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REVIEWS

Matrix Interference in the Determination of Elements in Biological Samples by Inductively Coupled Plasma–Mass Spectrometry and Methods for Its Elimination

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Abstract—The problems of detecting and accounting for spectral and nonspectral interferences arising in the determination of a wide range of elements in biological and pharmaceutical samples by inductively coupled plasma–mass spectrometry are discussed. We studied the features of the effect of matrix components on the accuracy of the results of analysis of biological fluids and pharmaceuticals using a quadrupole mass spectrometer. The processes that have the most significant effect on the suppression of an analytical signal measured by an Agilent 7500c quadrupole mass spectrometer are revealed. The main operational parameters responsible for minimizing the investigated nonspectral interference are determined. Various versions of the internal standard method are studied, which offer a decrease in the effect of the sample composition on the determination results. A direct relationship is found between various modes of operation of the instrument and the criteria for selecting an internal standard. Combined approaches to the complete elimination of nonspectral interference using external calibration are proposed. A method is proposed for decreasing the acidity of the test solution by adsorption separation of the analyte elements and acid.

Keywords: matrix interference, sample preparation, internal standard, inductively coupled plasma–mass spectrometry, biological and pharmaceutical samples

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Contemporary medicine is showing an increasing interest in the role of chemical elements in human's life. The focus is now on their integrated effect on the body functions. For example, over the years, clinicians and environmentalists have been actively conducting research into the determination of the risk of environmental exposure to public health. In the course of large-scale monitoring, they try not only to detect the presence of inorganic ecotoxicants in biological samples but also to trace changes in the concentrations of toxic and essential elements, depending on external factors. In addition, therapists, surgeons, and resuscitators seek to reveal a correlation between the levels of concentrations of chemical elements and the risk or the severity of the disease. The successful solution of this problem will contribute to the active development of medical diagnostic methods. It is necessary to note the rapidly developing area of clinical practice associated with the development of new drugs based on coordination metal compounds. Most of them are created for the treatment of tumor diseases of different aetiologies. When analyses of such complex samples as whole blood, serum, urine, hair, and bone tissue are performed for the clinical diagnostics and the development of new preparations, the key issue is the accuracy of determining a wide range of chemical elements. The best solution is to use inductively coupled plasma–mass spectrometry (ICP–MS).

Inductively coupled plasma mass spectrometry began to be used for a long time in clinical laboratories engaged in the analysis of biological samples and the development of new pharmacological preparations based on coordination metal compounds. The mass use of this method was limited by the high cost and complexity of the equipment. It was only in 2007 that an assumption was made that ICP–MS can gradually replace flame and electrothermal atomic absorption spectroscopy, the primary method in the clinical practice of that time. Today, ICP–MS already occupies a leading position in this field [1, 2], thanks to a significant progress of the instrumental base. This led to a significant reduction in the cost of ICP–MS spectrometers while retaining undoubted advantages: record low detection limits for chemical elements and isotopes (levels of ng/L and sometimes even pg/L); possibility of multielement analysis; broad (up to ten

Table 1. Standard parameters of the Agilent7500c mass spectrometer

Parameter	Value
Plasma	
Generator power, W	1400–1500
Gas flow rate, L/min	
Plasma gas	15–16
Auxiliary gas	0.89
Sample flow rate	1–1.2
Mass spectrometer	
Resolution, amu	0.7
Vacuum without plasma, torr	4×10^{-5}
Vacuum with plasma, torr	4×10^{-4}
Dynamic cell	
Gas	Helium
Flow rate, mL/min	2–10
Measurement parameters	
Measurement time at a point, s	0.1–0.5
Number of points per peak	3

orders of magnitude) dynamic range of linearity of the calibration characteristic; low level of instrumental measuring background (0–10 imp/s); the relative simplicity of spectra and interpretation of the data obtained; low consumption of the analyte; and the ability to perform analysis of samples of minimal volume (~0.1 mL) and mass (~0.1 mg).

However, like any other instrumental method, ICP–MS has its drawbacks, the most important of which are the so-called "matrix interference," both spectral and nonspectral. Their effect on the results of the determination and methods of dealing with this effect depend on the type of sample, the method of sample preparation, and the class of the device and its design.

This review is devoted to a brief summary of the results of our group for the development of the ICP–MS method for the analysis of biomaterials and pharmacological preparations performed in the last 5–7 years. Specific methods of analysis of biomaterials and pharmaceutical substances of various types were discussed; attention was paid to the problems of manifestation of matrix effects and methods of their accounting in order to obtain correct results for the determination of a wide range of analytes in biological samples and pharmacological preparations.

Equipment. The measurements were carried out on an Agilent 7500c inductively coupled plasma–quadrupole mass spectrometer (Agilent Technologies, United States) in the continuous injection mode (for aqueous solutions) and in the mode of measurement of nonstationary signals (for solutions based on ethyl alcohol).

The mass spectrometer is equipped with a Babington spray (or concentric spray) and a Scott spray chamber cooled by a Peltier element (to 2°C), a platinum sampler, and a skimmer. The ICP–MS ChemStation software (version G1834B) was used to control the instrument and process the results. For the formation of stationary plasma at atmospheric pressure, high purity argon was used (99.993%; Logika, Russia). The main parameters of the instrument are summarized in Table 1. Depending on the test sample and the specific analytical task, we developed procedures for ICP–MS determination of various sets of analytes.

Methods to eliminate matrix interference. The concentrations of various elements (vital, conditionally essential, toxic, and components of the active ingredients of potential antitumor drugs) in biological fluids during the course of the disease or treatment are of great interest to modern clinicians. However, ICP–MS procedures are somewhat complicated because of the need to take into account and eliminate the effect of the composition of the test sample on the results of analyte determination. We studied in detail the possibilities of more straightforward methods for eliminating matrix effects by selecting the optimal sample preparation procedure, varying the instrumental settings of the mass spectrometer, and optimizing the measurement technique and processing of analytical signals. We studied P, Mn, Co, Cu, Zn, Ga, As, Se, Cd, Pt, and Pb as analytes, that is, elements that were determined for specific tasks set by clinicians and drug developers.

It is known that spectral and nonspectral (matrix) interferences are the main limitations of the ICP–MS capabilities. The effect of the latter on the results of determination can usually be leveled by applying a calibration with the selection of a matrix of calibration solutions, internal standards (IS), or both at once [3, 4]. The introduction of the internal standard is the most popular approach, with various elements being used for this purpose. Before the start of our study, the general criteria for the selection of internal standards were not developed. Usually, different authors select an internal standard either by the proximity of the atomic masses of the standard and analyte or by the vicinity of their ionization potentials (IPs). In many studies, several internal standards are used to correct the analytical signals of a wide range of detectable elements [5]. It is important to note that internal standards used in the case of direct dilution are different from those used in the acid mineralization of whole blood.

Dilution of the sample. Because of the high concentration of organic and salt components in biological samples, their continuous injection into the plasma of an ICP–MS spectrometer is impossible. Before analysis, a sample preparation stage is necessary, which is

the most critical stage of any ICP–MS procedure. Various methods for preparing biological materials for ICP–MS analysis were discussed in detail in [6]. Currently, there are two widely used methods for the sample preparation of biological samples: (1) direct (simple) dilution with various reagents and (2) acid decomposition. The apparent advantages of dilution are simplicity, speed, and minimal risk of additional contamination of the sample. However, simple dilution cannot be used to convert solid samples, such as tissues, bones, or teeth, into a solution. In this case, acid mineralization is used.

Whole blood is the most complex biological fluid with a high concentration of both inorganic and organic substances. Simple sample dilution can be used as the sample preparation of whole blood for ICP–MS analysis. Usually, whole blood is directly diluted by 10, 20, or sometimes 50 times with various mixtures of aqueous solutions of Triton X-100, *n*-butanol, EDTA, ammonia, and nitric acid. To date, there is no generally accepted method for selecting a particular diluent composition. In [7, 8], eight different compositions of mixtures of the indicated components were investigated. Each component of this mixture plays a specific role. Triton X-100 protects burner injector from clogging, EDTA prevents precipitation and adsorption of metals on test tube walls, ammonia destroys red blood cells, *n*-butanol helps removing argon interference for selenium, and nitric acid stabilizes the forms of elements in solution. It is shown that a unique version of diluent cannot be selected. The fact is that some components of such mixtures adversely affect the signals of specific analytes. For example, the presence of 1-butanol in a solution leads to an overestimation of the P, As, and Se signals by almost twofold because of the charge transfer from the carbon ion. In ammonia solutions, the signal of platinum is also suppressed by a factor of 2, which is most likely because of the partial sedimentation of this element in an alkaline medium. In dilution with mixtures containing nitric acid, protein precipitation occurs in samples of patients with various kinds of inflammatory processes, leading to a loss of up to 15–20% of all the elements to be determined. For the sample preparation of samples of healthy volunteers, a mixture of Triton X-100–HNO_3 and Triton X-100–EDTA–HNO_3 (for all biological fluids) and diluted nitric acid (for urine) are recommended. The mixtures of Triton X-100–NH_3 and Triton X-100–EDTA–NH_3 are suitable for diluting patient samples if platinum is not a detectable element [7].

Acid mineralization is another way to reduce the effects of the organic matrix. This type of sample preparation is in demand, despite its shortcomings, namely, relative complexity, time and material costs, and increased risk of additional contamination of the sample. Usually, concentrated HNO_3 and hydrogen peroxide are used for decomposition. Microwave radiation is often applied to intensify the decomposition process; To solve each specific analytical problem, it is necessary to optimize the program of temperature and power of the furnace. In our experiments, microwave decomposition in an autoclave was used to eliminate the risk of contamination of the initial samples and reagents only of the Suprapur (Merck) brand.

The optimal conditions for the oxidative mineralization of biological fluids in a microwave field were selected by varying the ratio of reagents (nitric acid and hydrogen peroxide) and the time of maintaining the mixture at the maximum temperature. To assess the completeness of the oxidation of organic substances, we used additional processing of the mixture with perchloric acid. The determined concentrations of analytes coincide with each other regardless of the conditions of oxidative mineralization.

A comparison of the results of the analysis of whole blood and urine after different methods of sample preparation showed a discrepancy between the obtained results for P, Zn, As, Se, and Pt with a relatively high first ionization potential of ~9.5 eV (Table 2). With the oxidative mineralization of samples, the results of the determination of these analytes are underestimated on average by 20–25% compared with those obtained by direct dilution. Our study has shown that, depending on the sample (especially when analyzing whole blood and urine of patients in critical condition), this difference can reach 30–40%.

Apparently, in both methods of sample preparation and measurement, the elements under study are differentiated according to their ionization potential, which makes it impossible to obtain correct results for the entire set of analytes.

Selection of instrumental parameters. Significant discrepancies in the results obtained using different methods of sample preparation are mainly associated with the so-called "acid effect." Indeed, the residual concentration of HNO_3 in solutions after mineralization is 7–12%, while the acidity of standard solutions used for the calibration of the spectrometer and for its adjustment is 1–2% HNO_3. The manifestation of the acid effect can be explained by the fact that an aerosol formed from a solution with a high acid concentration can cool the plasma, because the total dissociation energy of an HNO_3 molecule is almost two times higher than that of an H_2O molecule. A possible decrease in plasma temperature causes a drop in the ionization efficiency of elements with high ionization potential, which we observed. The discrepancy between the results of determination is most noticeable for elements with relatively high ionization potentials (Zn, 9.39 eV; Se, 9.75 eV) in using an element with relatively low ionization potential (Rh, 7.46 eV) as an internal standard.

There are several ways to eliminate the acid effect. The most simple is the selection of a matrix of calibration solutions, similar in acidity to the matrix of the

Table 2. Results (μg/L) of determining elements in whole blood and urine of a practically healthy volunteer after different methods of sample preparation ($n = 3$, $P = 0.95$)

Element	Blood		Urine	
	dilution	mineralization	dilution	mineralization
P*	450 ± 10	340 ± 15	1100 ± 30	1000 ± 40
Mn	7.2 ± 0.2	7.5 ± 0.3	<0.1	<0.1
Co	0.9 ± 0.1	1.0 ± 0.1	0.65 ± 0.05	0.6 ± 0.1
Cu	950 ± 10	920 ± 20	15.0 ± 0.5	15.1 ± 0.8
Zn	5300 ± 100	4100 ± 50	890 ± 20	715 ± 20
Ga	101 ± 3	98 ± 4	101 ± 2	100 ± 4
As	9.0 ± 0.3	6.7 ± 0.2	11.6 ± 0.5	8.6 ± 0.6
Se	110 ± 5	80 ± 5	37 ± 3	29 ± 2
Cd	<0.03	<0.04	<0.02	<0.06
Pt	97 ± 3	78 ± 5	86 ± 2	70 ± 3
Pb	10 ± 1	9 ± 1	1.2 ± 0.1	1.0 ± 0.1

* Concentrations are given in mg/L.

test samples. This technique is inconvenient for the routine analysis of a large number of samples of different composition; it is necessary to evaluate the acidity of the analyte solution in each case and prepare calibration solutions with the same acid concentration. The evaporation of solutions after mineralization to a dry residue and its subsequent dissolution, which is also often used to solve this problem, can lead to additional sample contamination or the loss of volatile elements.

Another method of reducing the acid effect is to optimize the generator power and the flow rate of the carrier gas in analyzing samples of high acidity (searching for so-called "effective conditions") and selecting an appropriate internal standard [6]. This approach was proposed in [7, 8] and is the most versatile and simple for the routine analysis of biological samples of different compositions.

In everyday analytical practice, before performing a cycle of experiments, the operator sets up the spectrometer in the Autotune mode. With this setting, the standard program varies the main parameters of the device in order to obtain maximum sensitivity in the entire atomic mass range (from Li to U).

We showed that such a setting is not optimal for eliminating the acid effect [9, 10]. It was found that the selection of the so-called "stable parameters" (generator power and flow rate of the test solution by carrier gas) significantly decreased the effect of the solution acidity. The selection of the flow rate of the carrier gas is of primary importance in suppressing the acid effect. When the plasma generator power is 1450 W, the analyte signal intensity increases significantly with a decrease in the flow rate from 1.2 to 1.0 L/min. A further decrease in the flow rate to 0.8 L/min does not lead to a noticeable increase in intensity; however, the reproducibility of signals from almost all elements deteriorates significantly. Obviously, with an increase in the residence time of the aerosol in the plasma at low flow rates, the efficiency of the atomization of elements increases, and, consequently, the flux of ions into the mass spectrometer increases. Changing the parameters of the mass spectrometer affects the signal intensity of elements with low and high ionization potentials in different ways, but it is not possible to eliminate the acid effect entirely by selecting the effective operation conditions of the instrument. However, it was found that at a decreased flow rate of the carrier gas, the selection of the internal standard becomes not critical. In this case, one can use any and, most importantly, a single internal standard.

Thus, it was determined [9, 10] that in the "standard" mode of operation of a mass spectrometer using calibration without selecting a matrix after diluting and oxidizing mineralization of biological fluids, the correct results of determining all analytes from a single solution can be obtained only if several internal standards are used, selected on the basis of the proximity of the ionization potentials of internal standards and analytes. It is also shown that in the mode of "stable" parameters, the nature of the element—internal standard—is not fundamental, which means that any one internal standard can be used.

The accuracy of the determination of elements using the proposed approach was assessed [10] using the Seronorm Trace Elements Whole Blood, Level 2, PT-WB1, and IAEA-A-13 whole blood reference samples and the Seronorm Trace Elements Urine, Level 2 and Urine Control Lyophilized for Trace Elements Level II urine reference samples. The values of concentrations found after the application of various methods of sample preparation and the certified val-

Table 3. Results (μg/L) of analysis of standard reference samples of whole blood Seronorm Trace Elements Whole Blood, Level 2, and urine Seronorm Trace Elements Urine, Level 2, after applying various sample preparation methods ($n = 3$, $P = 0.95$)

Element	Whole blood			Urine		
	found		certified	found		certified
	dilution	mineralization		dilution	mineralization	
P*	190 ± 10	195 ± 15	191	530 ± 15	550 ± 20	543
Mn	30 ± 1	26 ± 1	29.9	11.2 ± 0.5	11.1 ± 0.4	10.9
Co	5.9 ± 0.3	5 ± 1	5.8	11 ± 1	11 ± 2	10.6
Cu	1450 ± 50	1480 ± 90	1330	23 ± 1	23 ± 2	22
Zn	6400 ± 200	6750 ± 350	6500	1270 ± 30	1260 ± 50	1338
Ga**	101 ± 3	99 ± 4	100	99 ± 3	100 ± 3	100
As	16 ± 2	16 ± 3	14.3	185 ± 10	185 ± 15	184
Se	120 ± 5	100 ± 10	112	75 ± 6	70 ± 6	70.1
Cd	5.5 ± 0.2	5.4 ± 0.6	5.8	5.0 ± 0.4	5 ± 1	4.9
Pt**	99 ± 3	100 ± 4	100	100 ± 3	100 ± 3	100
Pb	330 ± 10	320 ± 15	310	89 ± 4	90 ± 5	90.7

* Concentrations are given in mg/L.

** The addition of the element is equivalent to 100 μg/L.

ues of the contents for all elements coincide within the measurement error (Table 3).

Decreasing the acidity of test solutions. It was noted above that a significant problem of the ICP–MS method is quite severe restrictions on the composition of the solutions introduced into the plasma. In particular, with the introduction of solutions with an acid concentration of >3 wt %, the detection sensitivity decreases. To decrease the acid concentration, either the solution being analyzed is diluted, or it is evaporated, and the dry residue is dissolved in the acid of a desired concentration. Both methods have drawbacks, namely, when diluting a sample, the detection limits of elements increase; during evaporation, contamination of the sample or loss of the determined elements may occur. It was proposed to use the method of separation of acid and analytes when passing a strongly acidic solution through adsorbents of different nature [11]. This technique is successfully used to solve some technological problems: the recovery of rare-earth elements (REEs) from a phosphoric acid extraction agent [12] and the production of aluminum oxide from solutions of nepheline acid processing [13]. The possibility of using this method to determine the elements in bone tissue by ICP–MS was previously demonstrated [14, 15]. We conducted a systematic study of the separation of nitric acid and 39 metal ions using the AB-17 anion exchanger and Styrosorb 584 hypercrosslinked polystyrene. We determined Li, Be, Na, Al, K, Ca, Sc, Mn, Co, Ni, Cu, Zn, As, Se , Y, Zr, Nb, Mo, Ag, Cd, Sb, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Pb, Bi, Th, and U. When passing a solution with an initial HNO_3 concentration of 4 M, most of the elements quantitatively elute from the column with the anion exchanger by passing 2–4 mL of the test solution. The concentration of HNO_3 in this portion of the solution decreases 20-fold. We compared the separation of acid and REEs on the anion-exchange and neutral adsorbents. It is found that REEs and HNO_3 are separated more efficiently on neutral Styrosorb 584. Anomalous behavior of Ag, Pb, Th, and U on the AB-17 anion exchanger was observed. These elements are absent in the eluates, in which most of the elements and acid are accumulated. Ag, Pb, Th, and U are eluted only after passing four column volumes of deionized water. Figure 1 illustrates the anomalous behavior of Ag, Th, and U. It was found that Na, K, Fe, Al, and Li at a concentration of 50–1000 mg/L in the test solution do not affect the degree of extraction of REEs. The possibility of using the "acid retardation" method in the determination of impurities in HNO_3 (cp grade) and metallic silver by ICP–MS with direct injection of samples into the plasma without dilution is shown, and the associated increase in the detection limits is recorded. At the same time, the method of acid retardation is useless in analyzing strongly acidic solutions with a high concentration of matrix elements, for example, Li, Na, and Ca, as matrix and trace components are not separated. With a high concentration of matrix components, acid retardation should be combined with other separation methods.

Analysis of samples of different composition. Below are examples of decreasing and/or eliminating the effect of the matrix composition on the results of determination of analytes by ICP–MS in real samples.

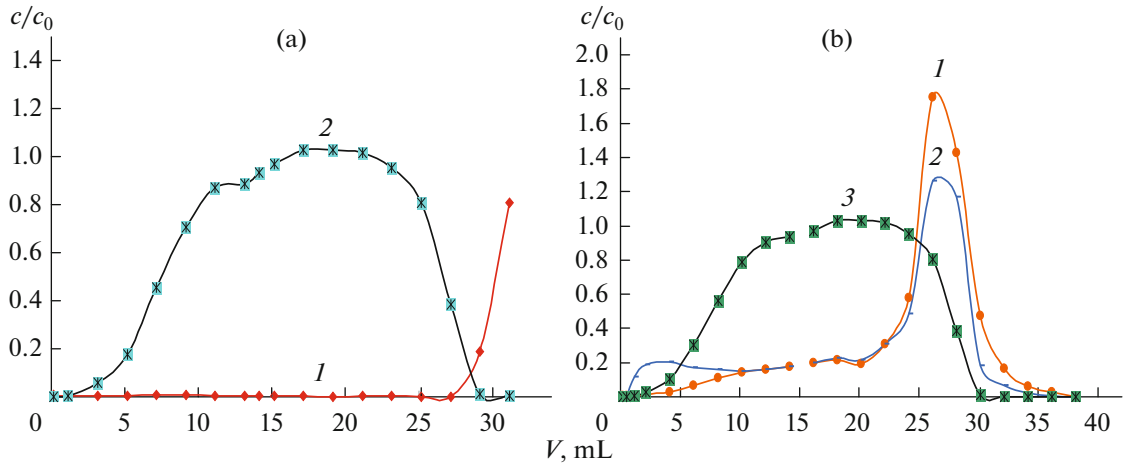

Fig. 1. Output curves of (a) (*1*) Ag and (*2*) HNO_3 and (b) (*1*) Th, (*2*) U, and (*3*) HNO_3 in the adsorption on AB-17 anion exchange resin in an NO_3^--form and desorption from the anion exchanger with deionized water; concentration of elements, 100 μg/L.

Blood and urine of infants. Together with the colleagues of Department of Children's Diseases no. 2, Pirogov Russian National Research Medical University, we analyzed the blood and urine of premature babies aged from several days to 2 months, suffering from renal failure. Clinically significant elements in the initial solutions for dialysis and in solutions obtained after dialysis in several patients were also determined [16, 17]. Analysis of the micronutrient composition of dialysate can be informative for assessing the micronutrient status of the patient's body as a whole. We studied two methods for eliminating the effect of organic components of the test solutions on the results of the determination of elements: microwave decomposition and simple dilution with 1% HNO_3 in deionized water. It was shown that such solutions can be analyzed after a simple dilution, avoiding the long stage of microwave decomposition [16, 17].

Urine, like dialysis fluid, is also a highly concentrated saline solution with a significant concentration of organic salts. We studied the need for mineralization and dilution of the sample in the analysis of urine. It is shown that urine can be analyzed without mineralization after diluting the sample with deionized water tenfold. To find the possible effects of polyatomic ions on the determination of elements by ion chromatography, the concentration of sulfate, chloride, and nitrate ions in the urine of children was determined. A high concentration of chlorides and sulfates is noted, which can interfere with the determination of ^{51}V, ^{53}Cr, ^{75}As, ^{65}Cu, ^{64}Zn, and ^{66}Zn. When the arsenic concentration in the sample is at the level of *n* μg/L, the chloride ions at a concentration of not more than 0.01 M do not affect the result of arsenic determination [16]. On the contrary, the accuracy of the results of determining chromium and vanadium is affected by the concentration of chloride ions already at a level of 10^{-4}–10^{-3} M. Sulfate at a concentration of not more than 0.05 M does not interfere with the determination of copper or zinc.

One of the options for eliminating matrix interference in ICP–MS is the use of a dynamic reaction cell. We compared the results of determining As, Cr, and V with such a dynamic cell and without it. With the arsenic concentration in the urine of >10 μg/L, the direct method of determination gives the same result as the method using the dynamic cell. However, the actual concentration of chromium and vanadium in the urine is significantly lower, that is, less than 10 μg/L. The use of a dynamic cell yielded accurate results for the determination of such low concentrations of these elements in the urine.

In [16, 17], the effect of high concentrations of matrix elements on the results of the determination of a number of elements in the blood, urine, and hair was studied. In urine, the matrix elements are sodium and organic acids; in the blood, these are elements of homeostasis (sodium, potassium, chloride); high concentrations of calcium and, especially, sulfur (up to 5%) are present in the hair. High concentrations of the matrix components may cause changes in the ionization conditions of the elements in the plasma and, accordingly, in the sensitivity curve. In this regard, in addition to the effect of polyatomic chloride-containing ions, we studied the effect of sodium and potassium (up to 150 mg/L), calcium and magnesium (up to 50 mg/L), and sulfur (up to 500 mg/L) on the intensity of the analytical signal of the elements being determined . Potassium and magnesium slightly overestimate the signals on the masses up to ^{53}Cr, and they do not affect the masses heavier than ^{53}Cr. Calcium at a concentration of 50 mg/L and sodium at a concentration of 150 mg/L overestimate the signals of light isotopes (Li, Be, B, Al, and V) by 20–30%. The effect of

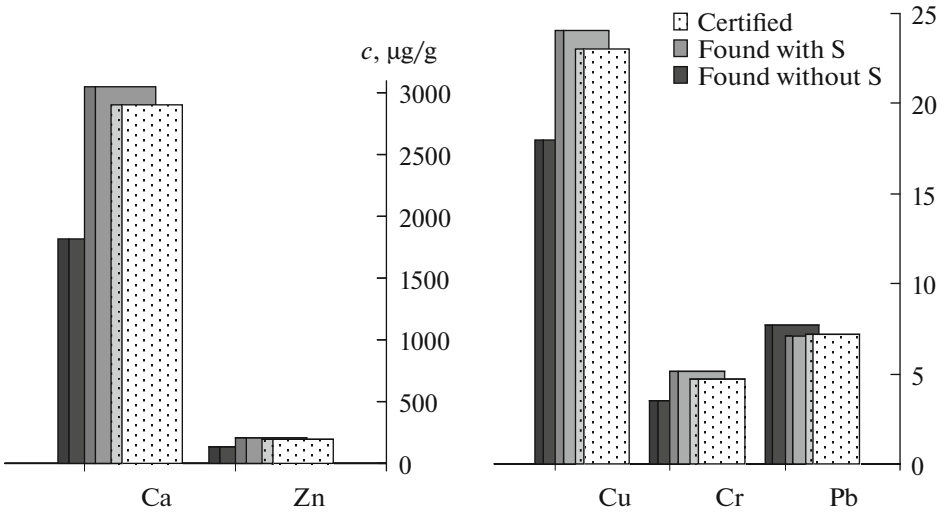

Fig. 2. Determination of elements in standard hair samples, using different methods to account for the matrix effect.

sulfur is most noticeable; it affects in different ways depending on the mass range. At a concentration of 500 mg/L, sulfur decreases the signals of a wide range of elements (from Li to Ba) on average by 30%.

The accuracy of the determination of elements in the hair was checked by analyzing standard samples of the composition [16, 17]. As the adverse effect of sulfur on the intensity of analytical signals of elements was revealed, the possibility of taking this effect into account using an internal standard was also studied. Sc, Y, and Tl were used as internal standards. None of these elements enabled us to take into account such a noticeable effect of sulfur. In the analysis of hair, the obtained concentrations of the elements turned out to be close to the certified values only with the introduction of 500 mg/L of sulfur in the calibration solutions (Fig. 2).

In this case, the measurements were carried out in the standard setting mode of the instrument. It is noted above that in such a mode of operation of the device, a single internal standard is not enough; it is necessary to use calibration solutions that are close in matrix composition to the samples to be analyzed. Figure 2 confirms this conclusion, that is, the introduction of sulfur in the calibration solutions yielded accurate results of the analysis.

Biologically active compounds. The approach proposed above expands the possibilities of ICP–MS and enables the determination of chemical elements in organic compounds. For example, Cu, Zn, Pd, Ag, Pt, and Au were determined in synthesized biologically active compounds in order to prove the absence of the catalytic effect of these elements in the reactions used for the synthesis of substituted indoles [18]. The weight of the substance was 1–2 mg. Using ICP–MS, we studied the distribution of melittin labeled with ruthenium in the tissues of mice [19]. Ruthenium-labeled peptides with $[(C_5H_5)Ru]^+$ are easily identified by mass spectrometry. This label is smaller and more stable than commonly used radioactive or fluorescent labels. Measuring the signal of ^{99}Ru, we studied the biological distribution of melittin in the organs and blood of mice.

It was stated above that the proposed approach is convenient for routine analysis because it offers analyzing various biological fluids without selecting a matrix of calibration samples. In [20], systems for delivery Pt-containing anticancer preparations into glioma cells were developed. The effectiveness of various transport proteins, as well as the safety and availability of the drug, was controlled by determining platinum in blood samples of rats with glioma. More than 300 blood samples were tested to verify the developed mechanism of drug delivery into the cell. Simple dilution of blood samples, the use of external calibration, and operations in the "stable parameters" mode significantly shortened the analysis time.

Gallium and platinum in pharmaceuticals. In recent years, increasing attention has been paid to the development and introduction of metal-containing anticancer preparations, an alternative to platinum chemotherapeutic agents. These drugs are synthesized, as a rule, using metal compounds that are absent in the human body, in particular, gallium(III). The most important stages of the clinical implementation of a promising gallium preparation based on a complex with 8-hydroxyquinoline are the study of its stability in biological media, toxicity, metabolism, pharmacokinetics, ways of excretion, etc. The determination of gallium in biological fluids, which is necessary for this purpose, requires the use of highly sensitive methods for reliable clinical monitoring. As part of a preclinical study of a new anticancer drug based on gallium 8-hydroxyquinolinate, the task is to develop an

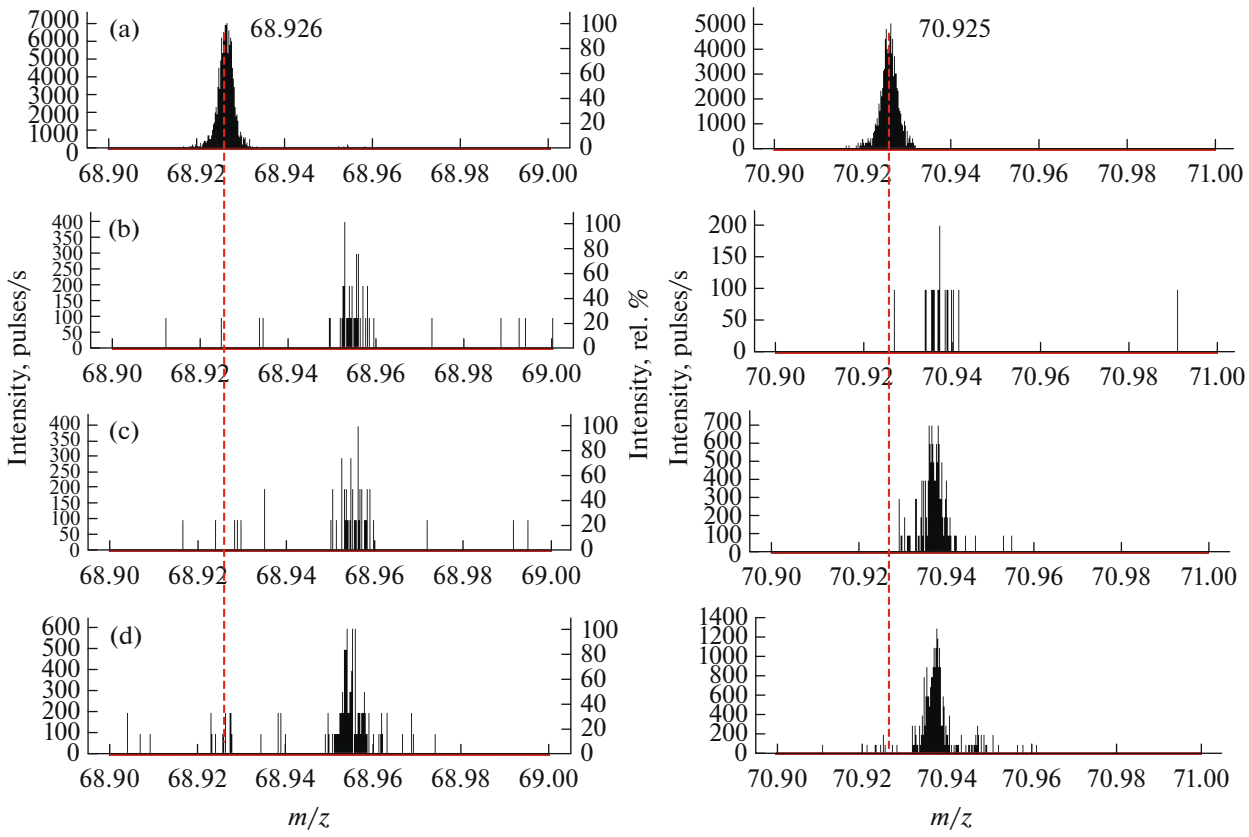

Fig. 3. High-resolution spectra near masses 69 and 71, recorded using an Element 2 instrument: (a) reference solution (1 μg/L of Ga), (b) high-purity water, (c) urine diluted 50-fold, and (d) a phosphate buffer solution diluted 50-fold.

approach to determine gallium in urine, serum, and model secretion of the small intestine (a phosphate buffer solution with the addition of pancreatin) by ICP–MS. Such a procedure should support clinical monitoring of the metal concentrations corresponding to different therapeutic doses of the drug.

The analytical signals of ^{69}Ga and ^{71}Ga are mainly affected by polyatomic complexes of sulfur, chlorine, and phosphorus, that is, elements, the concentration of which in human biological fluids is rather high. A procedure is developed for determining gallium in urine, serum, and small intestinal juice using quadrupole ICP–MS. The detection limits (c_{min}) of gallium, calculated by the 3σ criterion, were 60 ng/L for urine, 32 ng/L for a model solution of small intestine juice, and 50 ng/L for serum. The accuracy of the results is confirmed by the standard addition method [21, 22].

The issue of the nature of the background signal, which determines the value of c_l, is also essential. The mass resolution of the quadrupole ICP–MS does not allow a conclusion which of the factors limits the value of c_l: the background concentration of gallium in biological solutions or the effect of macro components of reagents on the magnitude of the signals of gallium isotopes. To solve this problem, an Element 2 high-resolution mass spectrometer was used. The background signal near the masses 69 and 71 recorded with the Element 2 instrument is shown in Fig. 3

The signals of a standard aqueous solution with a gallium concentration significantly exceeding the background level (1 μg/L) are located at masses 68.926 and 70.925. When determining the background signals in high-purity water, in urine diluted 50 times, and in a physiological buffer solution, the structure of the background signals is significantly different; that is, they are localized at masses 68.956 and 70.937. Thus, it is clear that the background signals in the test biological fluids are caused by the polyatomic molecules of the macro components of the samples rather than by the gallium concentration. The interpretation of the spectra showed that the signal at mass 69 is due to the presence of doubly charged ions Ba^{2+}, La^{2+}, and Ce^{2+}. Given the significantly lower concentrations of cerium and lanthanum compared to barium in biological fluids, it can be assumed that barium makes the main contribution. Barium at a concentration of 150 μg/L contributes to the signal at mass 69, corresponding to 9 μg/L of gallium. Additional confirmation of the main contribution of barium to the background signal at mass 69 is the linear dependence of this background signal on the concentration of barium in the solution. It is shown that the background signal is not deter-

mined by such diatomic ions as $^{33}S^{36}Ar^+$, $^{31}P^{38}Ar^+$, $^{29}Si^{40}Ar^+$, $^{51}V^{18}O^+$, $^{52}Cr^{17}O^+$, or $^{53}Cr^{16}O^+$.

The background signal near mass 71 may be due to $^{40}Ar^{31}P^+$ and $^{35}Cl^{36}Ar^+$ ions. The mass difference of these complexes is so small that even using a high-resolution device, it is impossible to determine the fraction of each in the common background signal at mass 71. On the other hand, ions $^{142}Nd^{2+}$, $^{142}Ce^{2+}$, $^{55}Mn^{16}O^+$, $^{53}Cr^{18}O^+$, $^{40}Ar^{15}N^{16}O^+$, and $^{40}Ar^{14}N^{17}O^+$ are excluded from the number of possible interfering ions with mass 71. According to the data obtained, it is the polyatomic complex $^{31}P^{40}Ar^+$ that is the primary source of background at mass 71. Thus, when using a quadrupole mass spectrometer, the detection limit of gallium in the biological fluids under study is determined by the interference of polyatomic ions formed by the matrix components of the samples rather than by the gallium concentration in the samples or reagents used.

To study the bioavailability of the preparation and assess the rate of its elimination from the body, the solubility of the gallium drug in a solution that simulates the juice of the small intestine and in the urine was determined. The found concentrations of gallium range from 0.35–0.50 to 1.3–1.5 mg/L for the active ingredient and the dosage form, respectively. The extraction of gallium into both biological fluids increases threefold during the transition from the complex to the dosage form; that is, the kinetics of dissolution of the drug depends on its form.

The binding of metal-containing anticancer substances with blood proteins affects the bioavailability and pharmacokinetic properties of drugs [23]. It is noted that the binding and transport of the preparation based on gallium occur with transferrin and albumin; however, the dependence of the formation of adducts on time and binding constant were not studied. We investigated the clinical aspects of the use of the drug based on gallium 8-hydroxyquinolinate [24]. We studied its binding to serum proteins—transferrin (in apo-form) and albumin—using ICP–MS. In the study of individual proteins and serum, it is found that the accumulation of the gallium preparation by albumin and serum occurs quickly, for less than 0.5 min. Transferrin quickly binds only 35% of gallium, and further accumulation lasts 48 h because of a stepwise process or the formation of various forms of metal adducts. Albumin binds 10% of gallium, and transferrin, 50%, while 94% of the introduced metal is found in serum after ultrafiltration. Other serum proteins are also involved in the transport of the drug. Based on these results, the protein–gallium drug adduct binding constants were calculated, namely, $(1.7 \pm 0.2) \times 10^4$ for transferrin and $(1.9 \pm 0.8) \times 10^3$ for albumin, which confirms our data that the delivery of a gallium-based drug to the tumor cells occurs more with transferrin than with other blood proteins [24].

Unlike gallium-based preparations, platinum complexes are primarily bound to albumin. Using ICP–MS after ultrafiltration of protein fractions, the characteristics of these drugs that are important for clinical studies were determined, such as the binding constant for the transport protein of the blood, the distribution in serum, and lipophilicity [25]. The albumin–metal drug adduct formation constants calculated from the results of measurements were from 10^4 to 10^6 for various platinum complexes. The affinity for the protein was shown to be varied depending on the halide included in the complex. For example, for the cis isomers, the binding constant decreases in the order of $I^- > Br^- > Cl^-$; for the trans isomers, such a pattern was not observed. The selection of the preparation that is most fully bound to the transport protein should prevent the removal of the active component from the body. To develop drugs of the new generation, it is necessary to understand the process of transfer of platinum complexes through the lipid layer of the cell. Based on the obtained values of platinum concentrations in the organic and aqueous phases, it was concluded that the compounds studied were lipophilic, with the cis isomers being more hydrophilic than the corresponding trans species. It is shown that 98% of the platinum complex is bound to albumin, and only minor amounts of the preparation are in the lipid phase [25]. Thus, the combination of the developed ICP–MS procedures for determining metals as part of complex anticancer preparations with a simple procedure for filtering the protein fractions is a high-performance tool for preclinical studies of new drugs.

CONCLUSIONS

The problems of the effect of sample composition on the results of determination of a wide range of analytes in biological and pharmaceutical samples by ICP–MS are discussed. The primary mechanisms are investigated, and methods for taking into account and eliminating this effect using an Agilent 7500c quadrupole mass spectrometer are proposed. The main operational parameters responsible for minimizing the investigated nonspectral interference are determined. Various versions of the internal standard method were studied, which offer decreasing the effect of the sample composition on the determination results. Combined approaches to the complete elimination of nonspectral interference using external calibration are proposed. A method is proposed for reducing the acidity of the test solution by the adsorption separation of the analyte elements and acid. Examples of solving specific analytical problems are given, namely, analysis of blood and urine in premature infants, analysis of medicinal anticancer drugs and mechanisms of transport of active drugs into the cell, and analysis of biologically active compounds.

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