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Application of ICP-MS for Evaluation of Fifty-One Trace Element Contents in Small Sample of Human Breast Tissue

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ABSTRACT

For the first time, a method of inductively coupled plasma mass spectrometry (ICP-MS) has been developed that makes it possible to evaluate the content of fifty-one trace elements (TE) in microsamples (mass from 10 mg) of breast tissues. Using the developed technique, the samples of tissue obtained from autopsies of 38 apparently healthy women aged 16-60 years who died suddenly was studied. The content of 23 TE: Al, As, B, Ba, Bi, Cd, Ce, Cr, Cu, La, Li, Mg, Mn, Nb, Ni, Pb, Rb, Sb, Sn, Sr, Ti, W, and Zn was determined in all or in most of samples. For these elements, all the main statistical characteristics were calculated, including the arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975. The content of 10 TE such as Co, Cs, Ga, Ge, Mo, Nd, Pr, Th, V, and Y was determined only in a small part of the studied samples of normal breast tissue; therefore, only the possible upper limit of their content was evaluated. The levels of 18 studied TE in normal breast tissue were below the detection limit (ppm): Be <0.001, Co <0.005, Dy <0.0005, Eu <0.0005, Er <0.0005, Gd <0.0005, Ge <0.005, Ho <0.0005, Lu <0.0005, Re <0.001, Se <0.1, Sm <0.0005, Te <0.003, Tb <0.0005, Tl<0.001, Tm <0.0005, U<0.002, and Yb <0.0005. It was shown that the content of Al, As, B, Cd, Cr, La, Li, Mn, Ni, Pb, Sn, Sr, and Ti in healthy breast tissue is many times higher than the level of these TE in blood serum, while the content of As, Bi, Cr and Pb is higher than in prostate and thyroid tissue. Particularly noteworthy is the ability of breast tissue to accumulate As, which, in our opinion, requires further detailed study.

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Introduction

Oncological diseases of the female breast have become one of the world's problems of recent decades, requiring an immediate solution. In the structure of oncological morbidity in women, breast cancer (BC) in almost all economically developed countries occupies the first place. More than 1 million cases of BC are registered annually in the world, and every ninth woman living today will develop breast cancer during her life [1].

The etiology of BC is unknown and is likely related to a combination of genetic factors, such as susceptibility genes (BRCA1 and BRCA2), and adverse environmental factors [2]. Despite numerous studies, adverse environmental factors have not been clearly identified, although many candidates have been found that increase the risk of BC [3]. Since the change in the human gene pool is rather slow, it can be assumed that the alarmingly rapid increase in the incidence of BC is primarily associated with the transformations taking place in the environment. The steady development of industry, industrial chemistry and technology in agriculture, food production, pharmaceuticals, medicine, cosmetics, especially over the past 100 years, has led to global changes in the quality of the human environment [4]. These changes also apply to the amounts of trace elements (TE) entering the human body.

The female breast is a specialized organ whose main function is to produce milk to feed the baby. The female breast is made up of mammary glands (glandular tissue) as well as stroma (adipose tissue and ligaments, surrounding ducts and lobules, blood and lymph vessels) [2]. The concentration of many TE in breast milk, such as cobalt (Co), chromium (Cr), iodine (I), manganese (Mn), molybdenum (Mo), nickel (Ni), rubidium (Rb), zinc (Zn) and some others are higher than in blood serum [5]. It was found that the mammary gland is able regulate these TE concentrations even with a significant change in the maternal diet or with various effects on the maternal condition [6]. This conclusion is in good agreement with our position on TE homeostasis in fluids and tissues of the human body, formulated by us about fifty years ago [4]. The ability of the glandular tissue during lactation to accumulate significant amounts of TE for milk production suggests a special elemental composition of this tissue during the dormant period of breast as well. In addition, it is known that adipose tissue is a pool for some TE [7].

Our previous studies have shown that TE homeostasis plays an important role in the normal and pathophysiology of human bones, thyroid and prostate glands [4, 8-39]. From this we can conclude that the specific physiological factors of the mammary gland associated with TE probably play a key role not only in the normal physiology of the glandular tissue, but also in the etiology of various diseases of the mammary gland, including breast cancer.

Despite the understanding of the important role of TE, surprisingly little is known about the involvement of TE in the normal and pathological physiology of the human breast.

There are few studies of TE content in the mammary gland of women using chemical and instrumental methods [40-51]. However, in the published data for almost all breast TE, there are large differences in the obtained mean values, which is probably due mainly to analytical difficulties.

The main objective of this study was to evaluate reliable values for the content of fifty-one TE in normal female breasts using inductively coupled plasma mass spectrometry (ICP-MS). The second goal was to evaluate the quality of the results obtained. The third objective was to compare the mean mass fractions of TE in normal breast tissue obtained in the study with data published in literature. The ultimate goal was to find differences between the results obtained for normal breast tissue and the reference data for the mass fractions of TE in blood serum, adipose tissue, and also tissues of the prostate and thyroid [2, 5, 52-56].

Material and Methods

Samples

A randomized sample of normal breast tissue was obtained from autopsies of 38 women (age from 16 to 60 years, Caucasian race, Caucasian lifestyle) who died suddenly. Autopsies were carried out in the forensic medical examination department of the Obninsk city hospital during the first day after sudden death. Typical causes of death for most women were car accidents and injuries. All of the dead were residents of Obninsk, a small town (about 120,000 inhabitants) in a non-industrial area 105 kilometers southwest of Moscow. Written informed consent was obtained from relatives of the victims during sampling. The study was performed according to the standards of the Institutional Ethics Committee and the Helsinki declaration of 1975, as revised in 1983, and was approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Tissue samples of mass about 10 g from all victims were taken in the right mammary gland in its lower inner quadrant. A scalpel made of high-purity titanium was used for sampling [57]. Available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, neoplasm, or other chronic disease that would interfere with normal breast development. None of the subjects received drugs that affect the morphology of the mammary gland and the content of TE in the gland.

Sample Preparation

One of the final goals of our studies of the content of TE in the mammary gland in normal and pathological conditions is the search for markers of BC and the development of new diagnostic methods by determining the content of TE in puncture biopsies of the lesion. When examining a patient with a single puncture biopsy, a material mass about 10-20 mg can be obtained. Therefore, we initially developed a technique for microwave (MW) autoclave acid digestion of breast tissue samples of small mass from 10 mg for subsequent determination of the TE content in them using ICP-AES and ICP-MS analytical methods [58]. We have made teflon mini vessels for placement inside a standard EasyPrep autoclave with a volume of 100 cm^3 (by CEM Corporation). The mini vessel is a cylinder with an internal volume of 3 cm^3 and an external diameter of 1.2 cm, closed by a stopper and then a pressure cap on the thread. Several options for the design of the stopper are provided. One variant of the plug provides for a hole with a diameter of 5 mm in which a teflon tube about 5 cm long is installed as a condenser of acid vapors and a kind of "separator" of the mini vessel volume and the EasyPrep autoclave one (Figure 1).

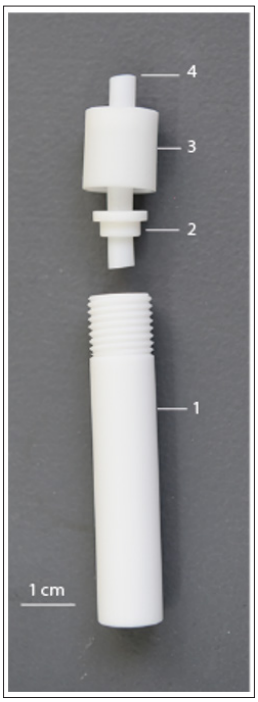

Figure 1: Photo of the Mini Vessel used for Samples Acid Decomposition (1- Cylinder for Sample with thread, 2-stopper, 3- threaded cap, 4- tube). All Parts are made from Teflon

The presence of a teflon condenser tube, on the one hand, avoids the loss of the contents of the mini vessel and minimizes the possible transfer of volatile components between samples. On the other hand, by creating a common atmosphere, the pressure inside the mini vessels is equal to outside one, which ensures adequate control of both pressure and temperature in the mini vessels using the standard means of the microwave system.

The mini vessels are located on the same level inside the autoclave, which ensures the identical intensity of the microwave field for all mini vessels. To create a pressure balance inside the system, an estimated amount of the same reagents is added to the autoclave as in the mini-vessels. The starting point is to select the required volume of acid sufficient to decompose the sample in the inserts. Next, the volume of acid added to the autoclave is calculated to ensure equal pressure. This condition is fulfilled when the ratio of the occupied volume (liner or autoclave) to the total volume (liner or autoclave) is equal. For details, see the ref [58]. The difference is that the reagents (acids) added to the autoclave may have a lower purity level compared to the acids used to decompose samples in mini vessels.

Thereby, the productivity of the samples preparation increases three times, extra-pure reagents are economically consumed and the volume of the sample after decomposition is minimized. The latter ensures the achievement of lower detection limits.

The analyzed samples of 10 mg or more mass were placed in mini-vessels. In each mini-vessel 1.4 ml of high-purity nitric acid was added. The mini-vessels were closed with a stopper, the stopper was fixed with a lid, and a teflon condenser tube was inserted into the common hole. Three assemblies of these mini-vessels were enclosed in autoclave. The nitric acid (12.5 ml) of pure for analysis

grade was added to the autoclave to provide a vapor pressure equal to the pressure of acids in mini-vessels. The autoclaves with mini-vessels were then placed on the microwave system rotor. One of the autoclaves contained temperature and pressure sensors, as well as a hollow fluoroplast cylinder, the volume of which corresponded to that of the enclosure. The samples were heated to 150°C for 15 min and hold for 20 min at this temperature. The radiation power in MW was 800 watts at a frequency of 2450 Hz. After cooling the vessels to 30°C the contents of the mini-vessels were quantitatively transferred into 10 ml test tubes and the solutions were adjusted to 10 ml with 2% HNO_3 solution.

ICP-MS Measurements

Deionized water distilled without boiling in a PTFE Subboiler ECO IR Maassen water and an acid purification system (Germany) and HNO_3 (65% for analysis, max 0.005 ppm Hg) from Merck (Germany) were used to prepare calibration solutions and in decomposition. The calibration dependences for the elements were determined using standard reference solutions manufactured by High-Purity Standards (High-Purity Standards, North Charleston, SC, USA): Trace Elements in Drinking Water Standard CRM-TMDW (26 elements), 68 Element Standards ICP-MS-68A (Solution A and Solution B) and single-element solutions of B, Mg, Al, Mn, Ni, Cu, Zn, Se, Rb, Sr, Cs, Ba.

Quadrupole mass spectrometer with inductively coupled plasma (X Series II made by Thermo Scientific, Germany) has been used. The mass spectrometer has been equipped with a concentric atomizer and a quartz cyclone atomization chamber cooled by a Peltier element (2°C).

Working calibration solutions for ICP–MS were prepared by sequential dilutions of the initial solutions of multielement ICP–MS-68 (10 mg/L) to 10, 25 and 50 μg/L for solution A, to 5 and 10 μg/L for solution B. Single-element solutions (10 mg/L) were diluted to 500 μg/L for B, Mg, Al, Mn, Ni, Cu, Zn, Sr, Ba and to 20, 75 and 100 μg/L for Se, Rb, Cs. In all cases, 2% HNO_3 was used as a diluent. The solutions were diluted before measurement in disposable polypropylene test tubes of the volume 10 or 50 mL (Litaplast-Med, Belarus). The gravimetric method was used to determine the degree of dilution. To control the signal drift and take into account the needed compensation during the processing of the results, indium was used as an internal standard, which was added to the sample solutions to get its concentration at 10 μg/L.

The parameters of the measurement procedure were as follows: generator output power 1400 W, plasma-forming gas (argon) consumption 13 L/min, auxiliary gas consumption 1.25 L/min, argon flow rate through the atomizer 0.88 L/min, plasma sampling depth 105 rel. units and sample flow rate 1 mL/min. Mass Spectra were Measured using two Scanning Modes: (1) panoramic (Survey Scan) with 5 passes from 5 to 244 m/z and (2) at points (Peak Jumping) with 1 channel per weight, the integration time of 20 ms, and with 25 passes. All measurements were performed using PlasmaScreen software. Subject to all the device settings, the level of oxide ions CeO+/Ce+ is no more than 2%, and the level of doubly charged ions (Ba2+/Ba+) is no more than 3%. For measurements of Zn, as and Se solutions obtained after MW decomposition of the samples were diluted in five times with distilled water. For measurements of the rest elements, the solutions were diluted twice with distilled water.

The ICP–MS data were processed using the iPlasmaProQuad software developed in the Vernadsky Institute [59]. This program was designed to process information output from the X Series II quadrupole mass spectrometer. The program involves all stages of processing from calibration to calculating element concentrations, including various corrections (the mass spectrometer is used only as an isotope mass detector). This approach gives complete control over the processing and estimation of the uncertainty of the measurement results, which is an essential performance characteristic both for the subsequent use of the results of analysis in other fields and for monitoring the quality of sample preparation.

International Reference Materials

To check the reliability of the results obtained, the Polish certified reference materials MODAS-5 (Cod Tissue) and MODAS-3 (Herring Tissue), as well as the reference material prepared by the International Atomic Energy Agency IAEA-153 (Powdered milk) were used.

Statistics

The main statistical parameters, such as the arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975 for mass fractions of TE (mg kg^{-1} of dry tissue). Were calculated using the MS Excel program,

Results

Table 1 depicts the mass fractions of determined elements in the three different international certified reference materials MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue) and IAEA-153 (Powdered milk) using the developed ICP-MS method.

Table 1: ICP-MS Data (Mean±SD) of Trace Elements Mass Fraction (mg kg^{-1}, dry mass basis) in Certified Reference Material MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue), and IAEA-153 (Powdered milk) Compared to their Certified Values

El	MODAS-5		MODAS-3		IAEA-153	
	Certificate	Our result	Certificate	Our result	Certificate	Our result
Ag	-	-	0.04±0.01	0.039±0.003	-	-
Al	-	6±1	-	14±1	-	-
As	1.64±0.27	1.7±0.1	9.24±0.81	8.8±0.4	-	-
B	-	0.34±0.05	-	9.0±0.3	-	2.03±0.07
Ba	0.162±0.028	0.18±0.02	2.71±0.28	2.6±0.1	-	0.67±0.04
Bi	0.007	0.006±0.001	-	-	-	-
Cd	0.005	0.0046±0.0004	0.33±0.03	0.32±0.01	-	-
Ce	-	0.006±0.002	-	0.021±0.008	-	-
Co	0.014	0.012±0.001	0.08±0.01	0.110±0.003	-	0.016±0.001
Cr	0.201	0.3±0.1	0.90±0.11	0.9±0.2	-	-
Cs	0.059±0.005	0.059±0.002	0.085±0.008	0.086±0.005	-	-
Cu	1.38±0.09	1.5±0.1	3.19±0.22	3.2±0.1	0.6±0.2	0.42±0.03
Ga	-	0.012±0.001	-	0.036±0.002	-	-
Ge	-	0.006±0.001	-	0.018±0.002	-	-
La	-	0.007±0.002	-	0.017±0.005	-	-
Li	0.026	0.030±0.002	0.90±0.11	0.76±0.03	-	0.034±0.005
Mg	1200±200	1178±38	3000±200	2739±75	1060±75	1023±19
Mn	0.92±0.08	0.89±0.05	5.78±0.61	5.3±0.1	-	0.22±0.04
Mo	-	-	0.13±0.02	0.14±0.01	0.3± 0.3	0.228±0.004
Nb	-	-	-	0.006±0.002	c	-
Nd	-	-	-	0.006±0.003	-	-
Ni	0.136	0.14±0.02	0.32±0.05	0.5±0.1	-	0.13±0.02
Pb	0.045	0.05±0.01	0.104±0.013	0.13±0.01	-	-
Rb	4.54±0.33	4.5±0.1	2.33±0.20	2.24±0.07	14.0±1.9	14.9±0.4
Sb	-	-	0.016±0.004	0.017±0.002	-	-
Se	1.33±0.1	1.2±0.1	2.63±0.2	2.8±0.1	-	-
Sm	-	-	0.0018	0.0015±0.0003	-	-
Sn	-	0.14±0.01	-	0.23±0.02	-	0.05±0.02
Sr	4.07±0.36	3.5±0.4	192±15	180±6	4.1±0.6	3.76±0.07
Ti	-	<0.9	-	<2.1		<0.2
Tl	-	0.0013±0.0002	-	0.0014±0.0005	-	-
U	-	-	0.075±0.008	0.063±0.002	-	-
V	-	-	0.78±0.11	0.62±0.01	-	-
W	-	0.024±0.008	-	-	-	-
Y	-	-	0.0096	0.009±0.003	-	-
Zn	20.1±1.1	21±1	111±6	114±3	39.5±1.8	33±1

El – Element, Mean - Arithmetical Mean, SD - Standard Deviation

Table 2 presents the main statistical parameters (arithmetic mean, standard deviation, standard error of the mean, minimum and maximum values, median, percentiles with levels of 0.025 and 0.975, as well as a possible upper limit of content for some TE) of mass fractions of determined TE in normal breast tissue in women aged 16 to 60 years.

Table 2: Basic Statistical Parameters of 33 Trace Elements Mass Fraction (mg kg^{-1}, dry mass basis) in the Normal Breast Tissue of Females between Ages 16–60 years

Element	DL	M(M_{max})	SD	SEM	Min	Max	Med.	P0.025	P0.975
Al	0.5	3.42	1.98	0.41	1.21	7.36	2.58	1.24	7.21
As	0.001	0.030	0.015	0.003	0.009	0.064	0.026	0.0095	0.0585
B	0.05	0.170	0.084	0.021	0.068	0.380	0.167	0.0684	0.351
Ba	0.02	0.174	0.148	0.031	0.027	0.601	0.140	0.0314	0.565
Bi	0.001	0.014	0.018	0.005	0.0010	0.0620	0.0072	0.00128	0.0558
Cd	0.002	0.047	0.033	0.006	0.0102	0.126	0.0365	0.0122	0.122
Ce	0.001	0.0066	0.0038	0.0008	0.0018	0.0160	0.0058	0.00204	0.0148
Co	0.005	(0.0067)	-	-	0.005*	0.0200	-	-	-
Cr	0.02	0.288	0.157	0.027	0.042	0.661	0.275	0.0649	0.630
Cs	0.001	(0.0036)	-	-	0.001*	0.0730	-	-	-
Cu	0.02	0.868	0.544	0.095	0.295	2.63	0.732	0.315	2.24
Ga	0.005	(0.0053)	-	-	0.005*	0.0160	-	-	-
Ge	0.005	(0.0055)	-	-	0.005*	0.0220	-	-	-
La	0.001	0.0062	0.0047	0.0010	0.0016	0.0240	0.0048	0.00171	0.0165
Li	0.005	0.0117	0.0047	0.0015	0.0057	0.0190	0.0116	0.00597	0.0189
Mg	0.05	20.8	13.0	2.3	10	79	18	10.0	44.9
Mn	0.02	0.128	0.138	0.025	0.042	0.774	0.092	0.0428	0.503
Mo	0.005	(0.0091)	-	-	0.005*	0.0250	-	-	-
Nb	0.001	0.012	0.014	0.004	0.0033	0.0551	0.0071	0.00333	0.0443
Nd	0.0005	(0.0013)	-	-	0.0005*	0.0130	-	-	-
Ni	0.02	0.144	0.087	0.015	0.027	0.319	0.112	0.0318	0.310
Pb	0.005	2.16	2.72	0.50	0.20	13.0	1.07	0.244	9.18
Pr	0.0005	(0.00057)	-	-	0.0005*	0.0030	-	-	-
Rb	0.005	0.368	0.360	0.063	0.092	1.71	0.250	0.096	1.38
Sb	0.001	0.0344	0.0357	0.0069	0.0090	0.145	0.0180	0.00965	0.131
Sn	0.01	0.113	0.114	0.023	0.035	0.564	0.068	0.0356	0.384
Sr	0.005	0.522	0.376	0.065	0.055	1.91	0.457	0.0954	1.49
Th	0.005	(0.00503)	-	-	0.005*	0.0060	-	-	-
Ti**	0.1	0.83	0.59	0.16	0.190	1.93	0.660	0.206	1.88
V	0.01	(0.012)	-	-	0.01*	0.060	-	-	-
W	0.002	0.080	0.104	0.030	0.0020	0.335	0.031	0.00384	0.298
Y	0.003	(0.0031)	-	-	0.003*	0.0060	-	-	-
Zn	0.05	4.00	2.87	0.50	1.58	14.9	3.49	1.58	12.6

DL – detection limit, M_{max} – arithmetic mean (see Eq.1), SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, Med. – median, P0.025 – percentile with 0.025 level, P0.975 – percentile with 0.975 level, * Detection Limit, **A scalpel made of ultra-pure Ti was used for sampling.

Comparison of our results with literature data in normal breast tissue of adult women is shown in Table 3.

Differences between the mean values of mass fractions of the determined elements in normal breast tissue of adult women (results of this work) and the reference values of these TE content in blood serum, adipose tissue, as well as in the tissues of the prostate and breast are presented in Table 4.

Table 3: Median, Minimum and Maximum Value of Means of Chemical Element Mass Fractions (mg kg^{-1}, dry mass basis) in Normal Breast Tissue of Adult Females According to Data from the Literature in Comparison with this Work Results

	Published data (Reference)			This work results
	Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	M±SD n=38
Al	3.5 (4)	0.103 (52) [40]	38.4 (20) [41]	3.4±2.0
As	0.48 (3)	0.095 (3) [42]	<5 (-) [43]	0.030±0.015
B	<0.16 (1)	<0.16 (-) [43]	<0.16 (-) [43]	0.17±0.08
Ba	3.1 (2)	0.030(-) [43]	6.24±0.59 (-) [44]	0.17±0.15
Bi	<0.06 (1)	<0.06 (-) [43]	<0.06 (-) [43]	0.014±0.018
Cd	0.034 (5)	0.0310 (8) [45]	<0.4 (-) [43]	0.047±0.033
Ce	0.0012 (1)	0.0012 (1) [46]	0.0012 (1) [46]	0.0066±0.0038
Co	<0.04 (3)	0.0360±0.0008 (18) 47]	0.06 (20) [41]	≤0.0067
Cr	0.088 (7)	0.0012(1) [46]	2.44±0.23 (-) [44]	0.29±0.16
Cs	0.0008 (1)	0.0008(1) [46]	0.0008(1) [46]	≤0.0036
Cu	2.56 (19)	0.4(1) [46]	2280±140 (-) [48]	0.87±0.54
Ga	0.005 (2)	0.004(1) [46]	<0.006 (-) [43]	≤0.0053
Ge	0.0004 (1)	0.0004(1) [46]	0.0004(1) [46]	≤0.0055
La	<0.6 (1)	<0.6 (-) [43]	<0.6 (-) [43]	0.0062±0.0047
Li	-	-	-	0.012±0.005
Mg	85.5 (4)	4.5±0.9 (-) [44]	680 (4) [49]	20.8±13.0
Mn	0.5 (7)	0.06 (-) [43]	3.74 (4) [49]	0.13±0.14
Mo	0.22 (4)	0.008(1) [46]	0.22 (20) [41]	≤0.0091
Nb	<0.3 (2)	0.0004(1) [46]	<0.6 (-) [43]	0.012±0.014
Nd	-	-	-	≤0.0013
Ni	0.16 (7)	0.01(1) [46]	1.14 (20) [41]	0.144±0.087
Pb	0.128 (6)	0.0081(1) [46]	3.21±2.15 (16) [44]	2.2±2.7
Pr	-	-	-	≤0.00057
Rb	626 (2)	0.2(1) [46]	2504 (4) [49]	0.37±0.36
Sb	0.044 (2)	0.030-0.044 (2) [42]	5.0 (-) [43]	0.034±0.036
Sn	0.52 (1)	0.52 (-) [43]	0.52 (-) [43]	0.11±0.11
Sr	0.2 (4)	0.12 (-) [43]	0.70±0.22 (16) [44]	0.52±0.38
Th	-	-	-	≤0.0050
Ti	0.13 (2)	<0.1 (-) [43]	0.16(1) [46]	0.83±0.59
V	<0.008 (1)	<0.008 (-) [43]	<0.008 (-) [43]	≤0.012
W	-	-	-	0.080±0.104
Y	-	-	-	≤0.0031
Zn	8.3 (17)	2.88 (46) [50]	27.8±5.0 (20) [51]	4.00±2.87

M - Arithmetic mean, SD – standard deviation,
(n)* – number of all references; (n)** - number of samples.

Table 4: The Comparison of the Means of Some Chemical Element Mass Fraction (mg kg^{-1}, wet mass basis) in Normal Breast Tissue of Adult Females (this work results) with those in Blood Serum, Muscle and Adipose Tissue (Reference data)

El	Our result*	Reference data				Ratios			
	Breast tissue I	Blood serum [5, 52] II	Adipose [2, 53] III	Prostate [54] IV	Thyroid [55,56]** V	I / I	I / III	1/IV	I/V
Al	1.70	0.23	0.79	7.0	1.86	7.39	2.15	0.24	0.91
As	0.015	0.005	-	0.005	≤0.001	3.00	-	3.00	-
B	0.085	0.030	-	0.20	0.105	2.83	-	0.43	0.81
Ba	0.082	-	-	0.29	-	-	-	0.28	-
Bi	0.007	<0.01	-	0.006	0.0045	-	-	1.17	1.56
Cd	0.024	0.0002	-	0.23	0.41	120	-	0.10	0.06
Ce	0.0033	-	-	0.006	0.0022	--	-	0.55	1.50
Cr	0.144	0.00015	0.201	0.11	0.136	960	0.72	1.31	1.06
Cu	0.434	1.25	0.28	1.9	1.02	0.34	1.55	0.23	0.43
La	0.0031	0.00044	-	0.017	0.0014	7.05	-	0.18	2.21
Li	0.0059	0.0005	0.011	0.0082	0.0038	11.8	0.54	0.72	1.55
Mg	10.4	21.7	-	210	74	0.48	-	0.05	0.14
Mn	0.064	0.001	-	0.27	0.33	64	-	0.24	0.19
Nb	0.006	<0.06	-	0.0011	0.16	--	-	5.45	0.04
Ni	0.072	0.00015	-	0.65	0.096	480	-	0.11	0.75
Pb	1.08	0.001	-	0.48	0.050	1080	-	2.25	21.6
Rb	0.184	0.2	-	2.9	1.57	0.92	-	0.06	0.12
Sb	0.0172	0.002	-	0.0083	0.027	8.60	-	2.07	0.64
Sn	0.057	0.001	-	0.064	0.028	57.0	-	0.89	2.04
Sr	0.261	0.03	0.044	0.51	0.95	8.70	5.93	0.51	0.27
Ti	0.415	0.07	-	0.57	0.93	5.93	-	0.73	0.45
W	0.040	0.0004	-	-	-	100	-	-	-
Zn	2.00	1.1	1.67	210	23.7	1.82	1.20	0.01	0.08

El - element
* We calculated these values using mean our data for water - 50% [60]
** We calculated these values using mean our data for water - 75% [61]

Figure 2 illustrates the possibility of assessing the uncertainty of the obtained individual values using the example of measuring Mn, Al and Ni.

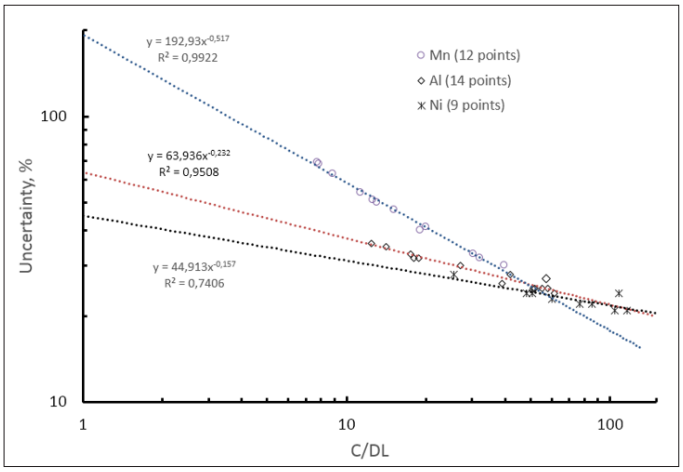

Figure 2: Dependence of Estimated Uncertainty of Measurement for the Single Sample on Ratio of the Found Concentration and Detection Limit for the Cases Mn, Al and Ni Approximated by Power Function and Extrapolated to the Value of C/DL=1.

Discussion

The developed ICP-MS method makes it possible to evaluate the content of fifty-one TE in breast tissues. Acceptable agreement between the obtained element's content values in the international certified reference materials MODAS-5 (Cod Tissue), MODAS-3 (Herring Tissue) and IAEA-153 (Powdered milk) with the data of the corresponding certificates (Table 1) proves the sufficient accuracy of analysis results accumulated in Tables 2-4.

The content of Al, As, B, Ba, Bi, Cd, Ce, Cr, Cu, La, Li, Mg, Mn, Nb, Ni, Pb, Rb, Sb, Sn, Sr, Ti, W, and Zn was determined in all or in most of the samples, therefore, for these TE, the detection limit, the mean value of the mass fraction (M), standard deviation (SD), standard error of the mean (SEM), minimum, maximum, median, and percentiles with levels of 0.025 and 0.0975 was calculated (Table 2).

The obtained values of M, SD, and SEM can be used to compare data for different groups of samples only under the condition of a normal distribution of the results of determining the content of TE in the samples under study. Statistically reliable identification of the law of distribution of results requires large sample sizes, usually several hundred samples, and therefore is rarely used in biomedical research. In the conducted study, we could not prove or disprove the "normality" of the distribution of the results obtained due to the insufficient number of samples studied. Therefore, in addition to the M, SD, and SEM values, such statistical characteristics as the median, range (minimum-maximum) and percentiles with the level of 0.025 and 0.0975 were calculated, which are valid for any law of distribution of the results of TE content in breast tissue.

When performing the analysis of single samples, it is important to assess the uncertainty of the results obtained. This will provide a statistically sound conclusion when interpreting the results of the analysis, for example, for attribution of the tissue sample (pathology vs norm). The iPlasmaProQuad program developed in the Vernadsky Institute is able to estimate the uncertainty of mass spectrometric measurement of concentration [59]. This uncertainty increases markedly when a decrease in the ratio of the found concentration and the detection limit, or, in other words, when the concentration approaches detection limit. Data was sampled for three elements Mn, Al and Ni, differing in the amount of data (samples) falling in the interval C/DL<=100. Figure. 2 shows the relevant data. The results are approximated by a power function and are linearized in logarithmic coordinates. It can be seen that the quality of approximation (R2) is largely it is determined by the number of results located closer to the detection limit. For the elements considered, this is the case manganese. Extrapolation of the approximating function for the case C/DL=1 gives an uncertainty value of more than 100%. Probably, the behavior of the dependence of the uncertainty value on the C/DL ratio is similar for other elements. Therefore, the data of the approximating function for manganese can be used to estimate the uncertainty of determining the concentration of other elements.

We have estimated the uncertainty for some data given in the Table 2 for Mn and Cu cases:
c_{min} = 0.04 ppm (70% uncertainty), c_{max} = 3.1 ppm (20% uncertainty) and c_{min} =0.04 ppm, (100% uncertainty), and c_{max} =2.6 ppm (22% uncertainty), respectively.

The Co, Cs, Ga, Ge, Mo, Nd, Pr, Th, V, and Y mass fractions in normal breast tissue were determined in a few samples (Table 2). The possible upper limit of the mean (M_{max}) for these TE was calculated as the average mass fraction, using the value of the detection limit (DL) instead of the individual value when the latter was found to be below the DL:

$$M_{max} = (\sum_{i}^{n_j} C_i + DL \times n_j)/n \qquad (1)$$

where C_i is the individual value of the TE mass fraction in sample -i, n_i is number of samples with mass fraction higher than the DL, n_j is number of samples with mass fraction lower than the DL, and $n = n_i + n_j$ is number of samples that were investigated.

The content of the following elements in all samples of normal breast tissue were under DL (ppm): Be <0.001, Co <0.005, Dy <0, 0005, Eu <0.0005, Er <0.0005, Gd <0.0005, Ge <0.005, Ho <0.0005, Lu <0.0005, Re <0.001, Se <0.1, Sm <0.0005, Te <0.003, Tb <0.0005, Tl<0.001, Tm <0.0005, U<0.002, and Yb <0.0005.

Most often, in studies of TE in the mammary gland, samples of visually intact tissue adjacent to the tumor are used. However, we have previously shown that the intact tissue adjacent to the thyroid tumors in terms of the level of TE content is not identical to the normal thyroid gland tissue of apparently healthy individuals [59,60]. Therefore, in our review of reported data, only results obtained from the study of normal mammary glands of apparently healthy women were used.

The results obtained for Al, B, Bi, Cd, Ce, Cs, Ga, La, Ni, Sb, Sn, Sr, V, and Zn were in good agreement with the medians of previously published means of TE contents (Table 3). Our results for Ba, Cr, Cu, Mg, Mn, Mo, Nb, Pb and Rb were within the reported ranges of means. At the same time, our data for As, Co, and Si means were below the range of reported means, while obtained result for Ti mean was higher. It is possible that the overestimated value of the mean for Ti obtained in the presented work is explained by the fact that a scalpel made of extra pure Ti was used for sampling. Literature data on Li, Nd, Pr, Th, W, and Y were not found (Table 3). However, it should be noted that the variations of published mean values for some of the studied TE are very large and amounts to several mathematical orders (Table 4).

The content of TE studied in healthy breast tissue were compared with the corresponding reference values in blood serum, adipose tissue, prostate and thyroid human gland (Table 4). Wherein, published data on the water content in breast tissue and the results of report No. 23 of the International Commission on Radiation Protection were used to recalculate the results for wet mass [2,5,52-56,60,61]. The comparison (Table 4) showed that the content of almost all TE in breast tissue are higher than in blood serum, excepting Cu, Mg, and Rb.

Thus, the ability of breast tissue to absorb Al, As, B, Cd, Cr, La, Li, Mn, Ni, Pb, Sb, Sn, Sr, Ti, W, and Zn from the interstitial fluid seems to be quite real. As noted above, breast tissue consists of a glandular component, adipose tissue, and stroma. On average, the ratio by weight of the glandular component and adipose tissue together with the stroma is approximately 1:1 [62]. From a comparison of the data obtained for the mammary gland with adipose tissue, it follows that Al, Cu, Sr, Zn accumulate mainly in the glandular tissue of the mammary gland (Table 4). Comparison of the TE content in the mammary gland with the content of the same TE in the prostate and thyroid gland shows that the mass fractions of As, Bi, Cr and Pb in the mammary gland are higher

than in other glands. It is despite the fact that approximately half of the breast tissue consists of adipose tissue, in which the content of TE significantly lower than in the glandular tissue (Table 4).

Conclusion

The developed ICP-MS method allows obtaining reliable data on the content of 23 TE: Al, As, B, Ba, Bi, Cd, Ce, Cr, Cu, La, Li, Mg, Mn, Nb, Ni, Pb, Rb, Sb, Sn, Sr, Ti, W, and Zn in breast tissue samples. The method also allows estimate the possible maximal mean of 10 TE, such as Co, Cs, Ga, Ge, Mo, Nd, Pr, Th, V, Y. In addition, using this method, researcher can additionally check the situation with the content of 18 TE, such as Be, Co, Dy, Eu, Er, Gd, Ge, Ho, Lu, Re, Se, Sm, Te, Tb, Tl, Tm, U and Yb, if the levels of these TE will be higher than the corresponding DL. An important advantage of the developed technique is the possibility of the TE content determination in samples with only a few milligrams mass, which makes it possible to use materials from puncture biopsy specimens of tissues for analysis.

The ability of breast tissue to absorb Al, As, B, Cd, Cr, La, Li, Mn, Ni, Pb, Sb, Sn, Sr, Ti, W, Zn from the interstitial fluid was found. The accumulation of such potentially toxic TE, as As, Bi, Cr and Pb, should be taken into account in further studies of the role of TE in the etiology of breast pathology, as well as in the development of methods for differential diagnosis diseases of this organ for example, benign and malignant tumors of the gland, based on the study of the TE composition of the lesion of the mammary gland.

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Conflict of Interest

The author has not declared any conflict of interests.

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