



SELECTION OF INTERNAL STANDARDS AT THE ANALYSIS OF BIOLOGICAL OBJECTS IN THE MASS-SPECTROMETER WITH INDUCTIVELY COUPLED PLASMA (ICP-MS)

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ABSTRACT

In work it was established that in a "standard" mode of a mass spectrometer when accounting all possible matrix effects, after a dilution of biological liquids the exact definition of all studied set of elements of one solution is possible only in case of application of several internal standards (IS) which are picked up proceeding from a proximity to their ionization potential (IP) to IP of analytes. It is also shown, the nature of the element acting as the internal standard is not basic, and so, reduction of set of the applied VS to unique is possible. The last approach is especially simple performed by that it is important for clinical practice.

KEYWORDS: matrix effect, biological liquid, internal standard, atomic weight, ionization potentials, mass-spectrometric analysis.

1. INTRODUCTION:

Inductively coupled plasma mass spectrometry (ICP-MS) has long been used in clinical laboratories dealing with the analysis of biological objects. Currently, ICP-MS is one of the main multi-element methods for analyzing various substances, due to the possibility of simultaneous recording of virtually all elements in a periodic system with detection limit of up to 10-13 at. %, good reproducibility of the analytical signal ($S \leq 0.01$) and high correctness of determination of the sample composition [1,2]. But on the other hand, as is known, the central problem is obtaining reliable quantitative data for plasma mass spectrometers, including ICP-MS. However, investigation shows that the results of the analysis by these methods do not always correspond to the true composition of the sample. The reason for this is the unequal yield of ions of different elements, related both to the features of the ion formation process in the plasma, and to their mass discrimination in the separation and registration system [3,4].

To take into account this discrepancy, else the 70s of the last century had used the so-called internal standard (IS) - element (s) with a known amount and composition introduced into the sample under analysis. The internal standard method is often used in ISP-MS to take into account instability of the device operation, correction of signal drift and elimination of matrix interference [5]. Among the main requirements for the element acting in the role of the IS, it should be noted [6].

- its complete absence or insignificant (undetectable) content in the analyzed samples;
- the absence of spectral cover affecting the signal of a given isotope in the matrix under consideration;
- the absence of interference caused by the internal standard for the analyzed elements;
- proximity to physical and chemical characteristics of the elements to be determined.

Table 1. Ranges of content of elements detected by ICP-MS in biological fluids

Element	Concentration, $\mu\text{g} / \text{l}$		
	Blood	Blood serum	Urine
Al	—	—	0 – 40
Fe	—	—	5 – 300
Co	0,5 – 2,5	0,45 – 1	0,25 – 2
Cu	750 – 1500	750 – 1800	10-70
Zn	5500 – 9000	750 – 1500	200 – 1000
As	0 – 90	0 – 10	0 – 350
Cd	0 – 5	0 – 0,15	0 – 1
Hg	0 – 50	—	0 – 10
Pb	0 – 100	—	0 – 5

Indeed, back in the late 1980s and early 1990s, two persistent, somewhat contradictory opinions emerged, according to which the main criteria for choosing an internal standard could be either the proximity of the atomic mass and the first ionization potential (FIP) [7], or the similarity of only the atomic mass of the IS and the analyte [8]. The key role of the last criterion in correcting the instability

of the device operation is primarily due to the fact that when measuring in a scanning mode two isotopes with similar masses are detected by the analyzer at approximately the same time, which helps to level out the effect of random fluctuations on the magnitude of their signals. For example, in investigations [9], the choice of the internal standard is based on the difference in the values of not only the FPI, but also the kinetic energy produced in the ion plasma, the binding energy of the atom in the oxide and hydride, and the electronegativity of the element. An even wider range of characteristics was studied in [10]: in addition to the atomic mass and the first ionization potential, attention is also paid to the second ionization potential, ion mobility and the thermodynamic properties of the solution being analyzed.

Of course, the imprint on the choice of the aircraft should be postponed not only by the nature of the analytes, but also by the operational settings of the mass spectrometer. It is known that when working with ISP-MS by an instrumental method it is possible to eliminate the influence of a matrix (and some concrete, rather than mixed) on the processes in the plasma, it is possible to use one of any internal standard. This is due to the fact that the effects generated inside the sample entry system during the formation and transport of the aerosol should act equally on all analytes, regardless of their physical and chemical properties. However, due to the fact that in ICP-MS the matrix noise is usually more deep and complex, due to, first of all, an additional ion extraction and focusing unit and the dependence of the mechanisms on the design and configuration of the device, the cases of applying one internal standard in the multi-element analysis of complex compositions matrices are rare. Moreover, there are examples in the literature when, it is possible to simplify the manifestation of matrix jamming up to one particular mechanism by the mass spectrometer adjustment, and, thus, to single out one single characteristic responsible for the choice of IS [5].

2. THEORETICAL PART:

In general, in multi-element analysis, the most common application of several IS (Table 2), selected according to one criterion or another. The determination of elements in biological fluids of the ISP-MS examples, one internal standard is used in cases of selection of a matrix of calibration solutions or calibration by the additive method [11] and / or, evidently, in the determination of 1 to 2 elements. The concentration of ions in the sample being analyzed is usually taken into account with the relative sensitivity coefficient (RSC) of the element to be determined with respect to the internal standard, an element with a known composition and quantity [3,12,13] introduced into the sample during sample preparation.

$$RSC_{IS}^x = \frac{J_x}{J_{IS}} \cdot \frac{n_{IS}}{n_x} \quad (1)$$

where J_x, J_{IS} - the measured analytical signals of the determined element and internal standard, n_x, n_{IS} - the concentration of the corresponding elements. At the same time, the concentrations of the elements sought were determined with respect to the internal standard. The thermodynamic approach that takes into account thermodynamic characteristics of individual substances in calculations [14] is used for the quantitative determination of RSC_{IS}^x . The determination the experimental values of the RSC, and to establish a correlation with the basic physicochemical properties of the elements being determined is its essence. Such correlation allows identifying the main processes in the plasma, responsible for the formation of ions and applying plasma or kinetic approaches to them [13].

The ratio of the intensity of the signal of analyte to the intensity of the signal IS in

the matrix under study compared with the comparison matrix is calculated by the formula:

$$R^* = [I(M)/I(IS)]_x / [I(M)/I(IS)]_{com} \cdot 100\% \quad (2),$$

The degree of change in the ratio of the signal intensity of the analyte to the intensity of the signal IS in the investigated matrix as compared to the comparison matrix calculated by the formula:

$$\Delta R^* = R^* - 100\% \quad (3),$$

where

$I(M)_x$ is the signal strength of the analyte in the investigated matrix;

$I(M)_{com}$ - the intensity of the signal of analyte in the comparison matrix;

$[I(M)/I(IS)]_x$ is the ratio of the intensity of the signal of analyte to the intensity of the signal IS in the investigated matrix;

$[I(M)/I(IS)]_{com}$ - the ratio of the signal intensity of the analyte to the intensity of the signal IS in the comparison matrix. 1% volume of HNO₃ is as the comparison matrix.

Table 2. Examples of MS-ISP analysis of biological fluids after oxidative salinity

Sample	Defined elements	Multiplicity of dilution	Conditions of mineralization	Internal standards
Blood	Al, As, Cd, Cr, Co, Cu, Hg, Mn, Ni, Pb, Sb, Se, Tl, Zn	10	1 ml sample – 1 ml HNO ₃ 10 h 180 °C	Ir, Rh, Sc
	Ca, Cu, Fe, Mg, Mn, Pb, Zn	20	1 ml sample – 2 ml HNO ₃ 1 h ~ 100 °C	In, Ir, Lu
	Al, As, Ba, Cd, Co, Cu, Li, Fe, Mn, Ni, Pb, Sb, Se, Sn, Tl, U, V, Zn	15	1 ml of the sample – HNO ₃ and H ₂ O ₂ heating to 95 °C and evaporation	Ge, Lu, Rh, Sc
Urine	Pd, Pt, Rh	20	5 ml sample – 1 ml HNO ₃ and 2 ml H ₂ O ₂ UV: 90 min 500 W	In
	Cd, Co, Cr, Ni, Pb, Rb, Sb, Se, V	10	5 ml of the sample – 1 ml of HNO ₃ and 2 ml of H ₂ O ₂ UV: 90 min 500 W	

In situations where a solution of the internal standard introduced into the sample can be a source of interference for analyte isotopes or simply cause contamination of the sample. It is sometimes recommended to use the polyatomic ions formed from the plasma-forming gas or matrix of the analyte as IS, such as Ar²⁺, ArO⁺, N²⁺, ClO⁺, SO⁺, MO⁺, where M is the atom of the element being analyzed. But even in these investigations, the application of such one internal standard is limited by the criterion of proximity of the atomic masses of the elements being determined. In addition, the difference in the mechanisms of formation and the distribution of similar ions in plasma can also impede the use of this approach in comparison with analyte ions [15].

One internal standard can also be used for additional mathematical correction of signals received during measurements within the framework of the so-called method of common analyte internal standardization (CAIS) [16]. Indeed, CAIS allows the use of any element as IS, regardless of its chemical or physical characteristics, both for accounting for instrumental drift of the mass spectrometer and for eliminating matrix interference. At the same time, it should be noted that for the leveling of simultaneously two effects, at least two IS are required, which differ from each other in atomic mass and IP. The basis of the method is the preliminary finding of a mathematical linear function that relates to each other the intensity correction factor of the analyte signal and the relative change in the intensity of the signal IS in a matrix with respect to water. For this, measurements are made in several (3 - 4) pre-prepared control solutions with different contents of the test components, but with the same concentrations of the elements being determined and the internal standard.

3. EXPERIMENTAL PART (METHODS AND MATERIALS):

The following reagents and solutions were used in the work:

- argon of high purity

- helium
- concentrated nitric acid (65%, "for analysis", Merck, Germany)
- solutions of Na, K, Mg, Ca, Ba, Mo, P and S, prepared from single-element standard solutions of 1000 mg / l (High-Purity Standards, USA)

The investigated objects and materials in the work used:

- samples of whole blood and urine;

The liquids were collected by means of mechanical dispensers with working volume ranges of 10 - 100 µl.

ISP - MS measurements were performed on an Agilent 7700 inductively coupled plasma quadrupole mass spectrometer (Agilent Technologies, USA) in continuous mode (for aqueous solutions). ICP-MS ChemStation software (version G1834B) was used to control the instrument and process the results. At the same time, samples were prepared using the microwave attachment "Ethos-one" from Milestone. The power was regulated in accordance with the temperature data throughout the entire mineralization process (tab.3).

Table 3. The values of the mass spectrometer operation parameters in the "standard" mode

The composition of the diluent
0.1 vol% Triton - 1 vol% HNO ₃
0.1 vol% Triton - 0.1 g / l DTA 1 vol % HNO ₃
0.1 vol% Triton - 0.5 vol % 1-butanol 1 vol % HNO ₃

Elements Li, Sc, Ge close atomic masses or IP to the analyte (subject to other general requirements) were used as IS when determining the elements Ca, Fe, Cu, Co, Zn Cr in the composition of whole blood and urine.

As follows from the review of the literature, elements with close atomic masses or IP to analyte, but containing stable isotopes, to avoid overlapping with isotopic peaks of unlabeled compounds most often are chosen as IS. The strong distinction of these parameters attracts to notable adjustment of sensitivity of the device according to the standard in comparison with an analyte. Thus, for example, the correction of the concentration of ⁷Li to ¹¹⁵In, which is often used as an internal standard, will be incorrect, because the effect of the matrix effect on the sensitivity of the device on ⁷Li and ¹¹⁵In is significantly different in magnitude. Also, ¹¹⁵In will not approach as the internal standard when determining tin owing to availability in the last of the isotope ¹¹⁵Sn creating isobaric imposing on ¹¹⁵In. In practice the ideal choice of the internal standard is not always possible therefore it is necessary to make a compromise. The corresponding fluctuations are shown in changes of an analytical signal for all analytes which are present at solution, and the concept of addition of the internal standard is one of ways of accounting of such changes of a signal. Signals of elements with high first potentials of ionization can be suppressed owing to presence of easily ionizable elements of a matrix, such as Na, K and Ca, in rather high concentrations. In this research the choice of internal standards for analytes, according to the standard approaches, was based on routine practice of selection of an element with the first potential of ionization and atomic weight as the perhaps close to an analyte. However at application of calibration in a synthetic matrix analytical results are quite close, regardless of what internal standard was chosen for an analyte. Besides, germanium is widely applied as the internal standard for several elements, including arsenic and selenium therefore it is necessary to show caution as it is known that it in the significant amounts is present at blood.

Blood exemplars usually dilute 10–50 - time to make possible the analysis of content of metals by means of ISP-MS. However organic components influence a flow rate and dispersion during analyses, especially if the analyzed blood exemplar partially coagulated with formation not of completely solubilized clots.

In this regard, monoisotope elements in all studied range of masses (from 9 to 209) with various PI: *Be, Co, As, Rh* and *Bi* (tab. 5, IS are highlighted in bold type) have been studied in this article.

Table 5. Values of the first potentials of ionization and atomic weight of elements

Element	PI, eV	Atomic weight, a.m.u
Be	9,3	9,0
Co	7,9	58,9
Cu	7,7	63,5
Zn	9,4	65,4
As	9,8	74,9
Se	9,8	79,0
Rh	7,5	102,9
Bi	7,3	209,0

When using Rh as IS it is possible to consider completely influence of compo-

nents of solution only for analytes from rather low, smaller 9,0 eV and the close to PI of the IS, ionization potential.

During the work in the "standard" mode, the value of atomic weight of IS cannot be criterion of the choice of the internal standard following from the data presented in tab. 6: the most apparent cases of failure to follow this rule are designated by the painted-over color of cells. At the same time the complete elimination of influence of components of a matrix is feasible due to selection of IS, proceeding from PI values. Really, minimum and insignificantly different from 0 values size ΔR^* accepts only in case of a proximity of potentials of ionization of an analyte and IS.

Table 6. ΔR^* values for the studied elements when using various IS matrix – 0,4% of NaCl

	ΔR^* , %				
	Be	Co	As	Rh	Bi
P	2	-23	-1	-20	-19
Mn	17	3	20	4	4
Co	20	–	18	3	1
Cu	19	-3	18	0	3
Zn	-5	-25	5	-20	-20
Ga	20	2	20	0	2
As	3	-20	–	-17	-18
Se	4	-23	4	-18	-19
Cd	3	-19	0	-16	-20
Pt	-2	-18	-1	-17	-20
Pb	15	4	20	3	2

Most likely, influence of components of the analyzed solutions on results of their definition, it is necessary to consider not on the one hand, considering only the processes happening immediately in plasma (evaporation, atomization, ionization), and from two parties, the processes proceeding in the system of input of an sample (enter of sample in the spray, education and transportation of aerosols). As the last directly depend only on physical properties of the analyzed solutions (viscosity, the interfacial tension, density and a volatility), but not from physical and chemical properties of analytes. The single element as IS regardless of its nature (atomic weight and PI) can be applied to elimination of such influence.

4. CONCLUSION:

Thus, it is established that in a "standard" mode of a mass spectrometer during the accounting of all possible matrix effects, using graduation without selection of a matrix, after both direct a dilution, and an oxidizing mineralization of biological liquids the exact definition of all studied set of elements of one solution is possible only in case of application of several IS which are picked up proceeding from a proximity of their PI to PI of analytes.

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