



Analytical Note

Determination of element levels in human serum: Total reflection X-ray fluorescence applications☆



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ARTICLE INFO

Article history:

Received 15 October 2015

Received in revised form 26 April 2016

Accepted 17 May 2016

Available online 18 May 2016

Keywords:

TXRF

Element concentration

Serum

Reference values

Sex

Age

ABSTRACT

Deficiency or excess of elements could disrupt proper functioning of the human body and could lead to several disorders. Determination of their concentrations in different biological human fluids and tissues should become a routine practice in medical treatment. Therefore the knowledge about appropriate element concentrations in human organism is required. The purpose of this study was to determine the concentration of several elements (P, S, Cl, K, Ca, Cr, Fe, Cu, Zn, Se, Br, Rb, Pb) in human serum and to define the reference values of element concentration. Samples of serum were obtained from 105 normal presumably healthy volunteers (66 women aged between 15 and 78 years old; 39 men aged between 15 and 77 years old). Analysis has been done for the whole studied population and for subgroups by sex and age. It is probably first so a wide study of elemental composition of serum performed in the case of Świętokrzyskie region. Total reflection X-ray fluorescence (TXRF) method was used to perform the elemental analysis. Spectrometer S2 Picofox (Bruker AXS Microanalysis GmbH) was used to identify and measure elemental composition of serum samples. Finally, 1st and 3rd quartiles were accepted as minimum and maximum values of concentration reference range.

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1. Introduction

It is known that elements have a significant role in numerous physiological processes of human organism and consequently affect human health and diseases. Deficiency or excess of elements could disrupt proper functioning of the human body and could lead to several disorders. Determination of their concentrations (not only Na, K, Ca and Fe but especially trace elements such as Cr, Cu, Zn, Se, Rb, and Pb) in different biological human fluids and tissues should become a routine practice in medical treatment. Therefore the knowledge about appropriate element concentrations and their proportions in human organism is required.

The aim of this study has been to define the reference values of element concentration determined in human serum (P, S, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br, Rb, Sr, Pb). To the described serum analysis a well-known method – the total reflection X-ray fluorescence (TXRF) method

– was used. The procedure used lets to simultaneously determine 13 elements in as a little as 800 µl of serum. Results presented in this paper are a continuation of research carried out in our laboratory and described in [1] (the number of samples tested was increased; the spectrometer used to analysis has a better detection limit).

Because the composition and quantity of elements in human fluids may vary depending on dietary habits, water and the environment, we discuss our results mainly in comparison with the ones presented in Polish literature.

The study was done for Analytical Laboratory of Holycross Cancer Center. The reference values of concentration calculated are the values, which one will be able to compare with the content of a given element determined in serum of a patient.

2. Experimental TXRF setup

The physical basis of total reflection X-ray fluorescence (TXRF) method is described shortly above and in details in many papers [2–4], therefore, we only present the experimental setup here. Serum analysis has been carried out using S2 Picofox spectrometer (Bruker AXS Microanalysis GmbH) equipped with 30 W X-ray tube with Mo anode, which works with parameters: high voltage 50 kV and electric current

☆ Selected paper from the Colloquium Spectroscopicum Internationale XXXIX (CSI 2015), Figueira da Foz, Portugal, 30 August–3 September 2015.

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Table 1

Acceptable range of concentration c_c in certified serum samples L1 and L2, analytical uncertainty u (95% confidence level), elemental concentration in reference serum sample c_r (in ppm units), and relative difference between them and measured values c_m . Abbreviations: a – approximate values, c – certified values, r – reference values, Δc_m – total experimental uncertainty (in ppm).

Element	Serum L-1 c_c (u)	Measured conc. $c_m \pm \Delta c_m$	$\frac{c_c - c_m}{c_c} * 100$ (%)	Serum L-2 c_c (u)	Measured conc. $c_m \pm \Delta c_m$	$\frac{c_c - c_m}{c_c} * 100$ (%)	Reference serum c_r	Measured conc. $c_m \pm \Delta c_m$	$\frac{c_c - c_m}{c_c} * 100$ (%)
K	126 ^a	110 ± 9	13	176–265 ^c (44)	193 ± 15	0			
Ca	88.3–103.7 ^c (7.7)	84.4 ± 6.8	0	95–143 ^c (24)	117 ± 9	0			
V							0.700 ^f	0.684 ± 0.096	2.3
Fe	1.31–1.51 ^c (0.08)	1.45 ± 0.12	0	1.72–2.58 ^c (0.43)	2.23 ± 0.18	0			
Co							0.700 ^f	0.733 ± 0.067	4.7
Cu	1.607–1.974 ^c (0.091)	1.75 ± 0.14	0	1.538–2.312 ^c (0.387)	1.94 ± 0.15	0			
Zn	1.660–1.824 ^c (0.082)	1.59 ± 0.12	4	1.223–1.840 ^c (0.308)	1.61 ± 0.12	0			
As							0.700 ^f	0.727 ± 0.063	3.9
Se	0.100–0.114 ^c (0.007)	0.102 ± 0.011	0	0.095–0.176 ^c (0.041)	0.112 ± 0.012	0			
Au	0.495–0.585 ^c (0.045)	0.448 ± 0.047	0	1.867 ^a	1.90 ± 0.15	0			

600 μ A, air cooled. The measurement system includes a Peltier-cooled XFlash® Silicon Drift Detector with area 10 mm², energy resolution <160 eV at 100 kcps (for Mn K α line) and multilayer monochromator with layer system made of 100 Ni/C duplicate layers with a spacing of 2.88 nm, set to Mo-K α line. S2 Picofox spectrometer allows measuring the characteristic X-rays of elements from Al to U (with exception of Zr to Ru). Software allows manual or automatic qualitative analysis of the spectrum and the automatic quantitative analysis of the content of the sample [5].

3. Material and sample preparation

Blood samples were taken from 105 normal presumably healthy volunteers from Świętokrzyskie Province: 66 women aged between 15 and 78 years old and 39 men aged between 15 and 77 years old. Among them there were 6 women and 6 male smokers.

The blood samples (about 1 ml of blood) were taken early in the morning from an elbow vein of each patient after 12 h without food and put into sterile tubes (disposable vacuum tubes BD Vacutainer) with blue stopper, for the analysis of trace elements, made of transparent plastic-polyethylene terephthalate (PET) – containing a clotting activator. Then, the blood samples were centrifuged in order to separate serum and red cells. In the centrifugation process, the laboratory

centrifuge MPW-350e was used, with time of centrifugation of 10 min and centrifugation rate of 1.669 \times g (RCF) (g – acceleration due to gravity). After that the serum samples were transferred to Eppendorf tubes and frozen in the temperature of about –25 °C and transported in this form in a special laboratory freezer to our laboratory. Before analysis the serum samples were thawed in room temperature. In the next step to specified amount of serum (800 μ l) the solution of gallium was added as the internal standard (50 μ l solution with a concentration of gallium equal to 100 ppm). Concentration of Ga in the sample was equal to 5.882 ppm. After that 2 μ l of solution was pipetted into silicon Synsil backing and this drop was dried using hotplate at a temperature of 38 °C. Eppendorf tubes and sample backings were previously washed in 10% nitric acid and repeatedly rinsed with deionized water and then were dried by centrifugation.

The study was performed with the approval of Bioethics Committee at Holycross Medical Chamber and according to the Helsinki Declaration of Human Rights and its revisions, and in accordance with Systems of Quality Management.

4. Measurement and spectrum analysis

Finally, each target was measured for 1 h and the spectrum of characteristic X-rays of the serum sample was collected and analyzed using

Table 2

Results presented good repeatability of analytical method used.

Element	Concentration measured (ppm) solution 1			Concentration measured (ppm) solution 2			Average concentration (ppm)	Standard deviation of average concentration (ppm)	Standard deviation of average concentration / average concentration * 100%
	Target 1	Target 2	Target 3	Target 1	Target 2	Target 3			
a) Results obtained by first person prepared serum solution.									
Ca	63.3	68.2	63.5	66.1	68.8	67.1	66.2	0.948	1.43
Cu	1.37	1.28	1.18	1.25	1.15	1.15	1.23	0.035	2.89
Zn	0.961	0.920	0.907	0.908	0.919	0.944	0.927	0.009	0.95
Se	0.057	0.058	0.059	0.058	0.059	0.060	0.059	0.0004	0.73
Rb	0.144	0.130	0.148	0.134	0.130	0.145	0.139	0.003	2.39
Pb	0.035	0.037	0.033	0.026	0.037	0.031	0.033	0.002	5.19
b) Results obtained by second person prepared serum solution.									
Ca	75.7	72.6	75.6	78.9	73.3	75.1	75.2	0.902	1.20
Cu	1.13	1.14	1.14	1.18	1.19	1.18	1.16	0.011	0.92
Zn	0.945	0.946	0.948	0.993	0.983	0.964	0.963	0.008	0.88
Se	0.060	0.060	0.064	0.063	0.065	0.068	0.063	0.001	1.98
Rb	0.133	0.140	0.144	0.139	0.152	0.153	0.144	0.003	2.22
Pb	0.031	0.028	0.031	0.036	0.031	0.037	0.032	0.001	4.35

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