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X-Ray Fluorescence Techniques in Medical Applications: Reference Values of Elements in Human Serum, Urine and Hair

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The aim of undertaken long-term studies of the elemental composition of human serum, urine, and hair is to define reference values of elements concentration. For this purpose the total reflection X-ray fluorescence method was applied to determination of several elements concentration in human serum, urine and hair (S, K, Ca, Fe, Cu, Zn, Br, P, Cr, and Rb in serum samples; Fe, Cu, Zn, Rb, Cr, Mn, and Sr in urine samples; S, K, Ca, Fe, Cu, Br, Zn, Cl, Ti, Cr, Mn, Ni, and Se in hair samples) in the range of concentration from ppb to several hundred ppm. The method of selection of the control group, the experimental setup and its calibration procedure are described. We also present sample preparation methods and procedure of measurements.

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1. Introduction

The elements, present in the human body, play an important role in its operation, both occurring in large quantities, as well as those in trace amount. As a result of human exposure to the environment, the elements appearing in the body can be harmful to it and can disturb its functioning. Therefore, the knowledge of the content of various elements in the human body can be helpful in determining its condition and during a successful treatment, as well the analysis of determination of element concentration is performed in many investigations. Consequently, many physical and chemical methods have been used to determine the concentration of elements in human biological materials. One of them is total reflection X-ray fluorescence analysis (TXRF), the main principle of which is based on the excitation of characteristic X-rays in the sample produced by radiation from X-ray tube. In the process of atom–radiation interaction, an inner shell electron from an atom in the sample is removed. Such an ionized atom constitutes an unstable system.

Another electron from the outer shell fills a hole and in accordance with the principle of conservation of energy, a photon is emitted. The Moseley law says that its energy is proportional to the atomic number of the atom. Therefore these photons are called "characteristic radiation" and it allows the identification of element. The main advantage of this method is the possibility to analyse the several elements simultaneously in a short time (100 s — 1 h) and a small quantity of material enough for analysis (μl, mg). This makes the TXRF method competitive with chemical methods.

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We have also performed in our laboratory a study of elemental composition of various medical samples (serum, malignant, and benign tissues [1, 2]) so the information about reference values of element concentration in human biological material is required. To summarize, in the present paper we have defined the range of optimal elemental concentration in serum, urine, and hair.

Just because the composition and quantity of elements may vary depending on dietary habits, water and the environment, we discuss our results with the ones presented in Polish medicine literature, only.

The experimental setup and its calibration procedure, sample preparation methods and procedure of measurement are described in detail below.

2. The experimental TXRF setup

The physical basis of the total reflection X-ray fluorescence method (TXRF) is well known and described in detail in many papers [3–5], so in this paper there is only the experimental setup and calibration procedure described, with particular emphasis on the need for calibration for each type of samples (serum, urine, hair). TXRF method is applied in our laboratory using two devices: TXRF module and Picofox spectrometer. Analysis of serum and urine samples was performed using TXRF module equipped with a 3 kW X-ray tube with Mo anode, working at the high voltage 50 kV with electron current 40 mA.

Hair analysis has been carried out using Picofox spectrometer equipped with 30 W X-ray tube works with parameters: high voltage 50 kV and electron current 600 μA. These devices allow to measure the characteristic X-rays of elements from Al to U (with exception of Zb to Ru). The measurements are performed in the air. Determined elemental concentration are from hundred ppb for TXRF module and single ppb for Picofox to 100%. Better detection value in the case of Picofox

spectrometer is achieved as a result of optimization of the setup geometry and using of multilayer monochromator. Spectrometer software allows manual or automatic qualitative analysis of the spectrum and the automatic quantitative analysis of the content of the sample.

Accuracy of the method was checked every day of measurement by measuring a sample with the elements of known concentration and the ratio of the difference between measured and certified values to the certified value was calculated. Expected discrepancy is up to 20%. The precision of the method was also checked in two different ways — both by repeatability (expected higher than 85%) and by inter-laboratory reproducibility (expected discrepancy is up to 10%).

3. Material and sample preparation

Analysed samples of serum were taken from 88 persons, who donated blood voluntarily, being the employees undergoing periodic examination of Holycross Cancer Center (HCC) in Kielce, Poland. Samples of urine (from 29 persons) and hair (from 43 women and 20 men) were collected from randomly selected adults. Procedure of sample preparation was elaborated and optimized separately for each kind of sample. To determine amount of serum (750 μl) internal standard was added. A determined amount of urine (3.3 ml; put in the morning) was mineralized in a close bottle with high purity HNO_3 (1 ml) and internal standard. Hair was washed according to the recommendations of the International Atomic Energy Agency, to remove impurities from the hair [6]. In next step 25 mg washed and dried hair was mineralized in a close bottle with high purity HNO_3 (1.25 ml) and internal standard. As internal standards, both solutions of gallium and yttrium were added. Yttrium or gallium was chosen because of the availability of technical equipment used quantitative analysis. Next, 2 μl of each kind of solution were pipetted into silicon Synsil backing and this drop was dried in infrared and finally measured during 1 h time. Figure 1 shows the example of typical X-ray spectrum of hair sample.

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. Analysis

Registered spectrum of characteristics X-rays gives both qualitative (energy of detected X-ray peaks) and quantitative (peaks area) information about the sample composition and elements concentration.

In the case of TXRF method, to calculate the concentration of elements, the calibration of setups must be done and calibration curves, which are dependence of relative intensity of emitted X-rays on element atomic number Z, must be obtained for K- and L series of X-rays independently. To calibration measurements there were selected elements which do not naturally occur in human serum, urine and hair or do occur in concentration lower than detection limit of method used (V, Co, As, Mn, Ga, Sc, Y, W, Ta, Gd, Ba, Pb, and Er for all kind of samples and additionally for serum: Pd, Sn, Tl, and Bi). The monoelemental and multielemental certified calibrating Merck solutions were used to calibration procedure. We would like to emphasize that there is no universal calibration curve. In medical samples biological matrix is different, which causes different X-ray scattering effect and consequently different peak to background ratio. Therefore three calibration curves were obtained for serum, urine, and hair, respectively. Figure 2 shows the example of calibration curves for K-series. It is clearly seen that relative intensity is different for different kind of samples.

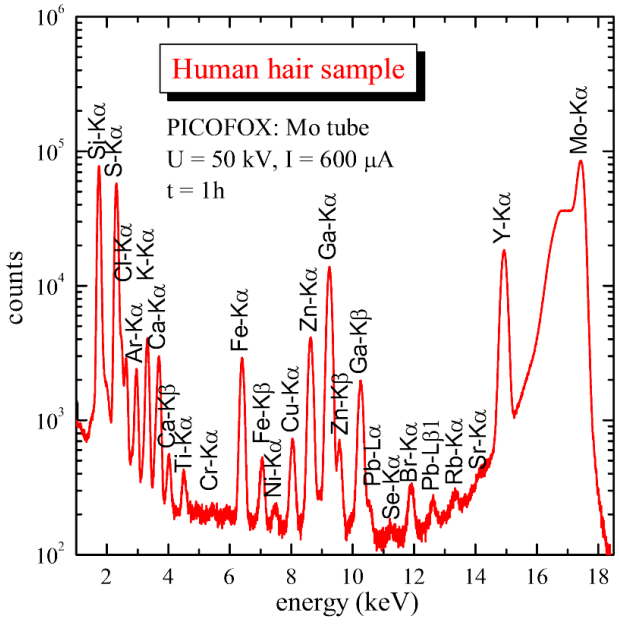

Fig. 1. Typical X-ray spectrum of hair sample.

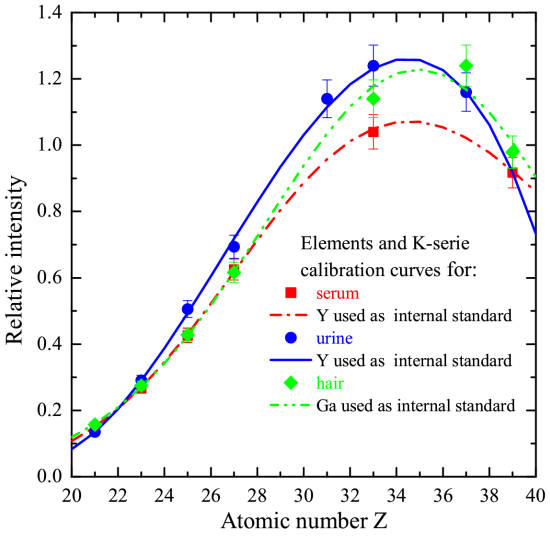

Fig. 2. TXRF calibration curves of K-series for serum, urine, and hair samples.

The spectra measured with TXRF module were analysed using Axil and QAES software. The fitting code Axil, assuming a Gausian line shape and polynomial/filter background was used for removing background level, for resolving the individual X-ray transitions and calculation of peak area. In next step in the case of TXRF module the QAES code was used for quantitative analysis where elemental concentrations were obtained on the basis of calibration curves. The concentration obtained from the QAES was converted on the weight of serum and urine, including the contaminations in solution of yttrium, gallium, and acid.

Picofox spectra were analysed using SPECTRA 7 application. Concentrations obtained from software were converted on the weight of hair, including the concentration of elements in solution of yttrium, gallium, and acid.

4.1. Problem of concentration below detection limit of analytical method

The applicability of X-ray fluorescence method used to trace elements determination is limited to concentration higher than ppb range. The concentration of an element can be determined only when the total number of counts in the corresponding X-ray line, I_{peak}, exceeds statistically significantly the corresponding background level, I_{bkg}. This procedure is also known as the "three standard deviation criterion". The minimal element concentration, which could be detectable by applied arrangement is defined as detection limit of the element (C_{DL}):

$$C_{\mathrm{DL}} = 3\sqrt{I_{\mathrm{bkg}}}c/I_{\mathrm{peak}},$$

where c is the concentration of element [7].

Consequently, concentration of some elements cannot be measured directly because the measurements yield is close to detection limit (for example Cr, Mn, Se, Rb). In this case in the set of samples the concentration is measured directly for some part of them and for the rest there is only the detection limit estimated. Solution of the problem how to include the information about estimated detection limit in data analysis, is the random left-censoring approach [8]. Using this statistical procedure one can estimate the distribution of concentration from the censored data, i.e. incomplete measurements, by using the Kaplan–Meier [9] estimator. The details of this statistical approach, accounting for detection limits in X-ray fluorescence analysis, are described in our previous paper [10, 11].

In order to determine reference values of element concentration in serum, urine, hair samples the following parameters of concentration distributions were calculated: 1st and 3rd quartile and median. Because of strong asymmetry of elemental concentration distribution, values of these quantiles reflect features of the distributions better than mean value and standard deviation. Therefore 1st and 3rd quartiles were accepted as minimum and maximum value of concentration reference range. In the case when 1st quartile could not be calculated, being below detection limit, as the beginning of the reference values range the minimum value of measured concentration was assumed.

5. Results and discussion

5.1. Serum

In serum samples the concentration of P, S, K, Ca, Cr, Fe, Cu, Zn, Br, Rb was determined. In the case of P, Cr, and Rb the censoring procedure was applied. Censoring level, defined as a ratio of the number of samples for which only detection limit was estimated to total number of analysed samples, was equal to 14% for P, 34% for Cr and 40% for Rb.Table I presents reference values of elements concentration and median in analysed serum samples.

TABLE I

Reference values of element concentrations in serum samples (in ppm units).

Element	Median	Reference values
S	527	452–693
K	97.4	86.6–125
Ca	65.5	56.2–93.5
Fe	1.48	1.25–1.76
Cu	1.03	0.827–1.34
Zn	0.928	0.690–1.10
Br	2.19	1.76–2.68
P	68.3	45.5–92.9
Cr	0.294	0.034–0.442
Rb	0.290	0.140–0.556

Serum concentration of Fe, Cu, and Zn are reported in Polish medicine literature (Fe 0.67–1.85 ppm; Cu 0.711–1.6 ppm; Zn 0.60–1.61 ppm) [12–15] and our results are in good agreement with these values. Concentration of Ca is lower than published by Szymańska et al. [15] (90–110 ppm).

5.2. Urine

In urine samples the concentration of Cr, Mn, Fe, Cu, Zn, Rb, and Sr was determined. In the case of Cr, Mn, and Sr the censoring procedure was applied. Censoring level was 83% for Cr, 79% for Mn and 62% for Sr.

Table II presents reference values of elements concentration and median in analysed urine samples. Lack of median value means that because of the high level of censoring the calculation was not possible.

TABLE II

Reference values of element concentrations in urine samples (in ppm units).

Element	Median	Reference values
Fe	0.194	0.157–0.251
Cu	0.025	0.020–0.031
Zn	0.735	0.503–1.29
Rb	1.110	0.640–1.83
Cr	–	0.015–0.033
Mn	0.016	0.016–0.024
Sr	0.072	0.147–0.163

Concentration of Cu is comparable with data presented by Raińska et al. [16]. Concentration of Mn is one order

higher than reported by Kucera et al. [17] (0.0092 ppm) and Jakubowski et al. [18] (< 0.003 ppm) and comparable with data by Seńczuk [19] (0.01 ppm). Chromium concentration determined by us is much higher than reported by Seńczuk [19] (0.0004–0.0007 with listed value 0.002 ppm) and by Kucera et al. [17] (0.00034–0.00131 ppm).

5.3. Hair

In hair samples the concentration of S, Cl, K, Ca, Ti, Cr (for men), Mn, Fe, Ni, Cu, Zn, Se, and Br was determined. In the case of Cl, Ti, Cr, Mn, Ni, Se, and Br (for women) the censoring procedure was applied. Censoring level was in the range from 10% for Cl to 79% for Mn and Se.

Table III and Table IV show reference values of elements concentration and median in analysed hair samples for uncensored and censored data, respectively (m — men, w — women). Lack of median value means that because of the high level of censoring the calculation was not possible.

TABLE III

Reference values of element concentrations in hair samples (in ppm units); m — men, w — women.

Element	S		K		Ca		Fe		Cu		Zn		Br
Sex	m	w	m	w	m	w	m	w	m	w	m	w	m
median	45135	45143	211	93.2	627	1965	33.2	39.5	12.4	17.7	172	181	2.59
reference values	44374–47314	43158–46178	134–497	49.2–132	460–917	915–3709	29.9–52.9	28.2–59.2	10.5–16.2	12.3–26.1	138–190	170–220	1.25–4.70

TABLE IV

Reference values of element concentrations in hair samples (in ppm units) for censoring case; m — men, w — women.

Element	Cl		Ti		Cr	Mn		Ni		Se		Br
Sex	m	w	m	w	m	m	w	m	w	m	w	w
median	758	–	2.75	8.08	0.585	0.862	–	0.784	0.706	0.314	0.157	1.33
reference values	223–2375	7.06–595	0.237–17.1	2.28–23.8	0.193–0.820	0.549–0.941	0.470–0.941	0.314–1.33	0.470–1.18	0.235–0.392	0.157–0.235	0.862–2.74

The concentration of Cr is in good agreement with values reported by Seńczuk in toxicology handbook [19] (0.2–2.0 ppm) and comparable with data reported by Markiewicz et al. [6] for women's hair (0.38 ± 0.29 ppm with minimum value 0.04 ppm and maximum value 1.47 ppm). Ni concentration is 100 times higher in our study than level reported by Seńczuk in toxicology handbook [19]. This effect must be carefully analysed. Elemental hair analysis is done by a number of commercial laboratories but they do not report what method is used and how the reference values are defined.

6. Conclusions

Our primary aim of undertaken long-term studies of the elemental composition of human serum, urine, and hair was to define reference values of elements concentration. The samples of serum, urine and hair were analysed using X-ray fluorescence method and distributions of element concentrations were obtained. We suggest accepting as the reference values of elemental concentrations those in the range between 1 and 3 quartiles.

The source of discrepancy observed between our and literature values may be due to the kind of population (home — industrial area or agricultural area, the kind of water and soil), eating habits, environmental pollution. In cited literature there is no information what population participated in study (number of people, gender, etc.).

We also would like to emphasize that comparison of our results with other could be complicated by the fact that we use censoring procedure. This procedure strongly influences on parameters value of concentrations distribution. Results of study, in which the concentration value is equal to zero is taken for calculations (not detection value, which definitely changes results of statistical analysis) could be different from ours. The discrepancy is seen in the case of chromium and manganese concentrations in urine, where the censoring level is high (83% and 79%, respectively). Similar problem is seen in the case of nickel concentration in hair.

The next step of our study there will be increase of the number of samples and separation of the results by gender. Results of this analysis might be helpful as a tool for monitoring the medical treatment process. Especially the composition of urine and hair can be valuable information because of the fact that urine and hair samples are relatively easy to take from the patient. After finding irregularities in these samples, the physician may order blood tests and perform other, more detailed research. We believe that such analysis might be helpful as a complementary diagnostic tool in medical treatment in cancer diagnosis, in therapy monitoring and in investigation of people's health as related to different aspects of environmental pollution.

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