Analytical Methods

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2019, **11**, 3401

Calibration strategies to correct for matrix effects in direct analysis of urine by ICP OES: internal standardization and multi-energy calibration†

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This study investigated internal standardization and multi-energy calibration (MEC) as strategies for direct analysis of urine by inductively coupled plasma optical emission spectrometry (ICP OES). Gallium, Ge, Pd, Sc and Y were evaluated as internal standards (ISs) for determination of Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Hg, K, Li, Mg, Na, Pb, Sb and Zn in urine. The accuracy and precision were evaluated by addition–recovery experiments and the best results were obtained when Ge or Pd was used as IS. Recoveries ranged from 80 to 120% and relative standard deviations for all elements and samples were lower than 7.5%, except in the case of the lowest analyte levels (0.025 and 0.050 mg L^{-1}). Recoveries varying from 80 to 120% were obtained only for the highest levels tested (>4.0 mg L^{-1}) when using MEC. The LODs obtained by internal standardization were lower than the LODs obtained by MEC. Both calibration methods were suitable to correct for matrix effects, making feasible the direct analysis of urine by ICP OES, without any sample preparation.

Received 29th April 2019
Accepted 13th June 2019

DOI: 10.1039/c9ay00907h

rsc.li/methods

1. Introduction

Human urine analysis is a convenient indicator for monitoring toxic element exposure from the environment or workplace. However, determination of elemental impurities in urine samples is challenging due to the sample complexity.[1] Urine usually contains high levels of total dissolved solids (TDS), such as urea, sodium, potassium, uric acid, bicarbonate and chloride.[2] These matrix constituents can cause severe matrix effects in elemental determination. Matrix effects are commonly intensified in direct analysis for spectrochemical techniques.[3]

Burden *et al.*[4] developed a method for Al determination by inductively coupled plasma optical emission spectrometry (ICP OES) in urine samples. Samples were 2-fold diluted with 0.22 mol L^{-1} nitric acid and warmed up to 40 °C. In another study, direct analysis and simultaneous determination of Ca, Cl, K, Mg, Na and P in human urine by ICP OES was further evaluated. However, these authors did not comment on the use of any strategy for matrix effect correction.[5]

Matrix effects can affect the nebulization step, transport processes and plasma energy. The difference in viscosity between the sample and aqueous standard solutions may cause transport interference. On the other hand, the presence of elevated concentrations of easily ionizable elements produces free electrons and affects plasma conditions.[6] Some of these effects may be corrected either by adjusting the instrument operational conditions or by tailored calibration strategies. The standard addition method (SAM), internal standardization and multi-energy calibration (MEC) are alternative calibration strategies with diverse applicability to correct for matrix effects.[3]

The SAM may correct for matrix effects since calibration solutions are prepared in the same matrix as the samples. However, it is a time-consuming method when considering the analysis of a high number of samples.[7] On the other hand, when using the recently proposed MEC method, only two calibration solutions are required per sample, of which one is composed of 50% v/v sample and 50% v/v standard solution containing the analytes and the other is composed of 50% v/v sample and 50% v/v analytical blank solution. Multi-energy calibration is performed by monitoring the instrument response at several wavelengths for each analyte, for example, by ICP OES[8] or microwave induced plasma optical emission spectrometry (MIP OES).[8,9] The multi-energy calibration method has been applied to different atomic spectrometric techniques and accuracies as good as those obtained using traditional external calibration, internal standardization and SAM methods were reached.[8] Six analytes were determined in complex samples by ICP OES, MIP OES and high resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS) applying MEC.[8] It was also applied for determination of As, Ba, Cd, Cr, and Pb in fertilizer samples.[9]

Another calibration method based on the same principles as those of MEC, named multi-isotope calibration (MICal), was applied to the determination of Ba, Cd, Se, Sn, and Zn in seven

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9ay00907h

certified reference materials. In this case, several natural isotopes of the same element were measured by inductively coupled plasma mass spectrometry (ICP-MS) and calibration was based on two solutions.[10] Recently, researchers described a calibration method, multispecies calibration (MSC),[11] based on the same principles as those of both MEC and MICal, used in combination with inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) to determine monoisotopic analytes such as As, Co and Mn, since MICal is limited for them. Using ICP-MS/MS, different chemical derivatives were generated in the reaction chamber and further separated and detected.[11]

Chan and Hieftje[12,13] proposed a cross-over point method to overcome matrix effects in ICP OES. The cross-over point can be determined by plotting the relative intensities obtained in undiluted and diluted samples (y-axis) at different observation heights (x-axis). According to the authors, at only one location is the extent of the matrix effect between the undiluted sample and diluted sample identical (zero) and this location is the cross-over point. Calculations will be performed at this cross-over point. The advantages of this method are the compensation for matrix effects occurring in the plasma and that there is no need to have prior information about the samples. However, this approach does not correct for transport effects.

Alternatively, using internal standardization, the intensity ratios between the analyte and IS spectral lines are used to plot the calibration curve. Ideally the IS should be affected by the same processes that the analyte is subjected to during the analysis, and for ICP OES, these processes are related to nebulization, transport, atomization, excitation and/or ionization. Consequently, matrix effects can be corrected.[3,6] The selected IS must be an element not present in the sample, so the selected IS is added to all samples, reference solutions and analytical blanks at known concentrations, preferably in the same concentration range as the analytes. It is expected that the IS will act as a control during sample nebulization, sample transport and plasma processes. Thus, ideally the ratio between analyte and IS signals corrects for possible fluctuations occurring during the analysis and consequently matrix effects are compensated for.[3,14] The use of Y as IS in ICP OES determinations is commonly reported in the literature.[15–19] Yttrium was useful in correcting for effects caused by drift in the plasma background level[15] and for signal compensation for determination of B and Ti in biological samples.[16] Yttrium was also effective as IS for accurate determination of Mn and Fe in bottled coconut water[17] and used as IS to compensate for transport interference in biodiesel analysis for determination of Ca, Cu, Fe, Mg, Mn, Na, and P by ICP OES.[18] However, other ISs have been used, such as Sc for Mn;[20] Ga for Al, Mg and Si; Cd for Ca and Fe; and Li for Na,[21] among others.

In this work, two calibration methods (internal standardization and MEC) were evaluated for direct determination of Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Hg, K, Li, Mg, Na, Pb, Sb and Zn in non-diluted urine samples. In order to ensure minimal matrix effects, the concentrations of standards applied in MEC and several ISs were studied for direct analysis of trace elements in urine.

2. Materials and methods

2.1. Instrumentation

Analyses were performed using an iCAP 6000 ICP OES (Thermo Fisher Scientific, Waltham, MA, USA) operated in axial view and under robust conditions. Argon (99.996%, White Martins-Praxair, Sertãozinho, SP, Brazil) was used in all measurements. The plasma operating conditions are described in Table 1 and the measured emission lines for each element are presented in Table 2.

2.2. Reagents, standard solutions and samples

Experiments were performed using HNO_3 (Synth, Diadema, SP, Brazil) purified in a sub-boiling distillation apparatus, Distillacid™ BSB-939-IR (Berghof, Eningen, Germany), and ultrapure water with resistivity >18.2 MΩ cm (Milli-Q®, Millipore, Bedford, MA, USA). Standard solutions used for ICP OES calibration and for addition and recovery experiments were prepared by diluting 1000 mg L^{-1} Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Hg, K, Li, Mg, Na, Pb, Sb, and Zn (Qhemis, São Paulo, Brazil) in 0.14 mol L^{-1} HNO_3 medium, as well as the ISs evaluated: Ga, Ge, Pd, Rh, Sc and Y.

The concentrations of the solutions used for calibration of Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Hg, Li, Pb, Sb, and Zn were 0, 0.025, 0.050, 0.10, 0.20, 0.40, 0.60 and 1.0 mg L^{-1}, and for Ca, K, Mg and Na, they were 0, 10, 20, 40, 60 and 100 mg L^{-1} prepared either in aqueous medium (0.14 mol L^{-1} HNO_3) or urine medium. In order to evaluate matrix effects, urine samples were not diluted for Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Hg, Li, Pb, Sb and Zn, 10-fold diluted for Na and K and 20-fold diluted for Ca and Mg. For constructing calibration curves in urine medium, intensities obtained in the analytical blank solution were subtracted from intensities obtained in the other calibration solutions. For the internal standardization method, 0.2 mg L^{-1} of each IS was added to the reference solutions, analytical blank and samples for Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Hg, Li, Pb, Sb and Zn and 40 mg L^{-1} of each IS for Ca, K, Mg and Na. Urine samples were provided by healthy individuals.

For the MEC method, calibration curves were obtained using two solutions.[8,9] Solution 1 was composed of 50% v/v urine sample and 50% v/v standard solution containing Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Hg, Li, Pb, Sb, and Zn prepared in 1% v/v HNO_3. Solution 2 contained 50% v/v urine sample and 50% v/v analytical blank, prepared in 1% v/v HNO_3. Taking into account the high levels of Ca, K, Mg and Na in urine samples,

Table 1 Operational conditions for ICP OES measurements

Instrument parameters	Operating conditions
Integration time (s)	15
Sample introduction flow rate (mL min^{-1})	1.0
Pump stabilization time (s)	5.0
RF applied power (kW)	1.2
Auxiliary gas flow rate (L min^{-1})	0.50
Nebulizer gas flow rate (L min^{-1})	0.50
Plasma gas flow rate (L min^{-1})	12

Table 2 Elements and wavelengths for ICP OES measurements using internal standardization and MEC[a]

Elements and wavelengths (nm)		
Analyte	Internal standardization	MEC
Al	167.079 II; 309.271 I	167.079; 185.593; 220.459; 226.910; 236.705; 237.312; 256.798; 266.039; 308.215; 309.271
As	188.979 I	188.979; 193.759; 197.262; 234.984
Ba	455.403 II	225.473; 230.424; 233.527; 234.758; 413.066; 455.403; 493.409
Be	313.042 II	234.861; 249.473; 265.045; 313.042; 332.134
Bi	223.061 I	190.234; 195.471; 222.822; 223.061; 306.770
Ca	317.933 II	183.801; 184.006; 315.887; 317.933; 318.128; 370.603; 373.690; 422.673; 431.865
Cd	228.802 I; 226.502 II	214.438; 226.502; 228.802; 326.106; 361.051
Co	228.615 II	195.742; 228.616; 230.786; 235.342; 237.862; 238.892
Cr	357.870 I; 283.563 II	205.560; 206.550; 266.602; 267.716; 276.654; 283.563; 284.325; 298.919; 318.070; 357.870; 359.349; 360.533; 425.435
Cu	324.754 I; 224.700 II	204.379; 211.209; 217.894; 219.958; 221.810; 224.700; 324.754; 327.396
Ga	294.363 I	NA
Ge	265.117 I	NA
Hg	184.949 I; 194.163 II	184.949; 194.163; 253.652
K	766.489 I	404.721; 511.225; 533.969; 578.238; 581.215; 583.189; 691.108; 766.489; 769.896
Li	670.784 I	323.263; 460.286; 610.362; 670.784
Mg	280.270 II	202.582; 279.078; 279.553; 279.799; 280.270; 285.213; 382.935
Na	818.325 I	314.928; 316.325; 318.979; 328.560; 568.819; 588.995; 589.594; 818.325
Pb	220.353 II	168.215; 182.205; 216.999; 220.353; 261.418; 280.199; 283.306
Pd	340.457 I	NA
Rh	233.477 II	NA
Sc	361.383 II	NA
Sb	206.833 I; 217.581 I	204.957; 206.833; 217.581; 252.852; 259.805
Zn	213.856 I; 202.548 II	202.548; 206.200; 213.856; 328.233; 330.259; 334.502; 472.216; 481.053
Y	371.028 II	NA

[a] Lines: I – atomic line; II – ionic line; NA: not applicable. A fixed emission line was kept for IS.

the urine samples used were 50-fold diluted for K and Na, and 10-fold diluted for Ca and Mg.

Accuracies were evaluated by addition and recovery experiments. For the internal standardization method, different concentration levels were evaluated: 0.025, 0.10, 0.20 and 1.0 mg L^{-1} for Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Hg, Li, Pb, Sb and Zn in urine samples without dilution and 10, 20, 40 and 100 mg L^{-1} Ca, K, Mg and Na in urine samples 100-fold diluted for K and Na, and 20-fold diluted for Ca and Mg. For MEC, solution 2 was prepared using 50% v/v urine sample with addition of different concentrations (0.10, 0.20 and 0.40 mg L^{-1}) of Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Hg, Li, Pb, Sb and Zn and 50% v/v analytical blank, as exemplified in Fig. 1. The mathematical treatment used to determine the analyte concentration in the sample considers the following relationships proposed by Virgilio *et al.*:[8]

$$I(\lambda_i)^{(\text{Sample + Standard})} = m(C^{\text{Sample}} + C^{\text{Standard}}) \quad (1)$$

$$I(\lambda_i)^{\text{Sample}} = mC^{\text{Sample}} \quad (2)$$

$$\frac{I(\lambda_i)^{\text{Sample}}}{C^{\text{Sample}}} = \frac{I(\lambda_i)^{(\text{Sample+Standard})}}{C^{\text{Sample}} + C^{\text{Standard}}} \quad (3)$$

$$I(\lambda_i)^{\text{Sample}} = I(\lambda_i)^{(\text{Sample+Standard})\left[\frac{C^{\text{Sample}}}{C^{\text{Sample}}+C^{\text{Standard}}}\right]} \quad (4)$$

$$\text{Slope} = \frac{C^{\text{Sample}}}{C^{\text{Sample}} + C^{\text{Standard}}} \quad (5)$$

$$C^{\text{Sample}} = \frac{\text{Slope} \times C^{\text{Standard}}}{(1 - \text{Slope})} \quad (6)$$

where $I(\lambda_i)^{\text{Sample+Standard}}$ and $I(\lambda_i)^{\text{Sample}}$ are the instrument responses at wavelength (i), m is a proportionality constant and C^{Sample} and C^{Standard} are the analyte concentrations in the sample and in the standard solution.

For Zn, two additional concentration levels were evaluated (2.0 and 3.0 mg L^{-1}). For Ca, K, Mg and Na, solution 2 was

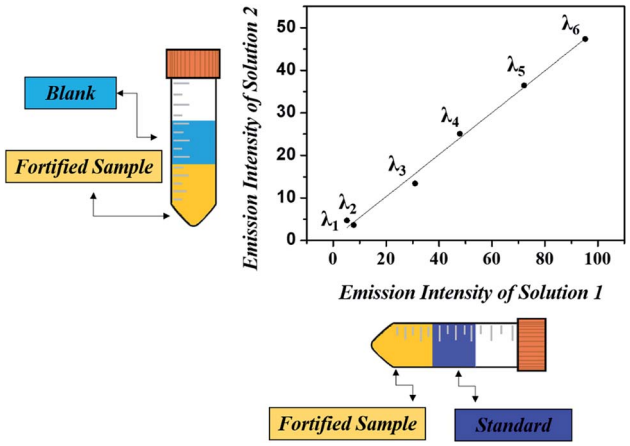

Fig. 1 Schematic representation of the addition and recovery experiments for MEC.

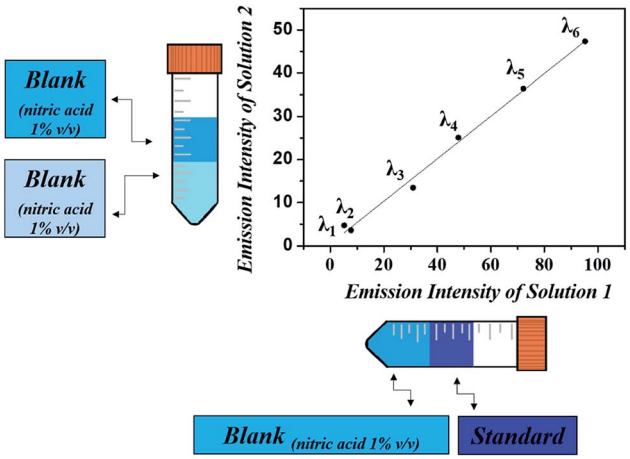

Fig. 2 Schematic representation of the experiments performed to calculate the LOD and LOQ for MEC.

prepared using 50% v/v urine sample diluted with additions of different concentrations (10, 20 and 40 mg L^{-1}) and 50% v/v analytical blank.

The limits of detection (LOD) and quantification (LOQ) were calculated according to IUPAC's recommendations considering three times and 10 times the standard deviation of 10 measurements from the blank for internal standardization and 10 estimated concentrations obtained from the blank solutions for MEC. For the calculation of LODs using the MEC method, ten calibration curves were constructed. Solution 2 was composed of 50% v/v blank plus 50% v/v blank, *i.e.* HNO_3 (1% v/v), and solution 1 was composed of 50% v/v blank plus 50% v/v standard solution containing 0.20 mg L^{-1} of all elements as exemplified in Fig. 2.[8,9]

3. Results and discussion

Usually for determinations using ICP OES, the maximum TDS allowed is about 3% m/v. However, when using sample introduction systems able to deal with high solids contents, it is possible to introduce up to 20% m/v.[22] However, effects on sample nebulization, aerosol transport, quartz torch, and plasma properties should be carefully assessed. Consequently, we evaluated the possibility of direct analysis of urine samples, since the TDS in urine samples is around 3% m/v.[2] On the other hand, the direct introduction of complex samples with high TDS can induce severe matrix effects. These effects were evaluated by obtaining analytical curves in both aqueous and urine media. Considering the values of slopes obtained in both media (Table 3), it is possible to point out that matrix effects occurred for all elements, except in the case of Ca and K, since it was observed that there were significant differences (Student's *t*-test at the 99.9% confidence level) among the slopes obtained in aqueous and urine media.

It was expected that the presence of high concentrations of EIEs would mainly affect elements with high ionization energies compared to elements with low ionization energies, as observed for As (9.8 eV), Be (9.3 eV), Bi (7.2 eV), Cd (8.9 eV), Hg (10.4 eV), Sb (8.6 eV) and Zn (9.3 eV), which showed significant differences in the slopes obtained in aqueous and urine media without IS; the differences were 1.1, 1.5,1.4, 1.5, 1.6, 1.5 and 1.5-fold, respectively (Table 3). However, some elements with low ionization energies also suffered from matrix effects. For example, Al (5.9 eV), Ba (5.21 eV) and Li (5.39 eV) showed significant differences in the slopes in aqueous and urine media (1.6, 1.1 and 1.6-fold, respectively).

Solutions with high carbon concentrations may cause changes in the plasma characteristics and consequently in the species distribution in the argon plasma. Grindlay *et al.*[23] showed that sensitivities for As, Hg and Sb were higher for carbon-containing solutions than for solutions without carbon. These authors explained matrix effects for these elements with charge transfer reactions among C^+ and each respective element in the plasma. Other elements, such as Cd and Pb, could also be involved in carbon based charge transfer reactions.[23,24]

However, Be and Zn showed similar differences between the slopes to As, Cd, Hg and Sb. Thus, it is supposed that matrix effects are related to physical differences between aqueous and urine media causing transport effects. The presence of either easily ionizable elements or carbon seems to cause major effects. In this context as previously mentioned, two calibration methods (internal standardization and MEC) were evaluated to correct for these matrix effects. For comparison purposes, application of internal standardization and MEC was also evaluated for Ca and K.

3.1. Internal standardization

For internal standardization, the choice of IS for ICP OES measurements is made by comparison of the physico-chemical properties, such as ionization energy, of the analyte and respective IS. It is supposed that, when similar, both will be affected similarly by the same processes during the analysis. Another point of discussion is the choice of either one atomic line of the IS for correcting for atomic lines of analytes or one ionic line to correct for effects on ionic lines. Thus, analytes and

Table 3 Slopes and linear correlation coefficients obtained in 0.14 mol L^{-1} HNO_3 and urine media by ICP OES with and without IS[a]

		Without IS			With selected IS	
Analyte – wavelength (nm)	Matrix	Slope ± SD	r	IS	Slope ± SD	r
Al – 167.079 (II)	Aqueous	0.508 ± 0.006	0.9992	Ge	$0.00217 \pm 3 \times 10^{-5}$	0.9989
	Urine	0.80 ± 0.01	0.9983		$0.00240 \pm 5 \times 10^{-5}$	0.9972
As – 188.979 (I)	Aqueous	$8.7 \times 10^{-4} \pm 1 \times 10^{-5}$	0.9990	Pd	$3.00 \times 10^{-4} \pm 3 \times 10^{-6}$	0.9989
	Urine	$9.9 \times 10^{-4} \pm 1 \times 10^{-5}$	0.9985		$3.30 \times 10^{-4} \pm 4 \times 10^{-6}$	0.9991
Ba – 455.403 (II)	Aqueous	262 ± 4	0.9986	Ge	1.09 ± 0.009	0.9995
	Urine	238 ± 1	0.9999		1.03 ± 0.008	0.9996
Be – 313.042 (II)	Aqueous	239.7 ± 0.8	0.9999	Pd	0.204 ± 0.002	0.9996
	Urine	155 ± 2	0.9991		0.191 ± 0.002	0.9994
Bi – 223.061 (I)	Aqueous	0.388 ± 0.003	0.9997	Ge	$0.00128 \pm 2 \times 10^{-5}$	0.9988
	Urine	0.526 ± 0.002	0.9999		$0.00122 \pm 1 \times 10^{-5}$	0.9989
Ca – 317.933 (II)	Aqueous	8458 ± 98	0.9993	Ga	0.642 ± 0.007	0.9997
	Urine	8561 ± 96	0.9993		0.661 ± 0.007	0.9997
Cd – 226.502 (II)	Aqueous	7.27 ± 0.07	0.9993	Ge	$0.0315 \pm 2 \times 10^{-4}$	0.9994
	Urine	10.8 ± 0.09	0.9995		$0.0335 \pm 2 \times 10^{-4}$	0.9998
Cd – 228.802 (I)	Aqueous	6.78 ± 0.08	0.9989	Ge	$0.0289 \pm 2 \times 10^{-4}$	0.9990
	Urine	9.8 ± 0.1	0.9993		$0.0299 \pm 2 \times 10^{-4}$	0.9995
Co – 228.615 (II)	Aqueous	5.36 ± 0.03	0.9997	Ge	$0.0178 \pm 1 \times 10^{-4}$	0.9996
	Urine	7.85 ± 0.03	0.9999		$0.0186 \pm 2 \times 10^{-4}$	0.9991
Cr – 283.563 (II)	Aqueous	0.948 ± 0.005	0.9997	Ge	$0.00129 \pm 3 \times 10^{-5}$	0.9956
	Urine	1.70 ± 0.02	0.9999		$0.00121 \pm 2 \times 10^{-5}$	0.9986
Cr – 357.870 (I)	Aqueous	3.89 ± 0.01	0.9999	Pd	$0.01300 \pm 7 \times 10^{-5}$	0.9998
	Urine	5.36 ± 0.02	0.9999		$0.0129 \pm 1 \times 10^{-4}$	0.9991
Cu – 224.700 (II)	Aqueous	1.587 ± 0.005	0.9999	Ge	$0.00523 \pm 4 \times 10^{-5}$	0.9997
	Urine	2.261 ± 0.007	0.9999		$0.00533 \pm 6 \times 10^{-5}$	0.9990
Cu – 324.754 (I)	Aqueous	6.15 ± 0.03	0.9998	Ge	$0.0204 \pm 2 \times 10^{-4}$	0.9995
	Urine	9.24 ± 0.05	0.9998		$0.0220 \pm 3 \times 10^{-4}$	0.9989
Hg – 184.949 (I)	Aqueous	1.091 ± 0.003	0.9999	Pd	$0.00144 \pm 2 \times 10^{-5}$	0.9989
	Urine	1.79 ± 0.02	0.9990		$0.00139 \pm 4 \times 10^{-5}$	0.9954
Hg – 194.163 (II)	Aqueous	0.467 ± 0.002	0.9998	Pd	$6.17 \times 10^{-4} \pm 8 \times 10^{-6}$	0.9989
	Urine	0.774 ± 0.004	0.9998		$6.02 \times 10^{-4} \pm 9 \times 10^{-6}$	0.9986
K – 766.489 (I)	Aqueous	25 846 ± 577	0.9982	Pd	0.61 ± 0.01	0.9991
	Urine	28 105 ± 532	0.9975		0.63 ± 0.01	0.9992
Li – 670.784 (I)	Aqueous	762 ± 14	0.9977	Pd	1.01 ± 0.01	0.9993
	Urine	1254 ± 5	0.9999		1.005 ± 0.009	0.9994
Mg – 280.270 (II)	Aqueous	103 370 ± 2053	0.9988	Pd	2.44 ± 0.07	0.9992
	Urine	94 726 ± 2930	0.9971		2.32 ± 0.07	0.9991
Na – 818.325 (I)	Aqueous	765 ± 20	0.9963	Ga	0.060 ± 0.003	0.9954
	Urine	898 ± 26	0.9955		0.061 ± 0.002	0.9970
Pb – 220.353 (II)	Aqueous	0.59 ± 0.02	0.9999	Ge	$0.00195 \pm 2 \times 10\text{-}^{5}$	0.9996
	Urine	0.80 ± 0.05	0.9998		$0.00191 \pm 2 \times 10^{-5}$	0.9989
Sb – 206.833 (I)	Aqueous	0.274 ± 0.003	0.9993	Ge	$8.6 \times 10^{-4} \pm 1 \times 10^{-5}$	0.9981
	Urine	0.411 ± 0.006	0.9986		$8.9 \times 10^{-4} \pm 2 \times 10^{-5}$	0.9965
Sb – 217.581 (I)	Aqueous	0.114 ± 0.002	0.9987	Pd	$8.6 \times 10^{-4} \pm 2 \times 10^{-5}$	0.9939
	Urine	0.088 ± 0.001	0.9987		$8.5 \times 10^{-4} \pm 2 \times 10^{-5}$	0.9944
Zn – 202.548 (II)	Aqueous	5.99 ± 0.02	0.9999	Ge	$0.0196 \pm 4 \times 10^{-4}$	0.9977
	Urine	9.13 ± 0.08	0.9994		$0.02192 \pm 6 \times 10^{-4}$	0.9941
Zn – 213.856 (I)	Aqueous	6.11 ± 0.04	0.9997	Ge	$0.0140 \pm 2 \times 10^{-4}$	0.9991
	Urine	4.24 ± 0.01	0.9999		$0.0143 \pm 4 \times 10^{-4}$	0.9937

[a] Lines: I – atomic line; II – ionic line.

IS with different ionization energies, as well as atomic and ionic lines, were evaluated.

The ISs which provided better similarities between the slopes of aqueous and urine media were Ga, Ge, Pd, Sc and Y for Al, Ba, Be, Cd, Cu and Zn (202.548 nm), and Ga, Ge and Y for Bi, Co, Cr (283.563 nm), Cu, Pb and Zn (213.856 nm). For Sb (206.833 nm), Ca, K, Mg and Na, the most suitable ISs were Ga, Ge and Pd and the best ISs for Hg and Li were Pd and Sc. Only Pd was efficient in correcting for matrix effects on Sb (217.581 nm). The slopes and linear correlation coefficients obtained in aqueous and urine media with the chosen IS are shown in Table 3.

Considering the values of ionization energies of analytes and ISs (Table S1†), and based on recoveries, the best IS for Bi 223.061 nm (I) and Co 228.615 nm (II) was Ge 265.117 nm (I), in agreement with the physico-chemical properties, since the

emission lines and ionization energies, 7.28, 7.88 and 7.89 eV for Bi, Co and Ge, respectively, are close. However, based on this statement, Y would be the best IS for Ba since both ionic lines (II) have ionization energies (6.21 and 5.21 eV) and wavelengths (371.028 and 455.403 nm) not very far from each other, compared to other ISs. However, based on recoveries, the best IS for Ba was also Ge. This is in disagreement with criteria based on similar physico-chemical properties between the analyte and the internal standard because the ionization energies (5.21 and 7.89 eV), wavelengths (455.403 and 265.117 nm) and even the type of process (ionic line *versus* atomic line) are quite different. Thus, it is possible to observe that there is no clear relation among physico-chemical properties, as well as no clear relation between atomic or ionic lines.

Chiweshe *et al.*[25] evaluated criteria adopted in the literature and they were not useful in identifying the best IS and consequently choices were made based on recoveries obtained from the analysis of certified reference materials. In agreement with these authors, the choice of the best IS was made taking into account recovery results.

Using internal standardization, no significant differences (Student's *t*-test at the 99.9% confidence level) were observed among slopes in aqueous and urine media for Al, Ba, Be, Bi, Ca, Cd (228.802 nm), Cr, Cu, Hg, K, Li, Mg, Na, Sb and Zn. Though close slopes were obtained in both media for As and Cd (226.502 nm), a significant difference (Student's *t*-test at the 99.9% confidence level) was observed due to the low standard deviation. However, good recoveries were obtained in addition and recovery experiments.

The accuracy of the analytical procedure was evaluated by addition and recovery experiments in a non-diluted (only diluted for Ca, K, Mg and Na) urine sample using external calibration (EC) and internal standardization both in aqueous solutions. Recoveries are shown in Table 4. Germanium was the best IS for Al, Ba, Bi, Cd, Co, Cr (357.870 nm), Cu, Pb, Sb (206.833 nm) and Zn. For As, Be, Cr, Hg, K, Li, Mg and Sb (217.581 nm), the best IS was Pd. Gallium was the best IS for Ca and Na. With few exceptions at the lowest concentration levels (0.025 and 0.050 mg L^{-1}), recoveries with IS ranged from 81 to 116%.

Recoveries without IS were outside this interval range, except in the case of Ca, K, Mg and Na. For Cr, Cu, Hg, Sb and Zn, with two emission lines evaluated, the best recoveries were obtained for the following lines: 357.870 nm (Cr), 224.700 nm (Cu), 184.949 nm (Hg), 206.833 nm (Sb), and 213.856 nm (Zn). Though matrix effects affected Mg and Na, recoveries from 80 to 120% were obtained without internal standardization. It is important to highlight that the urine sample for Ca, K, Mg and Na determination was diluted due to the presence of these elements at high concentrations (higher than 50 mg L^{-1}). Consequently, dilution led to lower matrix effects.

Table 4 Recoveries and relative standard deviations (%) for Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Hg, K, Li, Mg, Na, Pb, Sb and Zn in direct urine sample determined by ICP OES with and without IS[a]

	Recovery (RSD) (%)											
	Al – 167.079 nm		As – 188.979 nm		Ba – 455.403 nm		Be – 313.042 nm		Bi – 223.061 nm		Ca – 317.933 nm	
Level** (mg L^{-1})	Without IS	With Ge	Without IS	With Pd	Without IS	With Ge	Without IS	With Pd	Without IS	With Ge	Without IS	With Ga
1	162 (2)	122 (4)	<LOQ*	<LOQ*	126 (1)	95 (3)	145 (1)	94 (2)	<LOQ*	75 (8)	103 (4)	96 (3)
2	144 (1)	108 (3)	151.0 (0.7)	107 2)	124 (1)	93 (3)	152.0 (0.1)	93 (1)	130 (2)	88 (2)	106 (2)	98 (1)
3	153.0 (0.6)	114 (3)	154 (2)	107 (2)	126.0 (0.3)	93 (2)	150.0 (0.1)	96 (1)	129 (2)	95 (2)	109 (2)	101 (1)
4	149.0 (0.6)	112 (2)	158.0 (0.7)	109 (1)	126.0 (0.4)	95 (2)	155 (1)	93 (2)	134.0 (0.5)	92 (1)	105 (2)	101 (2)
	Cd – 226.502 nm		Co – 228.615 nm		Cr – 357.870 nm		Cu – 224.700 nm		Hg – 194.163 nm		K – 766.489 nm	
Level** (mg L^{-1})	Without IS	With Ge	Without IS	With Ge	Without IS	With Ge	Without IS	With Ge	Without IS	With Ge	Without IS	With Pd
1	145.0 (0.5)	109 (8)	140 (1)	105 (1)	74 (14)	51 (5)	132 (1)	98 (2)	92 (7)	52 (2)	90 (3)	84 (2)
2	145.0 (0.4)	108 (2)	148.0 (0.3)	103 (1)	131.0 (0.7)	90 (1)	143.0 (0.7)	99 (1)	137 (1)	82 (2)	106 (2)	98 (1)
3	144.0 (0.4)	107 (3)	145.0 (0.4)	108.0 (0.7)	133.0 (0.6)	99 (1)	141.0 (0.6)	104 (1)	152.0 (0.6)	97 (1)	109.0 (0.5)	101 (1)
4	145.0 (0.3)	109.0 (0.3)	145.0 (0.4)	100 (1)	140 (1)	97 (2)	142.0 (0.2)	97 (1)	162.0 (0.3)	99 (1)	105 (2)	101 (1)
	Li – 670.784 nm		Mg – 280.270 nm		Na – 818.325 nm		Pb – 220.353 nm		Sb – 206.833 nm		Zn – 202.548 nm	
Level** (mg L^{-1})	Without IS	With Pd	Without IS	With Pd	Without IS	With Ga	Without IS	With Ge	Without IS	With Ge	Without IS	With Ge
1	152.0 (0.6)	101 (1)	113 (4)	108 (2)	97 (9)	78 (12)	<LOQ*	<LOQ*	313 (4)	<LOD*	133 (1)	132 (1)
2	159.0 (0.1)	97 (1)	108 (3)	105 (1)	106 (8)	88 (12)	141.0 (0.4)	94 (2)	177 (2)	78 (3)	154.0 (0.1)	97 (1)
3	157.0 (0.2)	101 (1)	101 (2)	99 (2)	109 (6)	92 (12)	136.0 (0.4)	99 (1)	157 (2)	95 (3)	150.0 (0.3)	116.0 (0.6)
4	160 (1)	96 (2)	—	—	121 (7)	102 (12)	138.0 (0.4)	94 (1)	152.0 (0.5)	103 (1)	156.0 (0.4)	107 (1)

[a] *The LOD values are shown in Table 6. **Level 1: 10 mg L^{-1} for Ca, K, Mg and Na and 0.025 mg L^{-1} for the other elements. **Level 2: 20 mg L^{-1} for Ca, K, Mg and Na and 0.10 mg L^{-1} for the other elements. Level 3: 40 mg L^{-1} for Ca, K, Mg and Na and 0.20 mg L^{-1} for the other elements. Level 4: 100 mg L^{-1} for Ca, K, Mg and Na and 1.0 mg L^{-1} for the other elements. — outside the linear range. ND: not determined.

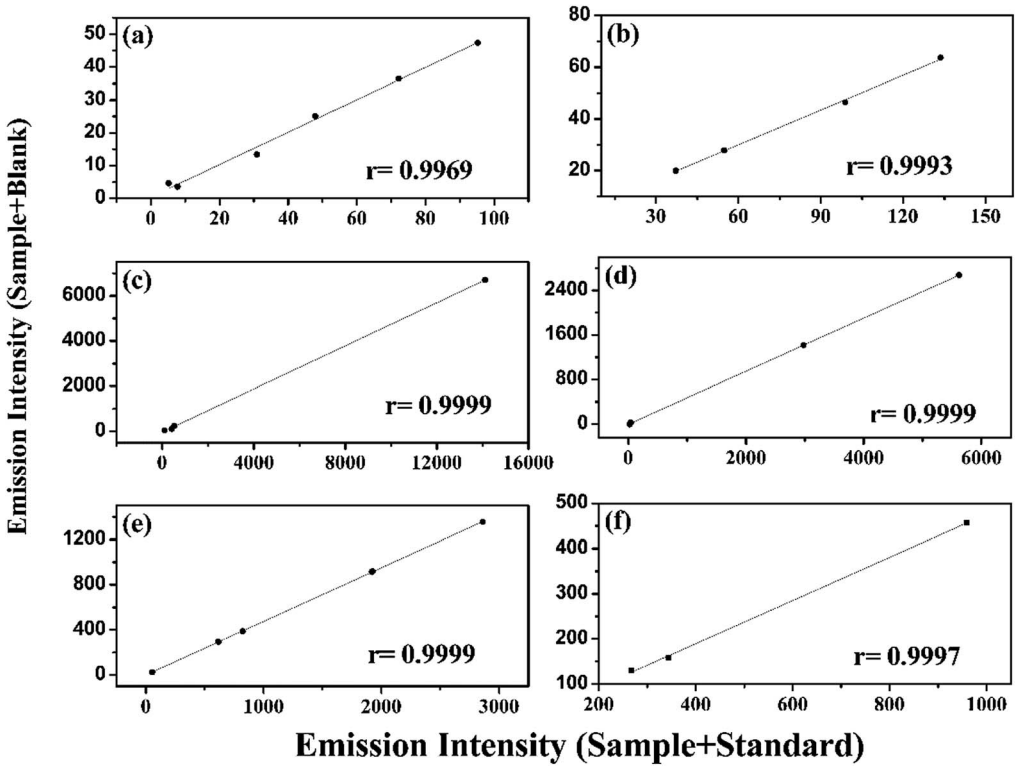

Fig. 3 Multi-energy calibration curves for (a) Al, (b) As, (c) Be, (d) Cd, (e) Co, and (f) Hg in urine sample obtained by ICP OES. Each point represents a different emission line for each respective analyte.

3.2. Multi-energy calibration

Multi-energy calibration was also tested to correct for matrix effects as a simple alternative to the SAM. As previously mentioned, MEC requires only two solutions for calibration. Since the sample matrix is present and constant in both solutions, matrix effects are corrected. Generally, application of MEC requires that the analyte presents at least three lines with good relative sensitivities and it is also important to have an estimate of the expected analyte concentration. This information allows the preparation of "Solution 1" with a concentration about two-fold the analyte concentration.[8] The MEC curves are shown in Fig. 3, S1 and S2.† The coefficients of correlation for all elements were higher than 0.99, even for elements with only three available lines (Hg and Li).

Multi-energy calibration allows the identification of spectral interference or lower intensity lines by observing anomalous points in the calibration plots. Hence, the measured wavelengths that showed this behavior were excluded for obtaining the analytical curves. Lower intensity lines were deleted for Al at 220.459 nm, Cr at 318.070 nm, Cd at 326.106 nm, Co at 195.742 nm, and Li at 323.263 nm. Also, emission lines for K at 533.969, 578.238, 581.215 and 583.189 nm and emission lines for Na at 316.325 and 318.979 nm were deleted. Possible spectral interferences for the deleted wavelengths is shown in Table S2.†

After optimization, the accuracy of the analytical procedure was evaluated by addition and recovery experiments and the results are shown in Table 5. For Ca, K, Mg and Na, recoveries from 80 to 120% were obtained at all evaluated levels. For the

Table 5 Recoveries for Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Hg, K, Li, Mg, Na, Pb, Sb and Zn in a urine sample determined by ICP OES using MEC[a]

	Recovery (RSD) (%)		
	Level (mg L^{-1})		
Analyte	Level 1	Level 2	Level 3
Al	<LOQ*	101 (6)	97 (8)
As	93 (4)	103 (6)	88 (5)
Ba	94 (2)	107 (1)	92 (2)
Be	91 (1)	106 (1)	92 (2)
Bi	131 (14)	126 (15)	105 (3)
Ca	94 (4)	105 (9)	120 (6)
Cd	96 (3)	105 (1)	93 (1)
Co	95 (2)	106 (1)	91.0 (0.5)
Cr	134 (8)	116.0 (0.6)	96 (3)
Cu	138 (1)	117 (3)	95 (2)
Hg	87 (2)	109 (2)	91 (2)
K	81 (2)	80 (6)	92 (1)
Li	102 (1)	111 (1)	98 (3)
Mg	117 (5)	119 (10)	117 (3)
Na	83 (1)	86 (7)	83.0 (0.2)
Pb	100 (7)	110 (6)	92.0 (0.5)
Sb	<LOQ*	114 (12)	96 (6)
Zn	465 (11)	303 (2)	177 (1)

[a] Level 1: 10 mg L^{-1} for Ca, K, Mg and Na and 0.10 mg L^{-1} for the other elements. Level 2: 20 mg L^{-1} for Ca, K, Mg and Na and 0.20 mg L^{-1} for the other elements. Level 3: 40 mg L^{-1} for Ca, K, Mg and Na and 0.40 mg L^{-1} for the other elements. *The LOD values are shown in Table 6.

other elements, recoveries varied from 87 to 102%, except in the case of Al, Bi, Cr, Cu, and Zn for a concentration of 0.10 mg L^{-1}. For the 0.20 mg L^{-1} concentration level, recoveries varied from 101 to 117% except in the case of Bi and Zn. Recoveries from 88 to 105%, except in the case of Zn, were obtained for the concentration level of 0.40 mg L^{-1}. For Zn, two additional levels of additions (2.0 and 4.0 mg L^{-1}) were tested due to the relatively high amount of this analyte in the sample (0.20 mg L^{-1}). In these cases, recoveries were 109 and 100%, respectively.

Best recoveries were obtained at all concentrations levels by IS. However, when adding a suitable analyte concentration in the MEC method, both calibration methods can be successfully used for direct analysis of urine samples by ICP OES. Similarly to the SAM, the MEC method requires the addition of a tailored analyte concentration to the sample. Usually this concentration is around the same as or higher than the analyte concentration roughly estimated for the sample. The IS method may be considered advantageous because of the use of only one analytical curve for the determination of each element for different urine samples. On the other hand, for MEC it is necessary to have one analytical curve for each element in each sample. Although just two solutions are used, posterior data treatment is necessary to construct analytical curves. It should be emphasized that the matrix matching capability is inherent to the MEC method and, on the other hand, the choice of a suitable IS is not straightforward.

As shown in Table 6, LODs obtained by internal standardization were generally lower than LODs obtained by MEC. Probably, LODs for the internal standardization method are frequently better because measurements were performed using the most sensitive emission line.

Table 6 Limits of detection (μg L^{-1}) obtained by three calibration methods: EC, IS and MEC

Analyte – wavelength (nm)	EC	Internal standardization	MEC
Al – 167.079	0.15	0.50	25
As – 188.979	9.2	11	26
Ba – 455.403	0.35	0.43	1.0
Be – 313.042	0.16	0.19	0.24
Bi – 223.061	15	8.1	21
Ca – 317.933	1.8	2.0	15
Cd – 226.502	0.65	0.69	2.8
Cd – 228.802	0.40	0.44	
Co – 228.615	0.42	0.13	2.9
Cr – 357.870	3.5	5.1	7.2
Cr – 283.563	22	48	
Cu – 224.700	3.6	3.6	5.7
Cu – 324.754	2.9	8.8	
Hg – 184.949	4.8	5.3	41
Hg – 194.163	3.1	1.2	
K – 766.489	3.4	3.0	70
Li – 670.784	0.22	0.23	0.23
Mg – 280.270	2.7	42	43
Na – 818.325	110	120	120
Pb – 220.353	17	18	19
Sb – 206.833	1.3	14	46
Sb – 217.581	9.2	13	
Zn – 202.548	1.7	2.4	9.8
Zn – 213.856	2.4	1.2	

4. Conclusions

Based on addition and recovery experiments, it was found that the choice of IS was not related to similar physico-chemical properties between the IS and the analyte, but it was chosen based on quantitative recoveries. Surprisingly, Ge and Pd were better ISs than Y, which is frequently used as IS for ICP OES measurements. Multi-energy calibration led to accurate results using only two calibration solutions and still allowed the identification of interference by observing anomalous points caused by interfering emission lines in the calibration curve. Best recoveries and lower LODs were obtained using internal standardization compared to MEC, and calculation is simpler because it is built into the ICP OES data acquisition software. However, the drawback associated with this calibration strategy is the difficulty of finding a suitable IS which is not present in the sample and whose emission line cannot generate spectral interference to emission lines of analytes. Multi-energy calibration is attractive because its matrix matching capability is inherent to its concept. However, its use will become more attractive if data treatment becomes incorporated into the built-in software. During all experiments, no salt deposit was observed in the nebulizer, nebulizer chamber and quartz torch, showing that direct analysis of urine by ICP OES can be performed without causing damage to the equipment's components. It can be concluded that both calibration strategies (internal standardization and MEC) can be used successfully to correct for matrix effects in direct analysis of urine by ICP OES.

Ethical statement

Urine was chosen as a model sample and this study neither addressed a comparison of batches of urine samples from human subjects nor parameters that could allow inferences about the physiological state. In addition, I testify that the urine used in the study was only collected from the authors and, therefore, it was understood that it was unnecessary to request authorization from the Committee on Research Ethics with Human Beings. However, all procedures were carefully adopted for manipulation of biological fluids.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – grants 141634/2017-0, 303107/2013-8, 305201/2018-2 and 428558/2018-6) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PNPD – Graduate Program in Chemistry, Federal University of São Carlos). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior –

Brasil (CAPES) – Finance Code 001. We also acknowledge the technical support provided by Analítica (São Paulo, SP, Brazil) and Thermo Scientific.

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