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**DETECTING ESSENTIAL AND TOXIC ELEMENTS IN URINE  
WITH THE HELP OF ICP-MS METHOD FOR DIAGNOSTIC  
STUDIES AND PUBLIC HEALTH RISK ASSESSMENT****T.S. Ulanova<sup>1</sup>, O.V. Gileva<sup>1</sup>, E.V. Stenno<sup>1</sup>, G.A. Veikhman<sup>2</sup>, A.V. Nedoshitova<sup>1</sup>**<sup>1</sup>FBSI "Federal Scientific Center for Medical and Preventive Health Risk Management Technologies", Russian Federation, Perm, 82, Monastyrskaya St., 614045<sup>2</sup>SBEI HPE "Perm State Pharmaceutical Academy", Ministry of Health in Russia, Russian Federation, Perm, 2, Polevaya St., 614990

**Abstract.** The publication describes a new method of detecting 12 elements in the urine using a quadrupole inductively coupled plasma mass spectrometer Agilent 7500cx (USA) with an octopole reaction system (ORS) in the reaction mode. Urine samples were analyzed immediately after dilution of 1/10 with 1% aqueous solution of HNO<sub>3</sub>. The validity of the results was confirmed by the standard analysis of urine samples Seronorm<sup>TM</sup> urine (Norway). The developed method can accurately and reliably determine vanadium, chromium, manganese, nickel, copper, zinc, selenium, strontium, cadmium, lead and titanium in urine samples. The detection limit for V, Cr, Mn, Ni, Sr, Cd, Tl, Pb is 0.1 g / dm<sup>3</sup> to 1.0 g Cu / dm<sup>3</sup> for Se 5.0 g/dm<sup>3</sup> for 50 Sr and Zn, 0 g/dm<sup>3</sup> with an error of maximum 25%.

The studies of biosubstrates of the children population living in environmentally safe areas of Perm Region, conducted with the help of the developed method of inductively coupled plasma mass spectrometry allowed to establish the baseline levels for the 12 elements as a standard of comparison for hygienic assessment, epidemiological studies, evaluations, and examinations.

**Key words:** method of mass spectrometry with inductively coupled plasma (ICP-MS), octopole reaction system, internal standard, urine.

Health risk assessment frequently requires verification of the obtained results using epidemiological, clinical, functional, and laboratory research methods [2, 3]. Chemicoanalytical studies aimed at determining the level of toxic and essential elements in human biological substrates are commonly used for the purposes of health risk realization evidence [1, 3, 6]. Lately, determination of the elements in biological medias has been more frequently used in hygienic studies, evaluations and examinations to establish causal relationships between environmental factors and public health [2,9,8,13-15]. It is the direct methods of determining the toxic compounds and elements in human biological medias which serve as conclusive evidence of hazardous technogenic effects, and combined with effect markers – of the caused harm to

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health [1, 15].

Today inductively coupled plasma mass spectrometry (ICP-MS) is widely used for a high accuracy and selective detection of a wide range of elements. Such characteristics as low limits of detection, low use of the analyzed substances, and possibility to work with minimum volumes determine a high information volume of the method when detecting micro- and ultramicro-levels in biological fluids [12].

Urine is an easily available biological media that frequently serves as a research target. At the same time, when using urine in their studies, researchers often encounter such issue as a high content of salts – chlorides, sulfates, phosphates – which significantly increases the matrix effect and results in an upward bias due to interference noise [5, 7, 11].

**The purpose of the study** is to develop a highly sensitive and accurate method of quantitative detection of V, Cr, Mn, Ni, Cu, Zn, As, Se, Sr, Cd, Tl, and Pb in urine as exposure markers in the conditions of a harmful environmental chemical impact.

**Materials and methods.** Quantitative detection of V, Cr, Mn, Ni, Cu, Zn, , Se, Sr, Cd, Tl, and Pb in urine samples was conducted on a quadruple Inductively coupled plasma mass spectrometer Agilent 7500cx (USA) with the use of an octopole reaction system (ORS). The use of ORS helps to get rid of the influence of polyatomic ions due to their collisions or reactions with the gas atoms filling the cell. Helium was used as a gas cell. The value of helium flow velocity was experimentally determined at 5.0 ml/min which allowed to significantly decrease the polyatomic overlapping while maintaining a high level of sensitivity. The power of the high-frequency signal equaled 1550 W. A double-pass Scott chamber was used for sample introduction. The temperature in the atomization chamber was 2.0 °C. The velocity of the sample flow into the atomization chamber was 0.4 ml/min. The speed of the detector was

<sup>3</sup> 100mcs per 1 ion. Helium was used as a reactant gas. The solution of <sup>7</sup>Li, <sup>59</sup>Co, <sup>89</sup>Y and <sup>205</sup>Tl в 2% HNO<sub>3</sub> with the concentration of 1 mcg/l for each of the elements were used for settings (Tuning Solution, USA). The ratios of <sup>140</sup>Ce<sup>160+</sup>/<sup>140</sup>Ce<sup>+</sup> totaled <1%, and for <sup>140</sup>Ce<sup>2+</sup>/<sup>140</sup>Ce<sup>+</sup> <3%. High-purity liquid argon 99,99% (TU-2114-005-00204760-99) was also used. The maximum flow velocity of argon totaled 20 l/min, pressure in the gas feeding tube totaled 700±20 kPa, T<sub>plasma</sub> = 8000-10000 K. The analysis automation was provided by an autosampler G3160B (Germany). A solution containing 27 elements with a concentration of 10 mg/l in a 5% aqueous solution of HNO<sub>3</sub> (Multi-Element Calibration Standard-2A, USA) was used as the main standard solution. Ultrapure HNO<sub>3</sub> (Sigma – Aldrich, USA) was used to prepare calibration solutions and samples. The concentration of the calibration solutions for the detection of Cd and Tl totaled 0.0; 0.1; 0.5; 1.0 mcg/l, for Mn, Ni, Cr, V, Se, and Cu – 0.0; 0.1;

0.5; 1.0; 5.0 mcg/l, for As, Sr, and Zn – 0.0; 1.0; 5.0; 10.0; 50.0 mcg/l. To prepare an internal standard solution (BC), we used a mixed standard solution  $^{209}\text{Bi}$ ,  $^{73}\text{Ge}$ ,  $^{115}\text{In}$ ,  $^6\text{Li}$ ,  $^{45}\text{Sc}$ ,  $^{159}\text{Tb}$ ,  $^{89}\text{Yc}$  with a concentration of 10 mg/l in a 5% aqueous solution of  $\text{HNO}_3$  (Internal Standard Mix, USA). We used  $^{159}\text{Tb}$  as an internal standard for the detection of Pb and Tl, for the detection of Cd we used  $^{115}\text{In}$ , and for other elements –  $^{72}\text{Ge}$ , due to the proximity of the ionization potentials and atomic mass.

The solutions were diluted with distilled water with specific resistance of 18.2 Mohm·cm, purified in Milli-Q Integral (Millipore SAS, France). The blank sample was prepared the same way as the working sample. We used an ultrasonic cleaning unit Elmasonic S 100H (Germany) to prepare the labware made out of glass, teflon, and polypropylene for the analysis. The dishes were kept for 20 min in bidistilled water at the temperature of 55 °C, then for 20 min in an aqueous solution of hydrogen nitrate (1:5) at 55 °C, then for 20 min – in distilled water at 55 °C.

The standard urine samples Seronorm<sup>TM</sup> urine (LOT 0511545, Sero AS, Billingstad, Norway) were diluted in 5 ml of distilled water.

Urine samples were collected in the morning and stored in sterile polypropylene 125 ml containers with screw caps (FLMedical Srl, Torreglia, Italy). For the subsequent mass-spectrometry study, 0.5 ml of urine was diluted with 4.45 ml of a 1% aqueous solution of  $\text{HNO}_3$  and 0.05 ml of an internal standard solution.

**Results and discussion.** The new method for the detection of 12 elements in urine with the help of inductively coupled plasma mass spectrometry differs from the current MUK 4.1.1483-03 [10] by a well-developed sample preparation procedure that minimizes matrix effect, selection of an internal standard for each of the detected element, use of a collision cell with helium for the correction of polyatomic interferences. The method was certified by the Certimet Center (№ 88-16374-102-01.00076) and registered at the Federal Information Fund for Provision of Uniformity of Measurements FR.1.31.2014.17064.

The measurement range of mass concentrations of V, Cr, Mn, Ni, Cu, Zn, As, Se, Sr, Cd, Tl, Pb and the limits of detection (LOD) are shown below in Table 1.

Table 1

**Measurement ranges in urine and limits of detection in solution, mcg/dm<sup>3</sup>**

Detected element	Mass of isotopes used in measurements	Measurement range in urine, mcg/dm <sup>3</sup>	Toxicity indicator (limits of relative error at P=0,95), ± d, %	Limits of detection (LOD) in the solution mcg/dm <sup>3</sup>
Vanadium	51	0.1 – 50.0	15-23	0.01
Chrome	53	0.1 – 100.0	14-23	0.01
Manganese	55	0.1 – 100.0	14-25	0.01
Nickel	60	0.1 – 100.0	15-22	0.01
Copper	63	1.0 – 200.0	14-20	0.1
Zinc	66	50.0 – 1000	13	5
Arsenic	75	1.0 – 100.0	14	0.1
Selenium	82	5,0 – 500.0	16	0.5
Strontium	88	50.0 – 1500	12	5
Cadmium	111	0.1 – 50.0	16-23	0.01
Thallium	205	0.1 – 50.0	15-19	0.01
Lead	206	0.1 – 500.0	15-23	0.01

The accuracy of detection of the 12 elements in urine was confirmed with the results of the analysis of the standard urine samples Seronorm™ urine (n=5, Fig. 1.).

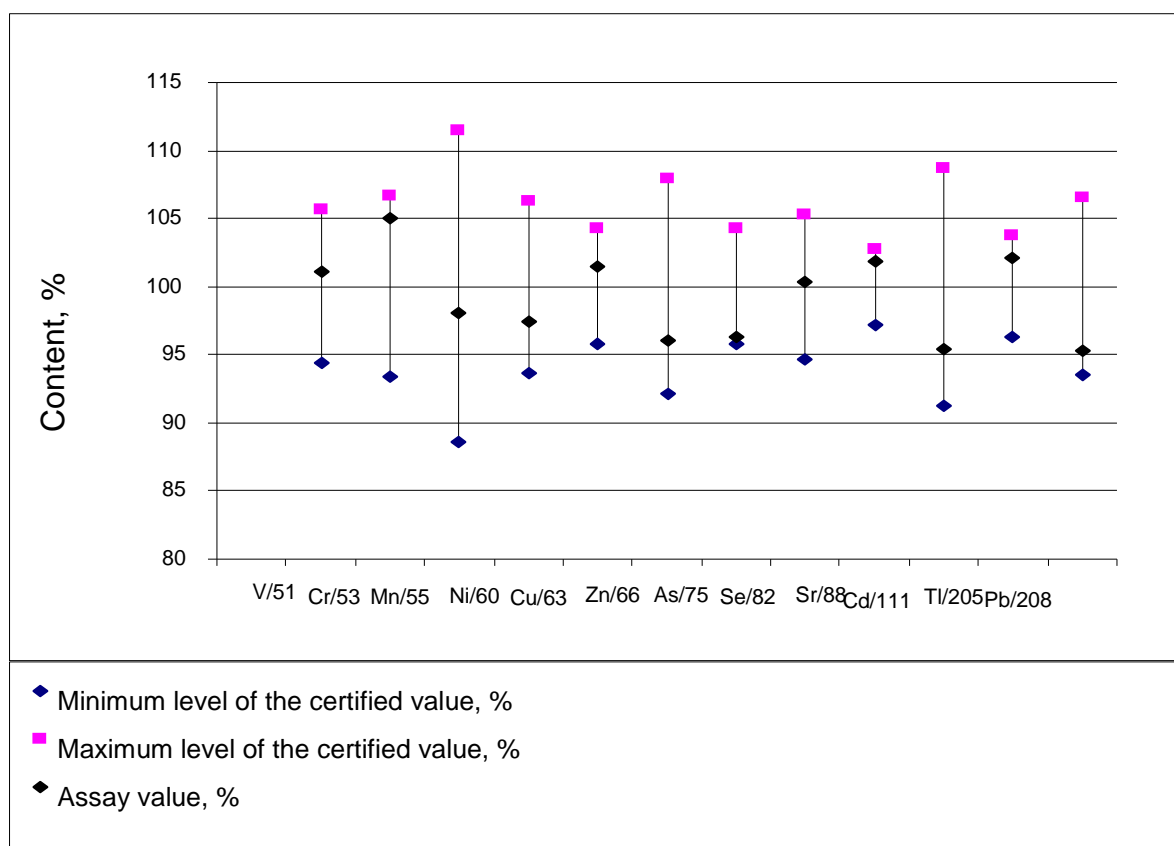


Fig. 1. Results of detection of microelements as compared to the certified values in Seronorm™ urine samples

The generalized results of the analysis of the standard urine samples (Fig. 1) obtained with the use of the developed method show that the detection error equals from 2% to 5%.

The developed detection method was tested in Perm Region with a minimal anthropogenic load. The test results are presented below in Table 2.

Table 2

**The background levels of the elements in urine of the children population of Perm Krai obtained with the use of ICP-MS, mcg/dm<sup>3</sup>**

Detected element	Background levels (n=73), M±m, mcg/dm <sup>3</sup>	Reference levels, mcg/dm <sup>3</sup> [11]
Manganese	0.93±0.3	0.5–10.0
Arsenic	13.0±2.5	5,0–50.0
Nickel	2.2±0.56	0.1–10.0
Lead	1.2±0.17	< 80.0
Strontium	166.0±26.0	20.0–350.0 [10]
Chrome	1.4±0.13	0.1–2.0
Copper	12.0±2.0	2.0-80.0
Zinc	240.0±40.0	180.0-850.0
Cadmium	0.2±0.2	0.5-4.7
vanadium	0,6±0.1	0.08-0.24

Based on the conducted studies of the urine samples of children, we can conclude that the developed method allows obtaining accurate data that matches the reference concentrations used as critical estimated value in clinical studies and evidence-based medicine.

**Conclusions.** Consequently, the proven sample preparation procedure, optimal elements of internal comparison, proven instrumental settings of inductively coupled plasma mass spectrometer, including octopole reaction system, allowed to minimize the matrix and interferential effects of the salt content of urine at the stage of sample preparation.

With the help of the developed method, one can accurately and reliably detect V, Cr, Mn, Ni, Cu, Zn, As, Se, Sr, Cd, Tl, Pb in urine samples, the limit of detection for V, Cr, Mn, Ni, Sr, Cd, Tl, Pb totals 0.1 mcg/dm<sup>3</sup>, for Cu 1.0 mcg/dm<sup>3</sup>, for Se 5,0 mcg/dm<sup>3</sup>, for Sr and Zn - 50.0 mcg/dm<sup>3</sup> with a detection error of less than 25%.

The analysis of urine of the children population of Perm Region residing in environmentally favorable areas conducted with the help of the developed method of inductively coupled plasma mass spectrometry method has established the background levels of V, Cr, Mn, Ni, Cu, Zn, As, Se, Sr, Cd, Tl, and Pb in urine as referent criteria for the purposes of epidemiological studies, examinations, and evaluations.

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