.5 $\mu\text{g/l}$ of Se prepared from the second-generation human serum (No. 29, #664) at (1+4) dilution. The results of $(97.2 \pm 1.4)\%$ were obtained from the mean of three determinations as listed in Table 3(c). Real serum samples (#1 to #4) were analyzed by using the proposed method and were found to contain 234.5 ± 5.2 , 248.6 ± 5.2 , 143.0 ± 3.1 , and 98.8 ± 0.5 $\mu\text{g/l}$ of Se in undiluted samples, respectively, from triplicates. The RSD (within-run, $n = 3$) is within 2.2%. The concentrations of Se measured in four serum samples from these four patients in Blackfoot Disease Treatment Center are in the normal range of 80–270 $\mu\text{g/l}$ suggested by the Clinical Guide Laboratory Test.⁷ However, the two higher serum Se values above exceed the range of values reported for healthy subjects by others (29–139 $\mu\text{g/l}$ ¹⁷ and 5–160 $\mu\text{g/l}$).²⁹ After spiking Merck Se standard solutions (9.60 and 19.2 $\mu\text{g/l}$) to serum sample #4 at (1+4) dilution, the average recoveries from triplicates were 98.4–102% with RSD within 2.9%. Thus, the proposed method can be applied easily, directly, and accurately to the measurement of Se in (1+4) diluted serum samples using Ni^{2+} (25 μg) + Pd (3.7 μg) + NH_4NO_3 (100 μg) as a chemical modifier.

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