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Cobalt as internal standard for arsenic and selenium determination in urine by simultaneous atomic absorption spectrometry

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Abstract

The effectiveness of internal standardization for simultaneous atomic absorption spectrometry (SIMAAS) was investigated for As and Se determination in urine. Co and Sn were selected as internal standard (IS) candidates based on the evaluation of some physico-chemical parameters related to the atomization. Correlation graphs, plotted from the normalized absorbance signals ($n = 20$) of internal standard (axis y) versus analyte (axis x), precision, and accuracy of the analytical results were the supportive parameters to choose Co as the most appropriate IS. The urine samples were diluted 1 + 2 to 1.0% (v/v) HNO_3 + 80 μg L^{-1} Co^{2+}. The mixture 20 μg Pd + 3 μg Mg was used as chemical modifier and the optimized temperatures for pyrolysis and atomization steps were 1400 and 2300 °C, respectively. The characteristic masses for As (47 ± 1 pg) and Se (72 ± 2 pg) were estimated from the analytical curves. The detection limits ($n = 20$, 3δ) were 1.8 ± 0.1 and 2.6 ± 0.1 μg L^{-1} for As and Se, respectively. The reliability of the entire procedure was checked with the analysis of certified reference material from Sero AS (Seronorm™ Trace Elements in Urine). The obtained results showed the matrix interference disallowed the instrument calibration with aqueous standards. The best analytical condition was achieved when matrix-matched standards were used in combination with Co as IS, which improved the recoveries obtained for As. Under this experimental condition, eight urine samples were analysed and spiked with 10 and 25 μg L^{-1} As and Se. The mean recoveries were 96 ± 6% (10 μg L^{-1} As), 95 ± 6% (25 μg L^{-1} As), 101 ± 7% (10 μg L^{-1} Se), and 97 ± 4% (25 μg L^{-1} Se).

Keywords: Internal standard; SIMAAS; Urine; Arsenic; Selenium; Cobalt

1. Introduction

Despite its usual monoelement mode of operation, electrothermal atomic absorption spectrometry (ETAAS) is recognized as a robust technique for trace and ultra-trace element determinations [1]. The low detection limits, the high selectivity, and the possibility to carry out an in situ thermal sample pretreatment are important characteristics of ETAAS, which can be regarded as one of the most attested techniques for elemental determinations in a wide variety of samples [1,3], mainly for biological materials of clinical interest [2].

Simultaneous atomic absorption spectrometry (SIMAAS), commercially introduced during the last decade [4,5], adjoined the multielement capability to the ETAAS reducing analytical time and costs associated with the replacement of the graphite parts. Moreover, the multielement capability allows the use of internal standard (IS) in atomic absorption spectrometry, which can improve precision and accuracy in determinations by SIMAAS [6–9]. This strategy can also expand the graphite tube lifetime owing to the constant analyte/IS absorbance signal ratio [7,9]. There are two works dealing with the use of IS for the direct Pb determination in wine [7] and blood, placenta, and urine [6]. Significant improvements in precision were observed when Bi was adopted as IS, correcting the damaging effect caused by the high concomitant concentration still contained in the untreated sample solution introduced into the atomizer

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[6,7]. The Se determination in sparkling drinking water was carried out using As as IS, showing the benefits of internal standardization for correcting the sampling errors which may occur for heterogeneous samples [9].

In spite of the successful use of IS to improve Pb and Se determination in complex samples, the multielement capability of the instrument was not explored to determine more than one analyte. On the other hand, the internal standardization approach can be more useful when compromise conditions are really imperative, i.e. when two or more analytes are simultaneously determined in a complex untreated sample. In this context, Ag was proposed as IS for the simultaneous determination of Cd and Pb in whole blood samples. The accuracy of the analytical results was improved after the correction with IS, showing the possibility to minimize the interference effects caused by the concomitants [8]. Moreover, the development of a continuum-source spectrometer will enhance the multi-element capability of ETAAS in the near future, and the studies involving the use of IS can be useful in this context.

The use of IS for analysing urine by SIMAAS was not investigated until now. In spite of being amenable to direct analysis, urine can cause intense matrix effects, damaging the accuracy and precision of the analytical results. For this reason, the objective of this work is evaluate the use of Co and Sn as IS for the simultaneous determination of As and Se in urine. They are important trace-elements and it is relevant to know their concentration in biological fluids accurately, to avoid misjudgement about the clinical diagnostics of deficiency or toxicity [10–13].

2. Experimental

2.1. Apparatus

All measurements were carried out by using a simultaneous line-source spectrometer (SIMAA-6000, Perkin-Elmer Life and Analytical Sciences, Shelton, CT, USA) equipped with a longitudinal Zeeman-effect background corrector, standard THGA tube with pyrolytically coated integrated platform, Echelle optical arrangement, and solid state detector. It was operated in four-element simultaneous mode using a hollow cathode lamp for Co ($\lambda = 242.5$ nm; $i = 25$ mA) and electrodeless discharge lamps for As ($\lambda = 193.7$ nm; $i = 380$ mA), Se ($\lambda = 196.0$ nm; $i = 290$ mA), and Sn ($\lambda = 286.3$ nm; $i = 325$ mA). The solutions were delivered into the graphite tube by means of a Perkin-Elmer AS-72 autosampler. Argon 99.996% (v/v) (Air Liquide Brasil S/A, São Paulo, Brazil) was used as protective and purge gas.

2.2. Reagents, solutions and samples

Only high-purity deionized water (18 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for preparing solutions and samples. Nitric acid (Synth, São Paulo, SP, Brazil) was purified by distillation in quartz sub-boiling stills (Marconi, Piracicaba, SP, Brazil). Analytical reference solutions were prepared by successive dilution of Titrisol stock solutions (Merck, Darmstadt, Germany) containing 1000 mg L^{-1} of arsenic (As_2O_5), cobalt ($CoCl_2$), selenium (SeO_2) and tin ($SnCl_4$). The chemical modifier solution was prepared from high-purity Suprapur salts (Merck): $Pd(NO_3)_2$ and $Mg(NO_3)_2$. All solutions were stored in decontaminated polypropylene bottles (Nalge Company, Rochester, NY, USA).

Fresh urine samples were collected from laboratory personnel directly in disposable polypropylene containers (Sarstedt, Nümbrecht, Germany) and kept at 4 °C for until 48 h prior to the analysis. Seronorm™ Trace Elements in Urine from Sero AS (Billingstad, Norway) was used to check the reliability of the entire proposed analytical method. Seronorm™ Trace Elements in Urine Blank was used to prepare the matrix-matched standards for calibrating the spectrometer. Both Seronorm™ urine materials were supplied in lyophilized form and reconstituted by dissolving the vial total content with high-purity de-ionized water.

2.3. Procedure

All glassware and polypropylene bottles were cleaned with detergent solution, soaked in 10% (v/v) HNO_3 for 24 h, rinsed with Milli-Q water, and stored into a closed polypropylene container. All solution and sample manipulations were conducted in a laminar flow bench (Veco, Campinas, SP, Brazil) to avoid the airborne contamination.

The instrumental conditions and the heating program for the graphite tube are showed in Table 1. The heating program optimization was carried out with a solution con-

Table 1
Spectrometer setup and atomizer heating program for the simultaneous determination of As and Se using internal standard

	Analytes		IS candidates	
	As	Se	Co	Sn
Spectrometer setup				
Wavelength (nm)	193.7	196.0	242.5	286.3
Bandpass (nm)	0.7	0.7	0.7	0.7
Lamp type[a]	EDL	EDL	HCL	EDL
Lamp current (mA)	380	290	25	325

Step	Temperature (°C)	Ramp (s)	Hold (s)	Argon flow rate (mL min^{-1})
Heating program for the atomizer				
Drying I	110	15	30	250
Drying II	130	10	10	250
Pyrolysis	1400[b]	10	15	250
Atomization	2300[b]	0	4	0
Cleaning	2450	1	4	250

Total program time: 99 s; sample volume: 10 μL; chemical modifier volume (20 μg Pd + 3 μg Mg): 10 μL; injection temperature: 20 °C.

[a] EDL: electrodeless discharge lamp; HCL: hollow cathode lamp.

[b] Parameters optimized after obtaining pyrolysis and atomization temperature curves.

taining 30 μg L^{-1} As(V) + 50 μg L^{-1} Se(IV) + 80 μg L^{-1} Co^{2+} + 100 μg L^{-1} Sn^{4+} in 1.0% (v/v) HNO_3, prepared in presence of a diluted urine sample (1 + 2).

Repeatability studies were carried out to verify the effectiveness of Co and Sn as IS. For this purpose, a diluted urine sample (1 + 2) spiked with 30 μg L^{-1} As(V) + 50 μg L^{-1} Se(IV) + 80 μg L^{-1} Co^{2+} + 100 μg L^{-1} Sn^{4+} in 1.0% (v/v) HNO_3 was used. Twenty consecutive measurements was obtained for As, Se, Co and Sn. For all elements, the absorbance values were normalized with respect to the first result of the consecutive measurements (n = 20).

Correlation graphs were obtained by plotting the normalized absorbance values for the IS (axis y) versus the normalized absorbance values for the analyte (axis x). The analysis of the correlation graphs can support the IS selection, which is done taking into account the linear regression parameters (correlation coefficient, intercept and slope), as suggested by Mermet and Ivaldi [14]. In addition, the comparison of As and Se relative standard deviation (R.S.D., n = 20) with and without IS correction was also used to verify the efficiency of the IS candidates.

The analytical reference solutions for the spectrometer calibration were prepared directly in the autosampler cups (total volume = 1200 μL), diluting 400 μL of Seronorm™ Trace Elements in Urine Blank with 800 μL of stock analytical reference solutions containing 0, 7.5, 15, 37.5, 75, 150 μg L^{-1} As(V) and Se(IV) + 120 μg L^{-1} Co^{2+} in 1.5% (v/v) HNO_3. The calibration curve range was from 5 up to 100 μg L^{-1} for As(V) and Se(IV). An Eppendorf micropipette (Brinkmann Instruments, Westburry, USA) was used to measure the volumes and to mix the diluted reference solutions. Another set of calibration standards, containing the same concentration of analytes (As and Se) and IS (Co), was prepared using water instead of urine, in order to estimate the interference caused by the matrix concomitants.

Eight urine samples were diluted (1 + 2) as described for the analytical reference solutions using a diluent containing only 120 μg L^{-1} Co^{2+} in 1.5% (v/v) HNO_3. These urine samples were also analysed after the addition of 10 μg L^{-1} As(V)/Se(IV) or 25 μg L^{-1} As(V)/Se(IV). The comparison of the obtained results, with and without IS correction, was used to evaluate the usefulness of internal standardization.

All measurements were based on integrated absorbance and made with at least three replicates, except for the repeatability tests (n = 20).

3. Results and discussion

3.1. Selection of Co and Sn as IS candidates

The effectiveness of internal standardization in SIMAAS depends on the suitable choice of the IS. It must present chemical and physical properties as similar as possible to those presented by the analytes [8]. Some relevant parameters for As, Se, Co and Sn are shown in Table 2. The presented data indicate the recommended conditions for the monoelement determination of As, Co, Se and Sn [15]. The comparison of the selected parameters suggests that the analytes (As and Se) and IS candidates (Co and Sn) present close electrothermal behaviors, and the set up of a compromise condition for determining all of them is possible by using Pd + Mg as chemical modifier (Table 2). However, it should be highlighted that the evaluation of the literature information represents only a first step towards the selection of an adequate IS. At last, the natural concentration of the element to be used as IS must not be detectable in the sample, to ensure that only the added amount is responsible for the absorbance signal. The values found in the literature for urine concentration of non-exposed individuals [16] ensure this condition for Co and Sn (Table 2).

Table 2
Recommended conditions for monoelement determination and expected concentration of As, Co, Se and Sn in urine

	T_p (°C)[a]	T_a (°C)[b]	Chemical modifier	m_0 (pg)[c]	Concentration (μg 24 h^{-1})
As	1200	2000	5 μg Pd + 3 μg Mg	40	~10–50
Co	1400	2400	15 μg Mg	17	~2
Se	1300	1900	5 μg Pd + 3 μg Mg	45	~30
Sn	1400	2200	5 μg Pd + 3 μg Mg	90	~10

[a] Pyrolysis temperature.
[b] Atomization temperature.
[c] Characteristic mass.

3.2. Heating program optimization

The pyrolysis and atomization temperatures were selected from the temperature curves obtained simultaneously for As, Co, Se and Sn in the presence of 20 μg Pd + 3 μg Mg (Fig. 1a). For this purpose, a urine sample was spiked with 30 μg L^{-1} As(V) + 10 μg L^{-1} Co^{2+} + 50 μg L^{-1} Se(IV) + 50 μg L^{-1} Sn^{4+} to evaluate the thermal behavior of the analytes and IS in the presence of the sample matrix.

The best pyrolysis temperatures obtained for As, Co, Se and Sn were 1600, 1800, 1400, and 1800 °C, respectively (Fig. 1a). Nevertheless, the setup of a compromise condition for the pyrolysis step requires taking into account the thermal behavior of the more volatile element to be simultaneously determined. As a consequence, the Se thermal behavior must be considered instead of As. In this context, the pyrolysis temperature obtained for the IS candidates (Co and Sn) did not impair the thermal sample treatment, because the pyrolysis temperature obtained for them (>1800 °C) is higher than the value observed for Se (1400 °C).

The adoption of a high pyrolysis temperature enhanced the effectiveness of the sample thermal treatment prior to the atomization step. The decrease of the background signal intensity was expressive (Fig. 1b), mainly for the analytes (As and Se). The concomitant volatilization is favored and some inorganic compounds, such as NaCl and KCl, can be eliminated when the pyrolysis step occurred at temperatures higher than 1100 °C [17–21].

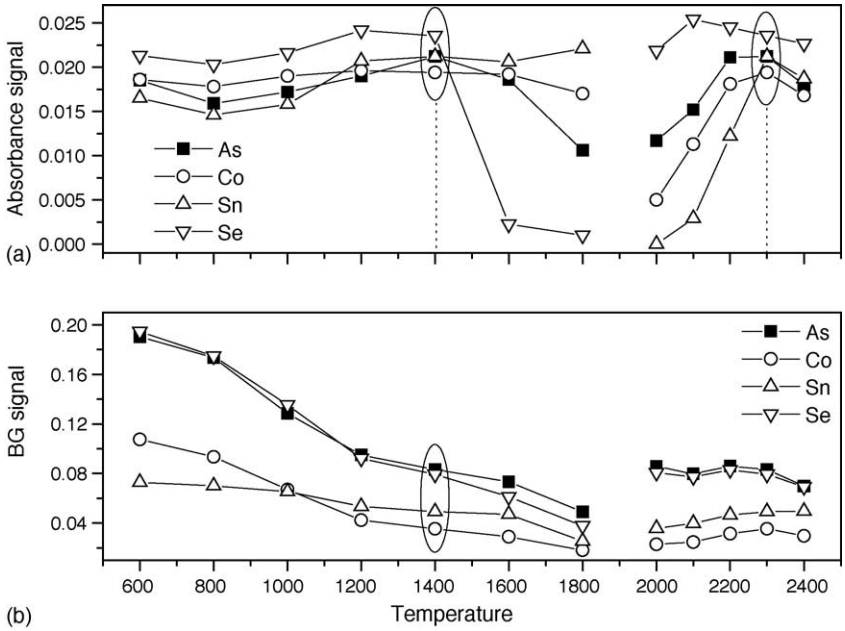

Fig. 1. Pyrolysis and atomization temperature curves simultaneously obtained for As, Co, Se and Sn in presence of 20 μg Pd + 3 μg Mg for a urine sample diluted 1 + 2: (a) atomic absorption; (b) background absorption.

The atomization temperature selection for multielement determinations by SIMAAS must consider the thermal behavior of the less volatile element to be simultaneously determined. In this context, As thermal behavior (2300 °C) is the limiting condition to select the atomization temperature (Fig. 1a). Once again, the presence of the IS candidates did not disturb the heating program optimization, considering the best value for the atomization temperature obtained for Co and Sn (2300 °C) is the same verified for As.

The compromise condition for determining As and Se in urine imposes 1400 and 2300 °C as temperatures for pyrolysis and atomization steps. It should be stressed that the more adequate thermal condition for both elements tested as IS (Co and Sn) can be fitted into the compromise condition set up for the analytes (As and Se). Therefore, the internal standardization efficiency can be evaluated under the best conditions for the analytes.

3.3. Performance evaluation for Co and Sn as internal standard

Repeatability tests were carried out in presence of a diluted urine sample (1 + 2) in order to check the efficiency of the IS candidates. Twenty consecutive measurements were carried out under optimised conditions (Table 1) and the relative standard deviations (R.S.D.s) with and without IS correction were compared (Table 3). The results observed for Sn indicated that its performance was not acceptable in presence of urine. The repeatability of the consecutive measurements obtained for Sn was worse (Sn: R.S.D. = 18%, $n = 20$) than those observed for the analytes without IS correction (Table 3). As a consequence, the R.S.D.s obtained for As and Se using Sn as IS were higher than the values observed without IS correction. On the other hand, improvements on R.S.D.s were observed when Co was used as IS for As and Se. In spite of decreasing the R.S.D.s for both analytes, the correction obtained for As after using Co as IS was more pronounced than for Se (Table 3).

A more accurate appraisal of the experimental data can be done by evaluating correlation graphs, in order to compare the performance of different IS candidates [8,14]. These graphs were used to verify the resemblance among the simultaneous measurements obtained for the analytes and IS by inductively coupled plasma optical emission spectrometry (ICP-OES), as proposed by Mermet and Ivaldi [14]. The judicious selection of IS by using correlation graphs improved the precision of the analytical results, and the real-time internal standardization was proposed for ICP-OES [14–23]. The use of correlation graphs for achieving the most suitable IS for multielement determinations by SIMAAS can be explored as well [8].

The evaluation of the performance for different IS can be carried out by comparing the parameters obtained from the linear regression of the correlation graphs with the ideal situation, which indicates the perfect matching between the analyte and IS signals: the correlation coefficient and the

Table 3
Relative standard deviations (R.S.D., $n = 20$) for As and Se with and without internal standardization in presence of diluted urine (1 + 2)

	IS	As (%)	Se (%)
No IS	–	9.4	7.5
With IS	Co	4.0	5.8
	Sn	14	17

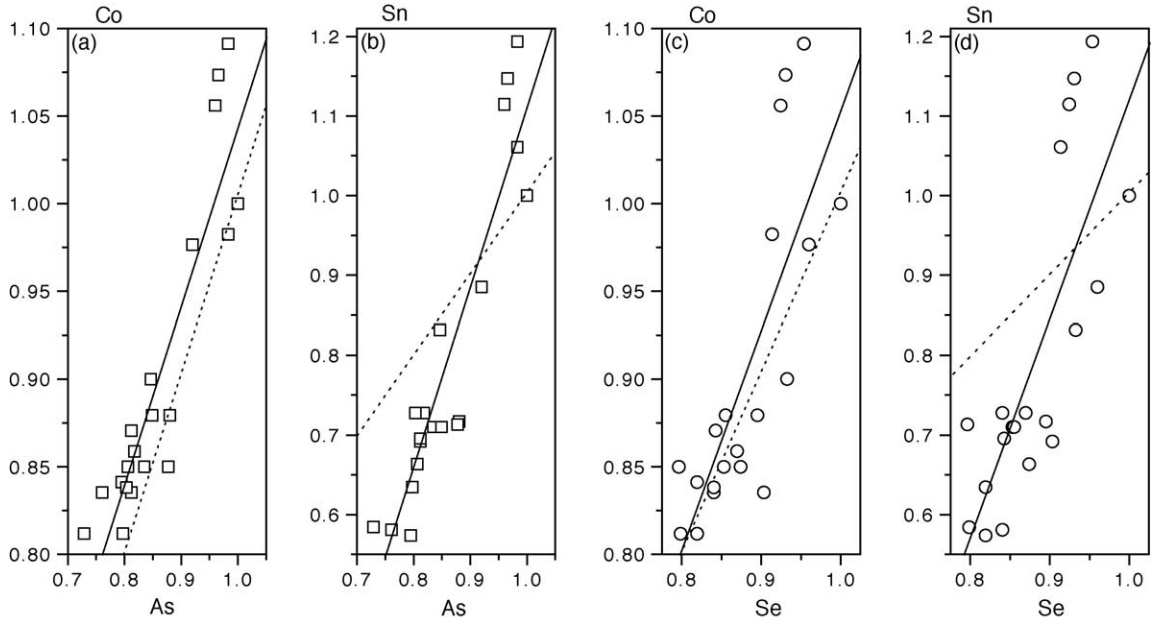

Fig. 2. Correlation graphs for As and Se using Co (a, c) and Sn (b, d) as IS for a diluted urine sample (1 + 2) in presence of 20 μg Pd + 3 μg Mg as chemical modifier (the broken line indicates the ideal condition, i.e., the perfect matching between the analyte and the IS).

slope should be equal to 1, and the intercept should be zero [8,14]. The correlation graphs obtained from the measurements made during the repeatability tests ($n = 20$) for Co and Sn as IS are shown in Fig. 2.

The results obtained for Co were close to the ideal condition in presence of diluted urine (Fig. 2a–c). The equations which describe the linear regression observed for Co are: $Co = 0.03 \pm 0.10 + 1.02 \pm 0.11$ As, $r = 0.91 \pm 0.04$ (Fig. 2a) and $Co = -0.19 \pm 0.20 + 1.24 \pm 0.23$ Se, $r = 0.79 \pm 0.06$ (Fig. 2c). In contrast, the correlation between Sn and the analytes was not satisfactory (Fig. 2b–d). Therefore, Co can be considered as the most appropriated choice to be used as IS for the simultaneous determination of As and Se in urine.

3.4. Co as IS for the simultaneous determination of As and Se in urine

Eight urine samples were analysed by using the optimized conditions (Table 1) and Co as IS. A certified reference material (Seronorm™ Trace Elements in Urine) were used to check the reliability of the proposed procedure and to evaluate the effectiveness of internal standardization for improving the analytical results obtained by SIMAAS.

Aqueous analytical reference solutions ranging from 5.0 to 100 μg L^{-1} for As(V) and Se(IV) and spiked Seronorm™ Trace Elements in Urine Blank were used for calibrating the spectrometer. The comparison of the calibration curves obtained from aqueous standards with those obtained from

Table 4
Comparison of the linear regression parameters of calibration curves obtained with aqueous standards and diluted urine solutions

IS	As			Se		
	b[a]	r[b]	b_{aq}/b_{sample}[c]	b[a]	r[b]	b_{aq}/b_{sample}[c]
Aqueous						
No	$(1.14 \pm 0.01) \times 10^{-3}$	0.9999 ± 0.0003	–	$(7.07 \pm 0.01) \times 10^{-4}$	0.9997 ± 0.0004	–
Yes	$(6.76 \pm 0.03) \times 10^{-3}$	0.9994 ± 0.0003	–	$(4.19 \pm 0.04) \times 10^{-3}$	0.9997 ± 0.0004	–
Urine 1 + 1						
No	$(7.51 \pm 0.06) \times 10^{-4}$	0.9998 ± 0.0005	1.52 ± 0.02	$(4.63 \pm 0.05) \times 10^{-4}$	0.9998 ± 0.0005	1.53 ± 0.02
Yes	$(4.03 \pm 0.03) \times 10^{-3}$	0.9995 ± 0.0006	1.68 ± 0.01	$(2.55 \pm 0.02) \times 10^{-3}$	0.9998 ± 0.0005	1.64 ± 0.02
Urine 1 + 2						
No	$(9.45 \pm 0.06) \times 10^{-4}$	0.9998 ± 0.0003	1.21 ± 0.01	$(6.09 \pm 0.03) \times 10^{-4}$	0.9999 ± 0.0004	1.16 ± 0.01
Yes	$(5.41 \pm 0.03) \times 10^{-3}$	0.9998 ± 0.0003	1.25 ± 0.01	$(3.49 \pm 0.01) \times 10^{-3}$	0.9999 ± 0.0003	1.20 ± 0.01

[a] Slope of the calibration curve.
[b] Regression coefficient.
[c] b_{aq}/b_{sample}: ratio between the slope obtained from aqueous standards and diluted sample solutions.

Table 5
Comparison of the results obtained for As with and without IS correction, when aqueous and matrix-matched standards were used for calibrating the spectrometer

	Calibration with aqueous standards								Calibration with matrix-matched standards							
	Without IS correction				With IS correction				Without IS correction				With IS correction			
	As ($\mu g\,L^{-1}$)	R.S.D. (%)	Rec 1[a] (%)	Rec 2[a] (%)	As ($\mu g\,L^{-1}$)	R.S.D. (%)	Rec 1[a] (%)	Rec 2[a] (%)	As ($\mu g\,L^{-1}$)	R.S.D. (%)	Rec 1[a] (%)	Rec 2[a] (%)	As ($\mu g\,L^{-1}$)	R.S.D. (%)	Rec 1[a] (%)	Rec 2[a] (%)
CRM[b]	84 ± 3	3.6	84	–	78 ± 2	2.6	78	–	93 ± 2	2.2	93	–	103 ± 2	1.9	103	–
A	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–
B	36 ± 1	2.8	87	77	35 ± 1	2.9	80	73	43 ± 1	2.3	105	82	52 ± 1	1.9	104	86
C	17 ± 1	5.9	70	79	17 ± 2	12	73	81	21 ± 2	9.5	82	95	22 ± 2	9.1	88	102
D	16 ± 3	19	63	57	17 ± 3	18	73	60	19 ± 3	16	100	94	21 ± 3	14	100	100
E	32 ± 1	3.1	80	79	34 ± 1	2.9	80	80	39 ± 1	2.6	87	88	45 ± 1	2.2	91	89
F	21 ± 1	4.8	67	64	21 ± 1	4.8	70	65	25 ± 1	4.0	77	74	32 ± 1	3.1	89	95
G	16 ± 4	25	63	63	16 ± 4	25	66	63	49 ± 4	8.2	87	91	53 ± 4	7.5	97	99
H	23 ± 1	4.4	60	61	24 ± 1	4.2	63	65	36 ± 1	2.8	79	79	39 ± 1	2.6	93	96
Mean	–	–	72 ± 10	69 ± 9	–	–	73 ± 6	70 ± 8	–	–	89 ± 10	86 ± 8	–	–	96 ± 6	95 ± 6

[a] Rec: recovery values obtained from spiked urine samples (Rec 1 = 10 $\mu g\,L^{-1}$ As(V) and Rec 2 = 25 $\mu g\,L^{-1}$ As(V)) and after comparing the results obtained for CRM with the recommended value.
[b] Recommended value: As = 100 $\mu g\,L^{-1}$ As.
[c] Less than the quantification limit (QL = 16 $\mu g\,L^{-1}$ As in undiluted urine).

Table 6
Comparison of the results obtained for Se with and without IS correction, when aqueous and matrix-matched standards were used for calibrating the spectrometer

	Calibration with aqueous standards								Calibration with matrix-matched standards							
	Without IS correction				With IS correction				Without IS correction				With IS correction			
	Se ($\mu g\,L^{-1}$)	R.S.D. (%)	Rec 1[a] (%)	Rec 2[a] (%)	Se ($\mu g\,L^{-1}$)	R.S.D. (%)	Rec 1[a] (%)	Rec 2[a] (%)	Se ($\mu g\,L^{-1}$)	R.S.D. (%)	Rec 1[a] (%)	Rec 2[a] (%)	Se ($\mu g\,L^{-1}$)	R.S.D. (%)	Rec 1[a] (%)	Rec 2[a] (%)
CRM[b]	27 ± 3	11	85	82	29 ± 3	10	80	79	33 ± 2	6.1	87	93	34 ± 2	5.9	90	96
A	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–
B	47 ± 5	11	67	71	48 ± 5	10	67	72	51 ± 2	3.9	93	95	52 ± 2	3.8	100	101
C	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–
D	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–
E	32 ± 3	9.4	77	81	30 ± 2	6.7	80	80	36 ± 2	5.6	104	95	48 ± 2	4.2	102	100
F	38 ± 2	5.3	70	64	40 ± 2	5.0	77	65	46 ± 3	6.5	104	94	47 ± 2	4.3	106	92
G	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–	<QL[c]	–	–	–
H	36 ± 1	2.8	77	73	38 ± 1	2.6	80	77	41 ± 3	7.3	110	103	43 ± 3	7.0	107	96
Mean	–	–	75 ± 7	74 ± 7	–	–	77 ± 6	75 ± 6	–	–	100 ± 9	96 ± 4	–	–	101 ± 7	97 ± 4

[a] Rec: recovery values obtained from spiked urine samples (Rec 1 = 10 $\mu g\,L^{-1}$ Se(IV) and Rec 2 = 25 $\mu g\,L^{-1}$ Se(IV)).
[b] There is no recommended value for Se.
[c] Less than the quantification limit (QL = 23 $\mu g\,L^{-1}$ Se in undiluted urine).

diluted urine (1 + 1 and 1 + 2) can be used to check the influence of matrix concomitants on the As and Se atomization. For this purpose, the slopes and regression coefficients obtained from the calibration graphs for aqueous solution and diluted samples, with and without the internal standardization, are shown in Table 4. In all situations, the regression coefficients (r) obtained were always higher than 0.999.

The comparison of the slopes (b) observed for the calibration graphs obtained from aqueous solution with those in presence of diluted urine can be use to estimate the effect caused by the matrix concomitants. In absence of matrix effect, the ratio between the slopes obtained from aqueous solution (b_{aq}) and diluted sample (b_{sample}) is equal to 1, and this condition ensure the suitability of using aqueous standards for calibrating the instrument.

The calculated b_{aq}/b_{sample} ratios indicated the presence of an intense matrix effect, impairing the atomization of both analytes. It was more severe when the urine was diluted only 1 + 1, considering the calculated ratios showed values higher than 1.5 (Table 4). The increase of the dilution factor to 1 + 2 reduced the matrix effect but it still forbid the calibration with aqueous standards, because the calculated ratios ranged from 1.16 up to 1.25 (Table 4). Moreover, the intense matrix effect caused by urine concomitants was not minimized by using Co as IS. The calculated b_{aq}/b_{sample} ratios with and without IS correction for urine 1 + 1 and 1 + 2 did not change significantly (Table 4).

Probably, the interference effect observed in presence of urine can be ascribed to phosphate and sulphate salts, which could not be decomposed or volatilized during the pyrolysis step. They cause severe interference on As and Se atomization, and pyrolysis temperatures as high as 1600 °C are indicated to minimize it [24,25]. On the other hand, the compromise condition for the simultaneous determination of As and Se restrains the pyrolysis temperature at 1400 °C and it is not enough to ensure the complete elimination of all inorganic matrix concomitants before the atomization step.

The expected amount of As and Se in urine samples for non-exposed individuals and the typical sensitivity obtained by ETAAS (Table 2) limited the dilution factor, which could not be higher than 1 + 2. In this context, the instrument calibration should be carried out using standards prepared in diluted urine (1 + 2). The matrix-matched strategy is indicated in this situation to avoid systematic errors [26], but the high chemical variability of different urine samples can still damage the analytical results. Therefore, the use of Co as IS can be useful to minimize the interference effect arisen from the chemical variability of different urine samples.

The results obtained after analysing the urine samples were presented in Tables 5 (As) and 6 (Se). The use of aqueous standards for calibrating the spectrometer caused a systematic error for both analytes. In spite of using Co as IS, the mean recoveries obtained for two different spikes in the samples (10 and 25 $\mu g\,L^{-1}$ As(V) and Se(IV)) ranged from 69 to 73% for As (Table 5) and from 74 to 77% for Se (Table 6). The matrix interference caused by urine concomitants seems to be slightly severe for As than for Se, considering the comparison of the recovery values. Hence, the simultaneous determination of As and Se cannot be carried out by using aqueous standards. On the other hand, the mean recoveries obtained when the calibration was done with matrix-matched standards were satisfactory for As and Se, considering the values ranged from 86 to 101% (Tables 5 and 6). Furthermore, the use of Co as IS improved the recoveries obtained for As: the mean values were between 86–89% and 95–96% without and with internal standardization, respectively (Table 5). The use of IS minimized the effect caused from the high chemical variability of different urine samples for As, which is susceptible to the matrix interference caused by the urine concomitants, mainly phosphate [24]. The results observed for Se, which is less affected by the matrix interference, did not benefit of the IS correction (Table 6).

3.5. Analytical figures of merit

The characteristic masses were calculated from the slope obtained with standards prepared in urine 1 + 2. They were 47 ± 1 pg for As and 72 ± 2 pg for Se. The detection limits (DL) were calculated based on the variability of the 20 measurements of the blank solution prepared in urine 1 + 2 according to 3 S_{blk}/b, where S corresponds to the blank measurement standard deviation and b the calibration curve slope. The found values were 1.8 ± 0.1 and 2.6 ± 0.1 $\mu g\,L^{-1}$ for As and Se, respectively. The quantification limit (QL) can be estimated from the DL values according to QL = kDL, where $k = 3$. Therefore, the QL values obtained for urine 1 + 2 were 5.4 $\mu g\,L^{-1}$ for As and 7.8 $\mu g\,L^{-1}$ for Se. These QL values are in agreement with the first standard adopted for the calibration curves (5.0 $\mu g\,L^{-1}$ for As and Se).

In spite of the time required to execute a complete heating cycle (99 s), the total time for carry out this analysis is shortened due to the fast sample pre-treatment (one dilution step) and the simultaneous capability of SIMAAS, which allows a two-fold increase in the analytical frequency compared with the monoelement ETAAS. Regarding the heating cycle time, it was possible to have 25 simultaneous determinations per hour. The atomizer presented good performance at least 700 heating cycles, and more than 1400 analytical results can be obtained, reducing costs associated with the replacement of graphite parts.

4. Conclusions

The use of Co as internal standard for determining As and Se simultaneously in by SIMAAS is useful for improving the accuracy of the results for As. The compromise condition limited the pyrolysis temperate to 1400 °C and impaired the volatilisation of some inorganic concomitants, mainly phosphate and sulphate salts. On the other hand, the matrix effects could be minimized for As and Se using matrix-matched stan-

dards for calibrating the spectrometer. Moreover, the internal standardization with Co improved the accuracy of the results obtained for As, which was more affected by the matrix interference than Se. This simultaneous method can be used for monitoring As and Se in urine for clinical purposes, with reduced instrument operation time and lower replacement of graphite tube, sample and reagent consumption.

The compulsory adoption of compromise conditions for simultaneous determinations by SIMAAS, in combination with high complex matrices from non-digested samples can deteriorate the accuracy and precision of the analytical data. In this context, the use of an adequate IS can attenuate the damages on the results. The selection of the most appropriate element to be used as IS can be supported by correlation graphs. Further studies involving internal standardization in SIMMAS should be carried out using other analytes with different IS candidates in other complex matrices.

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