

## SELECTION OF INTERNAL STANDARDS DEPENDING ON THE MODE OF OPERATION OF MASS SPECTROMETER WITH INDUCTIVE COUPLED PLASMA (ICP-MS)

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**Abstract-** The work was established a direct relationship between the various modes of device operation and the criteria for selecting the internal standard. Two equivalent combined approaches to eliminating matrix interference are proposed: 1) measurement in the "standard" operating mode of the device (after automatic adjustment for maximum sensitivity) with use internal standard (IS), selected based on the proximity of the first ionization potentials (*PI*) of the element and analyte, and 2) analysis in the regime of "stable" parameters using IS as a single element, irrespective of its atomic mass and *PI*.

**Keywords:** Matrix Effect, Biological Fluid, Internal Standard, Atomic Mass, Ionization Potentials, Mass Spectrometric Analysis.

### I. 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].

However, like any other instrumental method, mass spectrometry has its drawbacks, the most important of which are so-called matrix interference [6]. At the same time, their influence on the results of the determination and methods of controlling this effect depend on the type of sample and the method of sample preparation preceding the analysis. One of the ways to combat matrix effects is dilution, which is sufficient to level out their effects. However, the most common option is the use of the additive method [8, 9] or the selection of the matrix of calibration solutions [7].

Nevertheless, additionally an internal standard method is used to eliminate the matrix effects no matter how the calibration solutions are prepared. As one [8], as well as an entire group of elements [10] can act an IS. One single internal standard is used in the case of selection of a matrix of calibration solutions or calibration by the method of additives and / or, which is obvious, when determining 1 to 2 elements. In all other cases, several internal standards are applied simultaneously. At the same time, it is not entirely logical to use the IS group when selecting a matrix or grading by the additive method [11].

The criteria for selecting the element of the internal standard remain unclear, although in some cases it is explicitly indicated that this choice is based on the proximity of the atomic mass [12] and / or the first ionization potential [13] IS and analyte. In addition, the authors of Ref. [14] emphasize that the criterion does not always work: for example, when determining Li and Al, Rh is used as the internal standard, the atomic mass of which is much larger than the atomic mass of the analytes.

About the possibilities of leveling the matrix effects in the analysis of dilute biological fluids by the instrumental method, or rather due to the choice of so-called "robust conditions" of operational parameters are mentioned only in several works [12, 15]. The need to reduce the flow rate of argon through the atomizer, the

use of increased generator power for more efficient decomposition of the organic matrix and ionization of elements in the plasma is described in [15]. Adjustment of all parameters of the instrument using a diluted sample is recommended in [16]. In other cases, it is produced using a standard solution. It should be noted that even when operating in the «robust conditions» operating parameters, several IS being used at once. The use of one internal standard in [15] is connected, first of all, with a stronger dilution of samples.

The choice of IS should depend not only on the nature of the analytes, but also on the applied operational settings of the mass spectrometer. It is known that use of one of any internal standard it is possible to eliminate the influence of a matrix (and some concrete, rather than mixed) on the processes in the plasma, when working with ISP-MS by the instrumental method. This is due to the fact that the effects which 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 the matrix noise is usually more deep and complex in ICP-MS, first of all, due to, an additional extraction unit and ions focusing and the dependence of the mechanisms on the design and configuration of the device, the cases of applying one internal standard in a multielement analysis which complex by compositions matrices are rare. There are examples, in the literature where, it is possible to simplify the manifestation of matrix effects to one particular mechanism with the help of setting of mass spectrometer and thus, to single out one single characteristic responsible for the choice of the IS [5].

Development of approaches to eliminating matrix effects included the investigation of their minimization at the expense of only instrumental settings of the device or replacement of the nebulizer and the identification of a clear criterion for selecting an internal standard with the study of the possibility of application of one, and any IS.

Optimizing the settings of the mass spectrometer (search for so-called "stable conditions") can be one of the ways to eliminate matrix effect. The generator power and, most importantly, the flow rate of argon through the atomizer most often are the characteristic which varies in the course of this process.

Processes which occurring directly in the plasma, the effect of acid is highly depends on the values of the operating parameters of the device, primarily the flow rate of argon through the atomizer and / or generator power [17]. The use of the "standard" mode, when the setting of the mass spectrometer to the maximum sensitivity of the determination is carried out with a special solution of elements of different atomic masses in 2% by volume of HNO<sub>3</sub>, which always leads to the suppression of signals in samples with high acidity. This fact may be a possible explanation for the less efficient ionization of elements in these conditions, especially those that have a high *PI*, due to a greater plasma loading of nitric acid and, as a consequence, a decrease in its local temperature.

When switching to the so-called "robust conditions" regime, when the flow rate of argon is lowered relative to the "standard" characteristics, and the generator's used power remains the same or increases, elimination of signal suppression is observed by increasing the efficiency of evaporation, atomization and ionization processes. This is due to two reasons: first, the aerosol moves more slowly from the tip of the burner injector to the plasma with a decrease in the flow rate of argon, and secondly, the region of the sharp temperature difference (from one hundred to several thousand degrees) shifts to the tip of the injector; all this contributes to an increase in the residence time of the sputtered solution in the high-temperature zone [18].

## **II. EXPERIMENTAL PART (METHODS AND MATERIALS)**

The following reagents and solutions were used in the work:

- Argon of high purity
- Concentrated nitric acid (65%, "for analysis", Merck, Germany)

The investigated objects and materials in the work used:

- Samples of whole blood and urine of healthy people;
- The liquids were collected by means of mechanical dispensers with working volume ranges of 10 - 100 µl.

ICP-MS measurements were carried out 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.

A solution of the internal standard with a concentration of 5000 µg/l was prepared by diluting with the 1% by volume HNO<sub>3</sub> of the corresponding single-element standard solution of 1000 mg/l Rh. In all the test samples, the IS concentration equal to 25 µg/l has created by adding the required volume of the prepared solution.

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

Most often, elements were selected with close to analyte atomic masses or *PI* as IS, but which containing stable isotopes, in order to avoid overlapping with isotope peaks of unlabeled compounds. A strong difference in these parameters leads to a tangible adjustment of the sensitivity of the instrument in accordance with the standard in comparison with the analyte. Thus, for example, the correction of the concentration of <sup>7</sup>Li on <sup>115</sup>In, 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 <sup>7</sup>Li and <sup>115</sup>In is significantly different in magnitude.

Signals of elements with high first ionization potentials can be suppressed due to the presence of readily ionizable matrix elements, such as Na, K and Ca, at relatively high concentrations. In this study, the choice of internal standards for the analytes, according to conventional approaches, was based on the usual practice of selecting an element with the first ionization potential and the atomic mass as close as possible to the analyte. However, when applying calibration in a synthetic matrix, the analytical results are quite close, regardless of which internal standard was chosen for the analyte.

In the "standard" operating mode of the instrument, when using Rh as an IS, it is possible to fully take into account the effect of the solution components only for analytes with a relatively low, less than 9.0 eV and close to the *PI* of the IS itself, the ionization potential. The transition to "robust conditions" parameter values in the absence of an IS contributes only to a partial minimization of the matrix effect, but to the complete elimination of the differentiation of the analytes from the *PI*. In both cases, the question arises of recommendations concerning the criterion for choosing an internal standard based on the proximity of its atomic mass and / or *PI*, as well as the hypothesis of the possibility of using a single IS in the "robust conditions" parameters.

The most frequently used elements as IS being those with close atomic masses or *PI* to analyte. In this connection, monoisotopic elements in the whole investigated mass range (from 9 to 209) with different *PI*: Be, Co, As, Rh and Bi (Table 1, IS are in bold) were studied in this paper.

Table 1. The values of the first ionization potentials and atomic masses of the elements

Element	<i>PI</i> , eV	Atomic mass, 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 operating in the "standard" mode the value of the atomic mass of an IS cannot be a criterion for choosing an internal standard: the most obvious cases of non-fulfillment of this rule are indicated by colored cells which are presented from the data in Table 2. At the same time, the complete elimination of the effect of matrix components is feasible due to the selection of the IS, based on the values of the *PI*. Indeed, the value of  $\Delta R^*$  (the degree of change in the ratio of the intensity of the analyte signal to the intensity of the signal BC in the matrix under study) is minimal and insignificantly differing from 0) only in the case of proximity of the ionization potentials of the analyte and IS.

At transition to a mode of "robust conditions" parameters the criterion of a choice of the internal standard is absolutely simplified. In this case, as an IS, there can be one single element, and most importantly,

regardless of its atomic mass and *PI*. The described regularities are most clearly traced in the example of matrices 0.4% NaCl and 15 vol% HNO<sub>3</sub>.

Table 2. The values of  $\Delta R^*$  values for the elements under investigation when using different aircraft in both modes of operation of the device

matrix- 15% by volume HNO <sub>3</sub>					
"standard mode"			"robust conditions"		
	$\Delta R^*$ , %				
	Be	Co	As	Rh	Bi
P	<b>-3</b>	-30	<b>3</b>	-26	-32
Mn	35	<b>2</b>	40	<b>3</b>	-3
Co	37	-	42	<b>1</b>	-2
Cu	33	-2	40	-1	<b>2</b>
Zn	<b>-3</b>	<b>-30</b>	<b>-5</b>	-27	-37
Ga	35	<b>0</b>	42	<b>0</b>	<b>0</b>
As	<b>-2</b>	-27	-	-25	-30
Se	<b>2</b>	-33	<b>-3</b>	-23	-33
Cd	<b>1</b>	-25	<b>4</b>	-26	-31
Pt	<b>0</b>	-27	<b>1</b>	-25	<b>-30</b>
Pb	35	<b>1</b>	44	<b>1</b>	<b>3</b>

matrix- 0.4% by volume HCl					
"standard mode"			"robust conditions"		
	$\Delta R^*$ , %				
	Be	Co	As	Rh	Bi
P	<b>2</b>	-23	<b>-1</b>	-20	-19
Mn	17	<b>3</b>	20	<b>4</b>	<b>4</b>
Co	20	-	18	<b>3</b>	<b>1</b>
Cu	19	-3	18	<b>0</b>	<b>3</b>
Zn	<b>-5</b>	<b>-25</b>	<b>5</b>	-20	-20
Ga	20	<b>2</b>	20	<b>0</b>	<b>2</b>
As	<b>3</b>	-20	-	-17	-18
Se	<b>4</b>	-23	<b>4</b>	-18	-19
Cd	<b>3</b>	-19	<b>0</b>	-16	-20
Pt	<b>-2</b>	-18	<b>-1</b>	-17	<b>-20</b>
Pb	15	<b>4</b>	20	<b>3</b>	<b>2</b>

The effect of the components of the solutions being analyzed on the results of the determination should not be considered on the one hand, taking into account only the processes occurring directly in the plasma (evaporation, atomization, ionization), and from two sides, taking into account also the processes taking place in the input system sample (receipt of a sample in the nebulizer, formation and transport of aerosols). Since the latter directly depend only on the physical properties of the solutions under analysis (viscosity, surface tension, density and volatility), but not on the physicochemical properties of the analytes themselves, to eliminate such an influence, a single element can be used as the IS, regardless of its nature (atomic mass and *PI*).

For leveling the impact caused by the first processes, it is necessary either to use an IS with close to analyte ionization properties (*PI*, IS and analyte should be close), or use of special "robust conditions" values of operational parameters. The contributions of both processes, of course, depend on the matrix under study, but in the first approximation, as can be seen from the diagrams presented in Figure 1, they are quite commensurable or even equivalent.

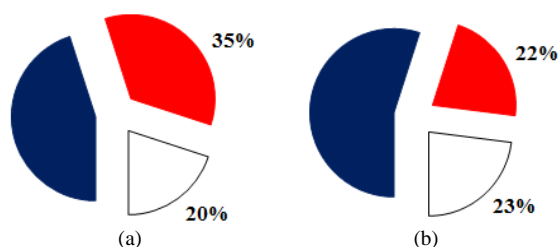


Figure 1. Distribution of contributions to underreporting of a signal by nature of origin: suppression due to processes occurring in the sample entry system (white color) and directly in the plasma (red color) - in matrices (a) 0.4 vol% NaCl (a), (b) 15 vol% HNO<sub>3</sub>

### III. CONCLUSIONS

Thus, it was found that in the "standard" operating mode of the mass spectrometer, taking into account all possible matrix effects, the correct determination of the whole set of investigated elements from a single solution is possible only if several IS being selected, based on the proximity of their *PI* to *PI* analytes. It is also shown that in the regime of "stable" parameters, the nature of the element acting as an internal standard is unprincipled, which means that it is possible to reduce the number of applied IS to a single one.

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