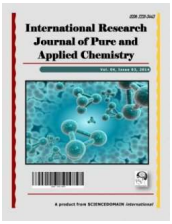

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Application of Reference Material IAEA A-13 as Ideal Substance for Comparative Studies of the Nuclear and Relative Analytical Method Precisions

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To estimate method's precision of five different analytical techniques when the chemical elemental contents in IAEA A-13 reference material were measured.
Methodology: The appraisal of using radionuclide energy dispersive X-ray fluorescence (REDXRF), instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR), instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) to estimate chemical elements contents in samples of human blood was carried out with reference material (RM) IAEA A-13 (Animal Blood). For estimation of repeatability of the results, no less than 7 sub-samples of RM were measured. Five different analyzing methods were employed and one sample per one labor shift.

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Results: Using results of method's precision for each available chemical element, the level of repeatability were estimated for single, duplicate, triplicate and more replications. It was shown that REDXRF analysis of duplicated samples of dried blood with the level of repeatability within ±10% allows estimation of mass fractions of only Br. INAA-SLR analysis under the same conditions provides information on 4 elements (Cl, K, Mn, and Na), ICP-AES – for 7 elements (Ca, Fe, K, Mg, Na, P, and Zn), ICP-MS - for 2 elements (Rb and Zn), and for INAA-LLR no element level could be determined within limits of repeatability ±10%.
Conclusion: At present all five methods combined allow estimation of mass fractions for only 11 elements (Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, Rb, and Zn) with repeatability <±10% if analysis of duplicated samples was used. Since the RM IAEA A-13 is virtually ideally homogenized material, the obtained estimates of the methods' possibilities may be considered at most optimistic.

Keywords: Chemical elements in blood; analytical uncertainty; nuclear and relative analytical methods.

1. INTRODUCTION

Since the times of the alchemists, chemical elements have been investigated in human organs, tissues and fluids. During the last decades the number of publications devoted to them increased considerably. Today, the number of published articles may be estimated at about twenty thousands, and the amount of monographs about hundred. At the end of the 20th century, a new scientific discipline appeared, focused on the role of chemical elements in human body under physiological and pathological conditions [1]. This new field of interdisciplinary study has been named: "Medical Elementology" (from lat. "medicina" and "elementum").

The basic information unit in medical elementology is the quantitatively expressed content of a chemical element in a studied biological object. Only this will allow a specific estimation of any changes during the development and existence. However, only in the last decades of the 20th century, really quantitative and metrologically provided (quality assured) methods for analysis of chemical elements in biological objects appeared.

Using such methods as radionuclide energy dispersive X-ray fluorescence (REDXRF), instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR), instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) the measurements of chemical elements contents in samples of normal bone [2-20], teeth [21-24], prostate [25-39], thyroid [40-44] and some other tissues and fluids of human body [45-48] as well as some foods and diets [49] were carried out by us. Moreover, the chemical elements contents in benign and malignant transformed tissues were also studied and a few chemical element as tumor markers were found [50-68]. In all these studies dozens of similar samples in each group were used. However, a potential application of chemical elements as tumor markers for cancer diagnostics opened a question about a measurement's uncertainty of each individual sample available in clinical practice.

The evaluation of new analytical methods is important tasks in laboratory medicine. Commercial publications declare wide-scale possibilities of new analytical methods and devices for assessment of chemical element contents in biological materials (as usual, almost all elements in the periodic table) but do not demonstrate uncertainties of available analytical data. However, real medical and biological tasks as usual raise very strict requirements to the result uncertainty especially if analytical data is used in diagnostic purposes. So, it is very important to know not only the list of chemical elements available for determination, but also the levels of result's uncertainties. Since the reference materials are virtually ideally homogenized substances, the obtained estimates of the methods' possibilities may be considered at most optimistic. Whole blood is the biological samples most often used in medical-biological researches.

This work had two aims. The first was to assess the means of chemical element mass fractions in certified reference material (RM) IAEA A-13 (Animal Blood) using data of five analytical methods (REDXRF, INAA-SLR, INAA-LLR, ICP-AES, and ICP-MS), obtained for group of RM

sub-samples every of which were measured by different operators during different days, months, and years. The second aim was to estimate the level of relative standard error of mean (±SEM, %) for each available chemical element obtained by single, duplicate, triplicate and more replications measurement by every method.

2. MATERIALS AND METHODS

2.1 Samples and Sample Preparation

For estimation of repeatability of the results no less than 7 sub-samples of RM IAEA A-13 (Animal Blood) were measured by five methods analyzing one sample per one labor shift during different days, months, and even years. No less than two operators carried out the measurements alternately. All samples were prepared from the RM according to the certificate recommendation. Unpainted plastic laboratory instruments and vessels were used during the sampling. Weighing was carried out on analytical electronic balance with the metering error no more than ±0,0001 g.

Each from 7 sub-samples of RM weighing about 8 mg was applied to a piece of adhesive tape, which served as a sample backing for REDXRF analysis. The 7 sub-samples of RM were used for chemical element measurement by INAA-SLR. Mass of each sub-sample was about 100 mg. The samples for INAA-SLR were sealed separately in thin polyethylene films washed with acetone and rectified alcohol beforehand. The sealed samples were placed in labeled polyethylene ampoules. The sample weighing about 50 mg was used for chemical element measurement by INAA-LLR. The collection included 20 sub-samples of RM. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

A dozen sub-samples of RM weighing about 100 mg for ICP-AES and ICP-MS were decomposed: 5 mL of concentrated HNO_3 (All-Union Russian State Standard 11125) was added to each sub-samples of RM, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Russia) and then heated for 10 min at 200℃ in a microwave oven Multiwave 3000 (PerkinElmer – A.Paar, Graz, Austria). After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (up to 10 mL) and transferred to plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without RM samples (only HNO_3 + deionized water), and the resultant solutions were used as control samples. For analysis, 1 mL of each solution was diluted with 0.5% nitric acid up to 10 mL. Deionized water was obtained by an electric distiller with combined membrane set DVS-M/1HA-1(2)-L (Mediana-Filter, Moscow, Russia).

To determine elemental contents in RM samples by REDXRF, INAA-SLR, and INAA-LLR biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [69]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as intra-laboratory standards. The BSS and intra-laboratory standards were treated and analyzed in the same conditions that RM sub-samples.

2.2 Instrumentation and Methods

Mass fractions of Br, Fe, and Zn were measured by REDXRF method. The facility for REDXRF included an annular ^{109}Cd source with an activity of 2.56 GBq, Si(Li) detector and portable multichannel analyzer combined with a PC. Its resolution was 270 eV at the 5.9 keV line of ^{55}Fe-source. The duration of each measurement was 60 min. Details of the sample preparation, the facility and method of analysis were reported in our previous publication concerning the REDXRF of chemical element contents in specimens of prostate tissue [25,26, 31,39,51,52,55,56,59].

The content of Cl, K, Mn, and Na were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor. The neutron flux in the channel was 1.7×10^{13}n cm^{-2} sec^{-1}. Ampoules with RM sub-samples, BSS, and intra-laboratory standards were put into polyethylene rabbits and then irradiated separately for 180 sec. Copper foils were used to assess neutron flux. The first measurement of each sample was begun 1 min after irradiation and the second one - 120 min after irradiation. The duration of the first and second measurements was 10 and 20 min, respectively. A coaxial 98-cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100), including a personal computer-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9-keV resolution at the ^{60}Co 1,332-keV line. Details of used nuclear reactions, radionuclides, and gamma-energies were presented in our earlier publications

concerning the INAA-SLR chemical element contents in human tissues and fluids [5-10,13-15,23,27,57,58,61-63].

A vertical channel of nuclear reactor was applied to measure the content of Ag, Co, Cr, Cs, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. The quartz ampoule with RM sub-samples, BSS, and intra-laboratory standards was soldered, positioned in a transport aluminum container and exposed to a 24-hour neutron irradiation in a vertical channel with a neutron flux of $1.3 \cdot 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$. Ten days after irradiation samples were reweighed and repacked. The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. The gamma spectrometer included the 100 cm^3 Ge(Li) detector and on-line computer-based MCA system. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line. Details of used nuclear reactions, radionuclides, and gamma-energies were presented in our earlier publications concerning the INAA-LLR chemical element contents in human tissues and fluids [11,13,14,28, 32,33,34,64-68].

Mass fractions of Al, Ca, Fe, K, Mg, Na, P, Si and Zn were determined by ICP-AES method using Optima 2000 DV spectrometer (Perkin-Elmer, USA) as well as mass fraction of Ag, As, Au, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Hg, I, Li, Mn, Mo, Ni, Pb, Pt, Rb, Sb, Se, Sn, Sr, Tl, V, W, Zn, and Zr were estimated by ICP-MS method using ELAN 9000 mass-spectrometer (PerkinElmer – Sciex, Concord, Ontario, Canada). Working regimens and spectral corrections of the both spectrometers followed recommendations of the manufacturers. Graduation of the spectrometers was carried out using multielement standard solutions, which were combined from mono-elemental Perkin-Elmer reference solutions of the series “Essentials”, mixed in corresponding proportions. Plotting of the graduation characteristic, processing and storage of the graduation results was provided by embedded software of the spectrometers. Details of the analytical methods and procedures used here such as wavelength, isotopes, spectrometer parameters and operating conditions were presented in our earlier publications concerning the chemical elements of human bone and prostate tissue [12,16-18,35].

2.3 Computer Programs and Statistics

A dedicated computer program of INAA mode optimization was used [70]. Using the Microsoft Office Excel software the arithmetic mean (Mean), relative standard deviation (RSD), and relative standard error of mean (RSEM) were calculated for all the chemical element mass fractions obtained.

3. RESULTS

Table 1 depicts certain statistical parameters (arithmetic mean; relative standard deviation; relative standard error of mean for single, duplicate, triplicate and more measurement) of the Br, Fe, and Zn mass fractions determined by REDXRF in seven sub-samples of RM IAEA A-13 Animal blood and the certified values of these elements.

Table 2 represents the aforementioned statistical parameters of the Cl, K, Mn, and Na mass fractions determined by INAA-SLR in seven sub-samples of RM IAEA A-13 Animal blood and the certified values of these elements, if any.

Table 3 shows the aforementioned statistical parameters of the Ag, Co, Cr, Cs, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions determined by INAA-LLR in twenty sub-samples of RM IAEA A-13 Animal blood and the certified values of these elements, if any.

Table 1. Precision and accuracy REDXRF method at determination of chemical element contents (mg/kg on dry mass basis) in the reference material IAEA A-13 animal blood (n_s=7)

Element	Results of determination					Reference sheet
	Mean	RSD (n_s=7) or RSEM n=1 %	RSEM n=2 %	RSEM n=3 %	RSEM n=7 %	Mean (95% confidence interval)
Br	19.8	9.9	7.0	5.7	3.7	22 (19-24)
Fe	2879	14.6	10.4	8.4	5.5	2400 (2200-2500)
Zn	9.81	26.3	18.7	15.2	9.9	13 (12.0-14.0)

Mean - arithmetic mean, RSD - relative standard deviation, RSEM - relative standard error of mean, n_s – number of sub-samples, n – number of each sub-sample measurements

Table 2. Precision and accuracy INAA-SLR method at determination of chemical element contents (mg/kg on dry mass basis) in the reference material IAEA A-13 animal blood (n_s=7)

Element	Results of determination					Reference sheet
	Mean	RSD (n_s=7) or RSEM n=1 %	RSEM n=2 %	RSEM n=3 %	RSEM n=7 %	Mean (95% confidence interval)
Cl	12978	3.9	2.7	2.2	1.5	-
K	2421	9.4	6.7	5.4	3.6	2500 (2100-2700)
Mn	0.776	10.5	7.4	6.1	4.0	-
Na	11966	5.4	3.8	3.1	2.0	12600 (11600-13500)

Mean - arithmetic mean, RSD - relative standard deviation, RSEM - relative standard error of mean, n_s – number of sub-samples, n – number of each sub-sample measurements

Table 3. Precision and accuracy INAA-LLR method at determination of chemical element contents (mg/kg on dry mass basis) in the reference material IAEA A-13 animal blood (n_s=20)

Element	Results of determination					Reference sheet
	Mean	RSD (n_s=20) or RSEM n=1 %	RSEM n=2 %	RSEM n=3 %	RSEM n=20 %	Mean (95% confidence interval)
Ag	0.0036	81.2	57.6	46.9	18.2	-
Co	0.0050	63.9	45.3	36.9	14.3	-
Cr	0.195	52.9	37.5	30.6	12.3	-
Cs	0.0147	87.4	62.0	50.5	19.6	-
Fe	2380	19.8	14.0	11.4	4.5	2400 (2200-2500)
Hg	0.0588	55.5	39.4	32.5	12.4	-
Rb	2.94	60.9	43.2	35.6	13.6	2.3 (1.7-3.1)
Sb	0.0187	113.0	80.1	65.3	25.2	-
Sc	0.0045	54.8	38.9	31.7	12.3	-
Se	0.159	98.5	69.9	56.9	22.0	0.24 (0.15-0.31)
Zn	13.2	53.1	37.7	30.7	11.9	13 (12.0-14.0)

Mean - arithmetic mean, RSD - relative standard deviation, RSEM - relative standard error of mean, n_s – number of sub-samples, n – number of each sub-sample measurements

Tables 4 and 5 represent the aforementioned statistical parameters of the Al, Ca, Fe, K, Mg, Na, P, Si, and Zn as well as Ag, As, Au, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Hg, I, Li, Mn, Mo, Ni, Pb, Pt, Rb, Sb, Se, Sn, Sr, Tl, V, W, Zn, and Zr mass fractions determined by ICP-AES and ICP-MS, respectively, in twelve sub-samples of RM IAEA A-13 Animal blood and the certified values of these elements, if any.

Table 4. Precision and accuracy of ICP-AES method at determination of chemical element contents (mg/kg on dry mass basis) in the reference material IAEA A-13 animal blood (n_s=12)

Element	Results of determination					Reference sheet
	Mean	RSD (n_s=12) or RSEM n=1 %	RSEM n=2 %	RSEM n=3 %	RSEM n=12 %	Mean (95% confidence interval)
Al	0.455	92.7	65.7	53.6	27.9	-
Ca	302	8.1	5.7	4.7	2.4	286 (226-332)
Fe	2187	4.9	3.5	2.8	1.5	2400 (2200-2500)
K	2883	6.0	4.3	3.5	1.8	2500 (2100-2700)
Mg	90.0	4.5	3.2	2.6	1.4	99* (81-139)
Na	13353	2.0	1.4	1.2	0.6	12600 (11600-13500)
P	837	7.9	5.6	4.5	2.4	940* (690-1120)
Si	2.44	44	31.2	25.4	13.3	-
Zn	11.7	6.8	4.8	3.9	2.1	13 (12.0-14.0)

*Mean - arithmetic mean, RSD - relative standard deviation, RSEM - relative standard error of mean, n_s – number of sub-samples, n – number of each sub-sample measurements, * - Information value*

Table 5. Precision and accuracy of ICP-MS method at determination of chemical element contents (mg/kg on dry mass basis) in the reference material IAEA A-13 animal blood (n_s=12)

Element	Results of determination					Reference sheet
	Mean	RSD (n_s=12) or RSEM n=1 %	RSEM n=2 %	RSEM n=3 %	RSEM n=12 %	Mean (95% confidence interval)
Ag	0.0104	127.0	90.1	73.4	38.3	-
As	0.0725	85.3	60.5	49.3	25.7	-
Au	0.0438	82.9	58.8	47.9	25.0	-
B	0.794	31.2	22.1	18.0	9.4	-
Ba	0.103	21.9	15.5	12.7	6.6	-
Be	0.0026	106.0	75.2	61.3	31.9	-
Bi	0.0434	114.0	80.9	65.9	34.3	-
Cd	0.0053	164.0	116.0	94.8	49.4	-
Co	0.0033	82.7	58.7	47.8	24.9	-
Cr	0.675	91.3	64.8	52.8	27.5	-
Cu	4.22	78.0	55.3	45.1	23.5	4.3 (3.7-4.8)
Hg	0.0099	198.0	140.0	115.0	59.6	-
I	0.831	76.4	54.2	44.2	23.0	-
Li	0,0522	18.0	12.8	10.4	5.4	-
Mn	0.0405	44.0	31.2	25.4	13.3	-
Mo	0.0300	76.8	54.5	44.4	23.1	-
Ni	0.0419	86.0	61.0	49.7	25.9	1* (0.6-1.4)
Pb	0.181	44.2	31.3	25.5	13.3	0.18* (0.15-0.29)
Pt	0.00296	102.0	72.3	59.0	30.7	-
Rb	2.28	4.4	3.1	2.6	1.3	2.3 (1.7-3.1)
Sb	0.00203	47.3	33.5	27.3	14.2	-
Se	0.551	33.8	24.0	19.5	10.2	0.24 (0.15-0.31)
Sn	0.0831	196.0	139.0	113.0	59.0	-
Sr	0.208	19.0	13.5	11.0	5.7	-
Tl	0.00220	153.0	109.0	88.4	46.1	-
V	0.180	98.2	69.6	56.8	29.6	-
W	0.0231	148.0	105.0	85.5	44.6	-
Zn	11.7	6.80	4.8	3.9	2.1	13 (12.0-14.0)
Zr	0.695	99.1	70.3	57.3	29.8	-

*Mean - arithmetic mean, RSD - relative standard deviation, RSEM - relative standard error of mean, n_s – number of sub-samples, n – number of each sub-sample measurements, * - Information value*

Information concerning the comparison of possibilities REDXRF, INAA-SLR, INAA-LLR, ICP-AES, and ICP-MS methods in the measurement of chemical elements mass fractions in blood with acceptable repeatability of results no more than ±10% is presented in Table 6.

4. DISCUSSION

The result's precision (P) is defined as a repeatability of results when a few sub-samples of homogeneous sample are measured one time each. It was accepted that P for i-element is

$$P_i = RSD_i \times 100\% \text{ or } P_i = (M_i / SD_i) \times 100\%,$$

where M_i is an arithmetic mean and SD_i – a standard deviation of mean. What does P_i mean? P_i means that, if a single measurement of one else sub-sample prepared from the same sample will be made during any other days, the result for i-element with probability about 67% will be inside range $M_i \pm P_i$ and with probability about 95% - inside range $M_i \pm 2\ P_i$. It is possible to improve a repeatability of mean $(RM)_i$ if to made measurements in duplicates, triplicates and so on. In this case $(RM)_i^n = (M_i / SEM_i) \times 100\%$, where SEM_i is standard error of mean and n – number of each sub-sample measurements. The $(RM)_i^n$ means that, if n measurements of one else sub-sample prepared from the same sample will be made during any other days, the result for i-element with probability about 67% will be inside range $M_i \pm (RM)_i^n$ and with probability about 95% - inside range $M_i \pm (2RM)_i^n$. As a rule, in clinical practice there are special recommendations of upper limit for P [71].

Table 6. Comparison of possibilities REDXRF, INAA and ICP methods in the analysis of chemical elements contents in blood with acceptable repeatability of results no more than ±10%

Method	Element	Single (P)	Duplicate (RM)[2]	Triplicate RM)[3]	n=10 (RM)[10]
REDXRF	Br	9.9	7.0	5.7	3.1
	Fe	-	-	8.4	4.6
INAA-SLR	Cl	3.9	2.7	2.2	1.2
	K	9.4	6.7	5.4	3.0
	Mn	-	7.4	6.1	3.3
	Na	5.4	3.8	3.1	1.7
INAA-LLR	Fe	-	-	-	6.3
ICP-AES	Ca	8.1	5.7	4.7	2.6
	Fe	4.9	3.5	2.8	1.6
	K	6.0	4.3	3.5	1.9
	Mg	4.5	3.2	2.6	1.4
	Na	2.0	1.4	1.2	0.6
	P	7.9	5.6	4.5	2.5
	Zn	6.8	4.8	3.9	2.2
ICP-MS	B	-	-	-	9.9
	Ba	-	-	-	6.9
	Rb	4.4	3.1	2.6	1.4
	Zn	6.8	4.8	3.9	2.2
Elements (All methods)		Br, Ca, Cl, Fe, K, Mg, Na, P, Rb, Zn	Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, Rb, Zn	Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, Rb, Zn	B, Ba, Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, Rb, Zn

For example, the recommendation of Russian Ministry of Health for the state's clinical laboratories regulates upper limit of P as ±10% [72].

The number of elements available for analysis in RM IAEA A-13 Animal blood samples by REDXRF was 3: Br, Fe, and Zn. The accuracy of obtained results for Fe and Zn may be estimated as unsatisfactory because the mean values of these element mass fractions are out of the certificate's 95% confidence intervals (Table 1). Using single measurement the result for Br only has the level of repeatability under ±10%. It is possible to reach such level of repeatability for Fe using measurement in duplicates.

The number of elements available for analysis in RM IAEA A-13 Animal blood samples by INAA-SLR was 4: Cl, K, Mn, and Na. The accuracy of obtained results for K and Na may be estimated as satisfactory because the mean values of these element mass fractions are within the certificate's 95% confidence interval (Table 2). Using single measurement the results for Cl, K, and Na have the levels of repeatability under ±10%. It is possible to reach such level of repeatability for Mn using measurement in duplicates.

The number of elements available for analysis in RM IAEA A-13 Animal blood samples by INAA-LLR was 11: Ag, Co, Cr, Cs, Fe, Hg, Rb, Sb, Sc, Se, and Zn. The accuracy of obtained results for Fe, Rb, Se, and Zn may be estimated as satisfactory because the mean values of these element mass fractions are within the certificate's 95% confidence interval (Table 3). It was found that using single measurement no one chemical element content can be estimated with the level of repeatability under ±10%. It is impossible to reach such level of repeatability for any other elements using measurement in duplicates or triplicates. Only the Fe mass fraction can be estimated with repeatability under ±10% using four measurements.

The number of elements available for analysis in RM IAEA A-13 Animal blood samples by ICP-AES was 9: Al, Ca, Fe, K, Mg, Na, P, Si, and Zn. The accuracy of obtained results for Ca, Fe,, Mg, Na, P, and Zn may be estimated as satisfactory because the mean values of these element mass fractions are within the certificate's 95% confidence interval (Table 4). Using single measurement the results for Ca, Fe, K, Mg, Na, P, and Zn have the levels of repeatability under ±10%. It is impossible to reach such level of repeatability for Al and Si even through measurement a group of 12 sub-samples.

The number of elements available for analysis in RM IAEA A-13 Animal blood samples by ICP-MS was 29: Ag, As, Au, B, Ba, Bi, Cd, Co, Cr, Cu, Hg, I, Li, Mn, Mo, Ni, Pb, Pt, Rb, Sb, Se, Sn, Sr, Tl, V, W, Zn, and Zr. The accuracy of obtained results for Cu, Pb,, Rb, and Zn may be estimated as satisfactory because the mean values of these element mass fractions are within the certificate's 95% confidence interval (Table 5). However, it was found that using single measurement only the mass fractions of Rb and Zn cam be estimated with the level of repeatability under ±10%. It is impossible to reach such level of repeatability for any other elements using measurement in duplicates or triplicates. Only the Li mass fraction can be estimated with repeatability under ±10% if a group of four sub-samples will be measured.

Nominally, all five instrumental methods combined allow detect in RM IAEA A-13 Animal blood 40 different elements: Ag, As, Al, Au, B, Ba, Bi, Br, Ca, Cd, Cl, Co, Cr, Cs, Cu, Fe, Hg, I, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pt, Rb, Sb, Sc, Se, Si, Sn, Sr, Tl, V, W, Zn, and Zr (Tables 1-5). However, analysis of single sample of CRM by the all five methods with the levels of repeatability within ±10% allows estimation of mass fractions of only 10 elements (Br, Ca, Cl, Fe, K, Mg, Na, P, Rb, and Zn), analysis in duplicate under the same conditions provides information on 11 elements (Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, Rb, Zn), analysis in triplicate under the same conditions does not increase the number of elements available for estimation, and analysis of group consists of 10 sub-samples under the same conditions provides information of mass fractions of only 13 elements (B, Ba, Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, Rb, Zn) (Table 6).

Since the RM IAEA A-13 Animal blood is virtually ideally homogenized material, the obtained estimates of the methods' possibilities may be considered at most optimistic. When investigating real samples of dried human or animal blood, one hardly can expect to achieve the same degree of homogenization, therefore the level of repeatability in this case can be even higher and, as consequence, the number of elements available for medical analysis – lower.

5. CONCLUSION

Using results of method's precision for each available chemical element, the level of repeatability were estimated for single, duplicate, triplicate and more replications of the analyses by REDXRF, INAA-SLR, INAA-LLR, ICP-AES and ICP-MS. It was shown that REDXRF analysis of duplicated samples of dried blood with the level of repeatability within ±10% allows estimation of mass fractions of only Br. INAA-SLR analysis under the same conditions provides information on 4 elements (Cl, K, Mn, and Na), ICP-AES – for 7 elements (Ca, Fe, K, Mg, Na, P, and Zn), ICP-MS - for 2 elements (Rb and Zn), and for INAA-LLR no element level could be determined within limits of repeatability ±10%. Thus, at present all five instrumental methods combined allow estimation of mass fractions for only 11 elements (Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, Rb, and Zn) with repeatability <±10% if analysis of duplicated samples was used. Since the RM is virtually ideally homogenized material, the obtained estimates of the methods' possibilities may be considered at most optimistic.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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