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ARTICLES

# Determination of the Elemental Composition of Dietary Supplements by Total Reflection X-Ray Fluorescence Spectrometry

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**Abstract**—Techniques for the determination of the elemental composition of dietary supplements (Multifort®, Komplivit®) are presented. It is proposed to use a state-of-the-art analytical method, total reflection X-ray fluorescence spectrometry. The differences in the sample preparation of encapsulated and not encapsulated dietary supplements are described. The effect of encapsulation on the results of the analysis is revealed. The results of qualitative and quantitative analysis are compared with the values certified by the manufacturing company.

*Keywords:* total reflection X-ray fluorescence spectrometry (TXRF), elemental composition, dietary supplements

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Dietary supplements (DSs) contain different inorganic elements possessing biochemical activity. The deficiency of these compounds in a body results in physiological disorders and also contributes to clinical characteristics of some somatic diseases and may cause of various diseases [1]. Several most known modern and traditional methods of pharmaceutical analysis have been documented; however, the optimum ranges and threshold permissible concentrations of elements in a body are not regulated by the State Pharmacopeia of the Russian Federation [2]. The analytical control of elemental composition and of its compliance to concentrations specified in instructions is a task of pharmaceutical analysis [3]. Concentrations of different elements in DSs can vary from several micrograms to dozens of milligrams per one gram of a preparation.

The elemental composition of DSs is determined by both state-of-the-art and traditional methods, such as inductively coupled plasma atomic emission spectrometry (ICP AES) [4], inductively coupled plasma mass spectrometry (ICP MS) [5–7], X-ray fluorescence spectrometry (XRF) [8] and atomic absorption spectrometry (AAS) [9]. A substantial amount of time in analysis by these methods is taken to transfer a sample to a solution. In addition, the methods are characterized by the high cost of analysis and require careful calibration for the elimination of matrix effects or do not allow multielement analysis [10].

In this work we propose the use of a state-of-the-art version of XRF, total reflection X-ray fluorescence spectrometry (TXRF). In TXRF, geometry 0.1°/90° is used (primary X-radiation arrives at a sample at a glancing angle of ~0.1°), in contrast to the geometry 45°/45° in classical XRF analysis. Therefore, the effect of total reflection is attained. In TXRF, the detector is placed very close to the surface of the sample holder, thus ensuring a wide space angle of the collection of radiation and a high counting rate. Primary radiation poorly penetrates into the substrate and is reflected in the direction to the absorber, without arriving at the detector and additionally exciting the sample. However, because of a thin sample layer and the monochromaticity of the exciting radiation, the analyst can achieve a reduced level of background radiation and lower the limits of detection (LODs), which in some cases are at a level of μg/kg (or μg/L). It is also possible to eliminate the phenomenon of the back absorption of X-radiation. On the reduction of the thickness of sample layer below a certain critical value, matrix effects are virtually not observed [1, 11].

The advantages of TXRF in comparison with the traditional energy-dispersive XRF are the low limits of detection, high signal-to-noise ratio, the absence of matrix effects, and also a possibility of quantitative analysis by an internal standard technique and no need in external calibration. The method was applied to the analysis of liquid samples [12], biological tissues, soils, and geological and archaeological samples [13].

The aim of this work was to develop techniques of sample preparation for DSs of different types and to determine the elemental composition of DSs by TXRF, and also to compare the results obtained with the certified data of the manufacturing company.

## EXPERIMENTAL

**Experimental procedure.** "Multifort" (LLC Santefarm, Russia) and "Komplivit" (JSC UfaVita, Russia) DSs were the test samples. Measurements were performed with a benchtop TXRF spectrometer S2 PICOFOX (Bruker Nano GmbH, Germany) using reflecting quartz glass carriers. The measurement time was 250 s. X-ray fluorescence was excited by Mo$K\alpha$ radiation (17.44 keV).

A pill of a Multifort DS was placed in a 100-mL volumetric flask and dissolved in distilled water. Then three aliquot portions of 1 mL were selected, and 5 μL of an internal standard was added to each portion. The internal standards for different aliquot portions were aqueous solutions of nickel, gallium (1000 μg/mL, Fluka Chemical, Great Britain) and yttrium (1000 μg/mL, ABCR, Germany). The concentrations of all internal standards were 5 μg/mL. The volume of an analyzed solution applied on a reflecting quartz glass carrier was 2 μL in each measurement. The sample applied on the substrate was dried in a vacuum desiccator (ISO Lab, Germany).

Pills of a Komplivit DS were grinded in a Pulverisette 7 planetary mill (Fritsch, Germany) in the solid state using an agate cup with agate spheres 10 mm in diameter at a rate of 700 rpm within 6 min. Then a homogenized sample of 1.0080 g, weighed with an accuracy of 0.1 mg on ED224S analytical balances (Sartorius, Germany), was placed in a 100-mL flask and dissolved in a mixture of 80 mL of distilled water with 20 mL of conc. $HNO_3$. For the complete dissolution of the pill, the mixture was shaken on an MS 3 digital laboratory shaker (IKA, United States) within 300 s. From the solution obtained, we selected 1-mL samples to three 1.5-mL vials, which were centrifuged on a MiniSpin centrifuge (Eppendorf, Germany) with a speed of 8000 rpm within 5 min. for the separation of the solution from the undissolved particles of pill's capsule. The internal standards were yttrium, nickel, and gallium solutions (1000 μg/mL, Fluka Chemical, Great Britain). A 2-μL sample applied onto a reflecting quartz glass carrier was dried in a vacuum desiccator.

We also tried to grind another pill in an agate mortar (Carl Roth, Germany); however, after the next sample preparation, the obtained results were characterized by low reproducibility and big errors. This was explained by the fact that at the manual grinding the sample was less dispersed and homogenized.

**Quantitative analysis.** The Spectra 7 software (Bruker Nano GmbH, Germany) allows the input of information on the concentrations of internal standards used to calculate the concentration of each of the elements to be determined. The intensities of characteristic lines of the present elements are automatically determined by this software. The concentrations of the elements to be determined can be calculated by Eq. (1) [11]:

$$c_i = \frac{c_{is} N_i S_{is}}{N_{is} S_i}, \quad (1)$$

where $c_i$ is the concentration of element $i$ to be determined, $c_{is}$ is the concentration of the internal standard, $N_i$ is the number of pulses in measurements of a spectrum of element $i$ to be determined, $N_{is}$ is the number of pulses in measurements of a spectrum of an internal standard, $S_i$ is the relative sensitivity of element $i$, and $S_{is}$ is the relative sensitivity of the internal standard element.

## RESULTS AND DISCUSSION

**Choice of a technique of sample preparation.** The procedure of sample preparation for Komplivit DS differs from that for Multifort DS, because pills of Komplivit DS do not completely dissolve in water. The coating, which consists of auxiliary compounds insoluble in water and possessing no biological activity, in the major part does not dissolve.

Sample preparation for the determination of elemental composition in the pharmaceutical industry was described in sufficient detail in [14]. In this work, the solvent was water or a mixture of water with nitric acid without organic solvents. The use of surfactants was also not required, which additionally simplified the sample preparation. Grinding of samples on a planetary mill ensured a rather high degree of homogeneity, which increased the throughput of analysis by one order of magnitude. For the proper choice of a technique of sample preparation, we considered differences in the types of the chosen DS, one of which (Multifort DS) was not encapsulated and water-soluble, and the other (Komplivit DS) was encapsulated and partially water-soluble.

**Choice of an internal standard.** First of all, the choice of an internal standard for the analysis of dietary supplements is explained by the elemental composition of the sample and also by a possible overlay of spectral lines of the internal standard and the sample. The most suitable internal standards for Multifort DS are Y, Ni, and Ga, because these elements were not detected at the stage of quantitative analysis. Selenium cannot be used as an internal standard, because it was declared in the composition of this DS by the manufacturing company. The internal standards Se, Ni, and Ga for similar reasons are suitable for the Komplivit DS. Cobalt cannot be used as an internal standard because the presence of this element in the DS was declared by the manufacturing company.

The use of several internal standards was necessary for obtaining comparative data, reflecting the accuracy of analysis. Peaks of various internal standards were presented in different energy ranges; therefore, partial spectral overlays with the lines of elements to be determined might be observed. In the region of characteristic lines of gallium, a constant background of

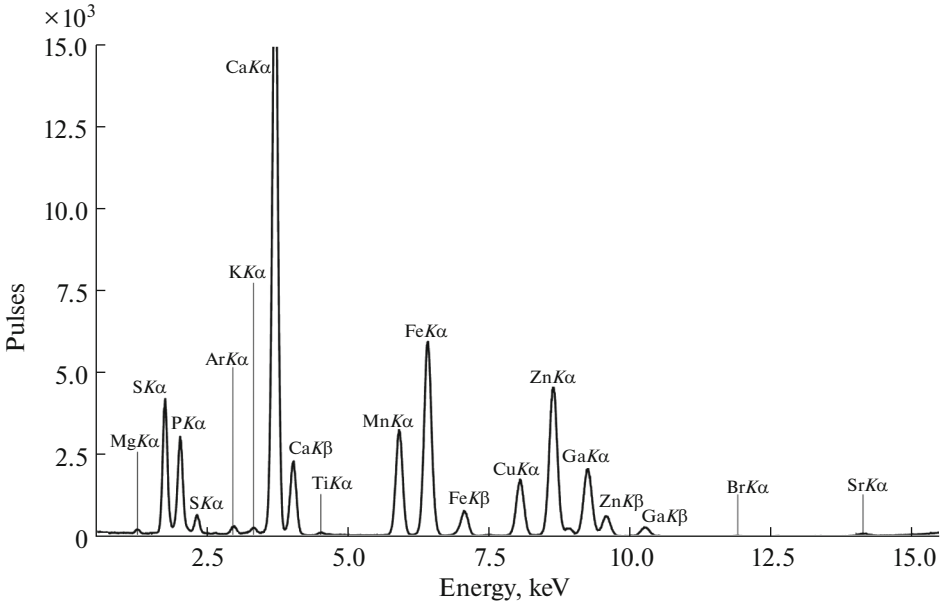


**Fig. 1.** A total reflection X-ray fluorescence spectrum of a sample of Komplivit DS with gallium as an internal standard.

scattered radiation was observed; therefore, it was the most preferable element as an internal standard. Gallium is absent in the majority of the studied samples. Taking into account the resolution of the detector (120–150 eV) in using Ni, Ga, Se, and Y standards, spectral overlays of Ni$K\alpha$ (7.480 keV) – Yb$L\alpha$ (7.416 keV), Ga$K\alpha$ (9.251 keV) – Ir$L\alpha$ (9.175 keV), Se$K\beta$ (12.497 keV) – Pb$L\beta$ (12.614 keV), and Y$K\beta$ (16.739 keV) – Nb$K\alpha$ (16.615 keV) were possible. Among the elements for which spectral overlays with elements of the internal standard were observed, there were no elements declared by DS manufacturers, which confirmed a possibility of using these internal standards.

**Elemental composition of samples.** A TXRF spectrum of a sample of Komplivit DS with a gallium internal standard is shown in Fig. 1. Samples of Komplivit DS contain P, S, K, Ca, Ti, Mn, Fe, Cu, Zn, Br, and Sr. The results of quantitative determination and a comparison of the elemental composition of the Komplivit DS with that declared by the manufacturing company are summarized in Table 1. The determination of magnesium, declared in the composition of the DS, by TXRF spectrometry was complicated. The presence of titanium among the found elements can be explained by the presence of its compounds in the composition of the pill's capsule. The presence of strontium was explained by its isomorphism with calcium; strontium compounds in almost all cases accompany calcium compounds. Among the elements there were also sulfur and bromine, which were present in biologically active compounds, the main macroelements of which were also specified in the composition of the vitamin supplements. The reason for the absence of cobalt among the found elements probably consists in the spectral overlapping of its line with the line of iron: Co$K\alpha$ (6.931 keV) – Fe$K\beta$ (7.059 keV).

A TXRF spectrum of a sample of Multifort DS with gallium as an internal standard is shown in Fig. 2. Samples of Multifort DS contain S, Cl, K, Ca, Cr, Mn, Cu, Zn, Se, Br, Rb, and Sr. The results of quantitative determination and a comparison of the elemental composition of the Multifort DS with that declared by the manufacturing company are given in Table 2. Among the declared elements, there was no iodine detected; the determination of iodine was complicated because of its high losses during the sample preparation. In [15], in the study of DS, iodine was determined with a rather high precision; however, it was the only found element and sample preparation was significantly simplified because of the absence of spectral overlays and impurities. The procedure of sample preparation differed in the fact that to obtain a DS solution, nitric acid was used instead of an ammonia solution and the amount of a sample applied on the substrate was greater. To obtain a thin film, instead of vacuum desiccators, the sample was dried in a laminar-flow cabinet at 40°C within 30 min. The internal standards were Y, Cd, and Ag solutions of different concentrations. An excess amount of potassium in the Multifort DS was explained by the fact that potassium compounds were used in the substrate material, possessing no biochemical activity. A significant difference in the concentration of selenium was observed because of its rather small concentration in the medicine and possible adsorption on the flask walls in the

**Table 1.** Determination of the elemental composition of a sample of the Komplivit dietary supplement by total reflection X-ray fluorescence spectrometry using different internal standards ($n = 5$, $P = 0.95$)

| Element | Experimentally found concentrations, mg/g | | | Element concentrations calculated for one pill ($m = 0.8663$ g), mg | | | Element concentrations declared by the manufacturer, mg |
|---|---|---|---|---|---|---|---|
| | Se | Ni | Ga | Se | Ni | Ga | |
| P | 65 ± 5 | 70 ± 8 | 65 ± 8 | 57 ± 5 | 61 ± 7 | 57 ± 7 | 60.00 |
| Ca | 49 ± 3 | 55 ± 5 | 48 ± 3 | 42 ± 3 | 47 ± 4 | 42 ± 3 | 50.50 |
| Mn | 3.0 ± 0.1 | 3.3 ± 0.3 | 2.9 ± 0.1 | 2.6 ± 0.1 | 2.9 ± 0.3 | 2.5 ± 0.1 | 2.50 |
| Fe | 4.5 ± 0.3 | 5.7 ± 0.5 | 6 ± 1 | 3.9 ± 0.3 | 4.9 ± 0.4 | 5 ± 1 | 5.00 |
| Co | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | 0.10 |
| Cu | 0.53 ± 0.05 | 1.3 ± 0.1 | 0.84 ± 0.06 | 0.46 ± 0.04 | 1.2 ± 0.1 | 0.73 ± 0.05 | 0.75 |
| Zn | 2.0 ± 0.1 | 2.2 ± 0.2 | 1.9 ± 0.2 | 1.7 ± 0.1 | 1.9 ± 0.1 | 1.7 ± 0.1 | 2.00 |

course of sample preparation. Selenium was also determined in dietary supplements and biological samples (blood, urine) in [16]. At the same time, the only distinction in the procedure of sample preparation was the use of aqueous suspension with a surfactant. In [16], the task was the determination of selenium in multielement samples. For the Multifort DS, the error of the results of analysis was smaller, and reproducibility was higher in comparison with those for Komplivit DS because of different processes of the production of pills and corresponding techniques of sample preparation.

* * *

Thus, techniques of sample preparation for DSs of various types are developed, qualitative and quantitative analyses of two dietary supplements by X-ray fluorescence are performed, and the results obtained are compared with the declared concentrations. The internal standards of Ni, Ga, Se, and Y, suitable for the quantitative analysis of DSs are chosen. The reproducibility of the results of analysis of the not encapsulated Multifort DS is better in comparison to those for the encapsulated Komplivit DS. The proposed versions of

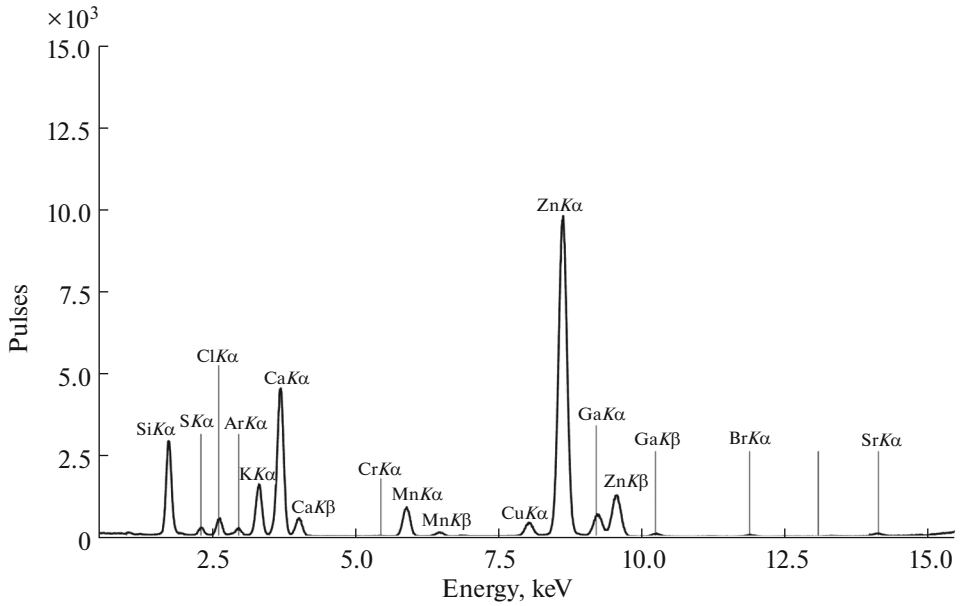


**Fig. 2.** A total reflection X-ray fluorescence spectrum of a sample of Multifort DS with gallium as an internal standard.

**Table 2.** Determination of the elemental composition of a sample of the Multifort dietary supplement by total reflection X-ray fluorescence spectrometry using different internal standards ($n = 5$, $P = 0.95$)

| Element | Experimentally found concentrations, mg/L | | | Element concentrations calculated for one pill ($m = 3.9252$ g), mg | | | Element concentrations declared by the manufacturer, mg |
|---|---|---|---|---|---|---|---|
| | Y | Ni | Ga | Y | Ni | Ga | |
| K | 98 ± 4 | 103 ± 6 | 109 ± 3 | 10.0 ± 0.1 | 10 ± 3 | 11.2 ± 0.3 | 2 |
| Ca | 198 ± 2 | 200 ± 70 | 219 ± 15 | 20.2 ± 0.2 | 20 ± 7 | 22 ± 2 | 20 |
| Cr | 0.18 ± 0.04 | 0.2 ± 0.1 | 0.16 ± 0.06 | 0.018 ± 0.007 | 0.02 ± 0.01 | 0.017 ± 0.004 | 0.02 |
| Mn | 14.9 ± 0.3 | 15 ± 4 | 16.3 ± 0.5 | 1.52 ± 0.03 | 1.5 ± 0.4 | 1.66 ± 0.06 | 1.6 |
| Cu | 3.51 ± 0.08 | 3.5 ± 0.2 | 3.7 ± 0.9 | 0.36 ± 0.09 | 0.36 ± 0.02 | 0.37 ± 0.07 | 0.4 |
| Zn | 79.2 ± 0.7 | 80 ± 14 | 87 ± 3 | 8.08 ± 0.06 | 8 ± 1 | 8.8 ± 0.3 | 8 |
| Se | 0.10 ± 0.07 | 0.1 ± 0.3 | 0.1 ± 0.3 | 0.010 ± 0.008 | 0.01 ± 0.03 | 0.01 ± 0.03 | 0.02 |
| I | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected | 0.15 |

sample preparation can be used for the determination of the elemental composition of DSs of various types.

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