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Determination of chromium and selected elements in multimineral and multivitamin preparations and in pharmaceutical raw material

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Abstract

The content of elements in pharmaceutical preparations is one of the indispensable factors of the evaluation of their quality. In the present work, the following macro- and microelements Ca, Cr, Cu, Fe, Mg, Mn, Mo, P, Se and Zn were determined in multimineral and multivitamin preparations and in pharmaceutical raw material. Inductively coupled plasma mass spectrometry (ICP-MS) and electrothermal atomic absorption spectrometry (ET AAS) were used throughout the study. The examined samples were dissolved in a high-pressure microwave system using concentrated nitric acid. The effect of the carbon residue in the digest solution on the determination result was eliminated by introducing an equation correcting the ArC^+ interference with ^{52}Cr.

Keywords: ICP-MS; ET AAS; Trace elements; Determination of chromium; Spectral interferences; Pharmaceutical preparations

1. Introduction

Chromium is one of the trace elements indispensable for the proper function of living organisms. It is an active component of glucose tolerance factor (GTF) [1]. The mechanism of the GTF action and the role of chromium in glucose metabolism are, since many years, the objective of intense scientific investigations. As the most probable, a mechanism, in which GTF is strengthening the action of insulin through the stimulation of the action of its receptor—tyrosine kinase. The relation between chromium and the activity of insulin is not fully defined as yet. Despite of this, it is considered that chromium is the element, which influences the action of insulin and, at the same time, the metabolism of carbohydrates, fats and proteins.

The daily intake, necessary for the normally functioning human, is about 33 μg of chromium,

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and the limit of daily safe intake is estimated to be 250 μg [2]. The accurate intake for chromium is difficult to fix, because it is distinctly depending on the food consumption. Excessive consumption of sweets increase excerption of chromium with urine, and consequently increases the demand for this element. It was found that chromium contained in the normal diet satisfies daily needs of man [1,2]. In the case of other microelements, a proper diet composed of food of different origin ensures sufficient quantity and adequate proportions of trace elements necessary for the normally functioning organism. The deficiency of microelements, including chromium, could be caused by particular health conditions of humans such as intestinal disorder, neoplastic diseases, cirrhosis of liver. This deficiency may also appear in dialyzed patients or during convalescence [3].

In order to supplement the deficiency of chromium in the diet, a number of pharmaceuticals containing this element has appeared on the market. These are mainly multimineral and multivitamin medicines recommended for the application in prophylactic and treatment of deficiency of these microelements.

In the natural environment, chromium is occurring in two oxidation states, Cr(III) and Cr(VI). Chromium(VI) is considered as a procarcinogen, which in the presence of biological reductans easily transforms into Cr(III). The products of chromium metabolism, i.e. Cr(V), Cr(IV), Cr(III) and free radicals could directly interact with DNA, this feature being connected with the genotoxicity of chromium compounds. The toxicity of Cr(III) compounds is about a 100-fold lower than that of Cr(VI), and the mutagenicity is a feature almost exclusively of Cr(VI) compounds. From the differences in toxicity of chromium compounds in various oxidation states, it follows that speciation analysis in the case is advisable [4].

Considering the toxicity of chromium compounds, inspection of its content in food and medicines is a necessity. Because chromium is applied in chemical reactions as a catalyst, also its content in pharmaceutical raw materials must be determined.

Taking into account the low content of chromium in the studied material, analytical methods of appropriate determinability should be used. The AAS method is recommended by several authors for the determination of trace amounts of chromium in environmental [5], biological [6–8] and food samples [9,10]. The Inductively coupled plasma mass spectrometry (ICP-MS) and ICP-AES methods allow the multielemental analyze of environmental [11–14] and food samples [15]. On the other hand, electrochemical methods usually are applied in speciation analysis of chromium [16–18].

In the present study, the content of chromium and other elements—components of multimineral and multivitamin pharmaceuticals—was determined in tablets, capsules and in diacerein. ICP-MS and electrothermal atomic absorption spectrometry (ET AAS) were used throughout the experiments.

2. Experimental

2.1. Apparatus

A mass spectrometer with inductively coupled plasma VG PlasmaQuad 3, an atomic absorption spectrometer Z-5000 from Hitachi, an atomic absorption spectrometer AA 660 from Shimadzu, hollow cathode lamps, graphite tubes, graphite tubes pyrolytically coated, a microwave mineralizer from Plazmatronika were used throughout.

2.2. Samples

Preparations containing $Cr(III)Cl_3 \cdot 6H_2O$—Centrum Junior A+Zinc from Whiehau-Much (one tablet contained the following amounts of the elements: Cr, 12.5 μg; Mo, 12.5 μg; Se, 15 μg; Cu, 0.40 mg; Mn, 0.50 mg; Zn, 4.00 mg; Fe, 4.40 mg; Mg, 60.0 mg; P, 125 mg; Ca, 162 mg); Multitabs Classic from Ferrosan (one tablet contained: Cr, 50 μg; Se, 50 μg; Cu, 2.0 mg; Mn, 2.5 mg; Fe, 14.0 mg; Zn, 15.0 mg; Mg, 75 mg); Materna from Wyeth-Ayerst (one tablet contained: Cr, 25 μg; Mo, 25 μg; Se, 25 μg; Cu, 2.0 mg; Mn, 5.0 mg, Zn; 25.0 mg; Mg, 50 mg; Ca, 250 mg); Witagin from Labofarm (one tablet contained 30 μg of chromium); preparations contain-

ing other chromium compounds—Redusan from Biokraft Pharma (one tablet contained 4.16 μg of chromium organically bounded in yeast); Ovulavit from Bional (one capsule contained 62 μg of chromium in the form of chromium orotate); diacerein (4.5-bis(acetyloxy)-9,10-dihydro-9,10-dioxo-2 anthracenecarboxylic acid); a substance, permissible content of chromium, 5 μg g^{-1}.

2.3. Standards and reagents

Standard solutions of calcium, chromium, cobalt, copper, iron, magnesium, manganese, molybdenum, phosphorus, selenium and zinc at a concentration of 1 mg ml^{-1} from Merck; nitric acid from Merck; reagents of ICP-MS purity; redistilled water, additionally purified in the Nanopure Deionization System from Barnstead; argon 99.999% (v) from Praxair Kędzierzyn.

2.4. Sample preparation

An average weight from ten tablets or an average content from ten tablets was determined. Weighed samples of about 0.2–0.6 g of tablet mass and 0.16 g of capsule content were placed in a Teflon crucibles and 3 ml of nitric acid was added. Mineralization with the use of microwaves was conducted in a closed system, in a four-steps system at a maximum microwave power of 280 W. The maximum pressure and temperature during mineralization were 45 at and 155 °C. Mineralization was performed in 110 ml Teflon vessels. The mineralizates were transferred into volumetric flasks and were made up to 100 ml with water. Then, successive dilutions were performed in such a way as to obtain the foreseen concentration of the elements to be determined in an optimal measuring range.

Weighed samples (about 0.2 g) of determined substance were placed in Teflon crucibles and the procedure used was the same as described above. The mineralizates were transferred into 100 ml volumetric flasks, 100 μl of the solution of internal standard of concentration 10 mg l^{-1} was added and made up to volume with water.

2.5. Determination of Cr and selected elements in multimineral and multivitamin preparations by the ICP-MS method

Determination parameters: excitation power of plasma 1380 W; the flow rate for: plasma (12.6–13.01 min^{-1}), nebulizer (0.74–0.80 l min^{-1}) and auxiliary (0.7–0.8 l min^{-1}) gases; the base line of the background below 10 cps; the amount of doubly charged ions 70/140 Ce^{2+}/Ce and 69/138 Ba^{2+}/Ba below 3.0%; the amount of oxide ions 156/140 CeO/Ce below 3% and that of 154/138 BaO/Ba below 0.2%; the aspiration time of a sample was 180 s; the measurement time was 15 s in 3-fold repetitions. As an internal standard in the determinations, cobalt at a concentration of 10 μg l^{-1} was used.

2.6. Determination of Cr in diacerein by the ET AAS method (spectrometer AA-660)

The determination of Cr was performed in a graphite tube pyrolytically coated. The background Smith-Hieftje correction was applied at a current intensity of the lamp 8 and 480 mA. The measurements were performed at a wavelength of 357.9 nm, the slit width being 0.25 nm.

2.7. Determination of Cr in the certified reference material Green Algae by the ET AAS method (spectrometer Z-5000)

The determination of Cr was performed in a graphite tube pyrolytically coated. The background Zeeman correction was applied at a current intensity of the lamp 6 mA and a voltage of 427 V. The measurements were performed at a wavelength of 359.3 nm, the slit width being 1.3 nm.

2.8. Determination of Ca and Fe in multimineral and multivitamin preparations by the ET AAS method (spectrometer AA-660)

The determination of Ca was performed in a graphite tube, Fe in pyrolytically coated. The background Smith–Hieftje correction was applied at a current intensity of the lamp 12 and 480 mA

for Ca, 10 and 320 mA for Fe. The measurement of Ca were performed at a wavelength of 422.7 nm; the measurement of Fe were performed at a wavelength of 248.3 nm; the slit width being 0.25 nm. The determination parameters of chromium, calcium and iron are presented in Table 1.

3. Results and discussion

In this study, chromium and other selected elements were determined in pharmaceutical preparations and in raw material by the methods of ICP-MS and ET AAS. The optimum measuring range for chromium and selected elements as well as the limit of detection in the applied analytical methods are presented in Table 2.

Statistical evaluation of the obtained results from chromium determination is given in Tables 3 and 4. In Table 3 results are given of chromium determination in three preparations containing various chromium compounds and Table 4 contains results for diacerein. From the data presented in Table 3, it follows that the developed analytical procedure can be used to the determination of chromium occurring in both inorganic and organic compounds as well as in complex matrices. In Fig. 1 examples are shown of multielement determination in the tablets Centrum Junior A+Zinc, Materna and Multitabs Classic. The content of chromium and selected elements was in the accepted tolerance limits in all the preparations studied.

The examined tablets contained both macroelements (Ca, Mg, P) and microelements (Cr, Cu, Fe, Mn, Mo, Se, Zn), vitamins and multimolecular auxiliary substances such as: cellulose, magnesium stearate, shellac, starch, gelatin. Prior to the determination, the tablet and capsule masses were dissolved with the use of microwave energy. However, during microwave dissolution of the complex matrices a danger arises that traces of carbon remain in the obtained mineralizates. Only in the case of chromium determination an effect of carbon residues in the mineralizates on the final results was observed. The ^{12}C and ^{13}C atoms originating from the matrix can form with argon from the plasma an ArC^+ ion interfering with the

Table 1
The parameters for the determination of chromium, calcium and iron by the ET AAS method

Step number	Operation	Spectrometer Z-5000			Spectrometer AA-660				
		Cr			Time (s)	Gas flow rate (l min^{-1})	Temperature (°C)		
		Temperature (°C)	Time (s)	Gas flow rate (l min^{-1})			Ca	Cr	Fe
1	Drying	140	40	0.2	20	1.5	120	120	120
2	Pyrolysis	800	20	0.2	20	1.5	700	600	500
3	Atomization	2700	5	0	4	0	2400	2400	2300
4	Cleaning	2800	4	0.2	5	1.5	2700	2800	2500

Table 2
Optimum determination range and detection limit of the selected elements in the ICP-MS and ET AAS methods

Element	Method	Optimum determination range (μg l^{-1})	Equation of calibration curve	Regression coefficient	Limit of detection (ng l^{-1})[a]
^{52}Cr	ICP-MS	0.5–10.0	$Y = 4.51717 \times 10^2 + 1.59151 \times 10^4 X$	1.0000	48.3
^{53}Cr			$Y = 0.82442 \times 10^2 + 1.87175 \times 10^3 X$	0.9998	5.0
Cr	ET AAS, Z-5000	0.5–10.0	$Y = 1.15852 \times 10^{-2} X + 1.70959 \times 10^{-3}$	0.9999	1.2
	AA-660	1.0–10.0	$Y = -2.657 \times 10^{-6} X^2 + 6.898 \times 10^{-3} X$	0.9998	87
Ca	ET AAS	40–80	$Y = 2.109 \times 10^{-6} X^2 + 3.4 \times 10^{-3} X$	0.9997	537
Fe		25–50	$Y = 8.535 \times 10^{-6} X^2 + 3.57 \times 10^{-3} X$	0.9998	429
^{65}Cu	ICP-MS	0.5–10.0	$Y = -1.12296 + 4.62233 \times 10^3 X$	1.0000	47.1
^{24}Mg		0.5–10.0	$Y = 4.4298 \times 10^2 + 1.94915 \times 10^4 X$	0.9999	24.3
^{55}Mn		0.5–10.0	$Y = 8.33765 \times 10^2 + 1.80094 \times 10^4 X$	1.0000	9.0
^{95}Mo		0.5–10.0	$Y = 0.10335 + 3.59088 \times 10^3 X$	0.9997	43.2
^{31}P		1.5–10.0	$Y = 0.70386 + 1.37899 \times 10^3 X$	0.9992	750
^{82}Se		0.5–10.0	$Y = -6.24824 + 2.74359 \times 10^2 X$	0.9999	24.0
^{66}Zn		1.0–10.0	$Y = 6.63591 \times 10^2 + 3.85339 \times 10^3 X$	0.9999	109

ICP-MS; Y, counts per second; X, concentration (μg l^{-1}).
[a] Three standard deviations of blank test.

^{52}Cr and ^{53}Cr isotopes, which leads to positively falsified results (Fig. 2) [19,20]. The natural abundance of the chromium isotopes ^{52}Cr and ^{53}Cr equal to 83.8 and 9.5%, respectively. Also the two remaining chromium isotopes ^{50}Cr and ^{54}Cr (abundance 4.3 and 2.4%) are not free of the interference. For ^{50}Cr the interferences come from Ti, SO, ArN, V, ArC and for ^{54}Cr from ArN (Fig. 2).

Because the chromium concentration in the examined samples exceeded 18 μg g^{-1}, prior to quantitative determination consecutive dilutions of the mineralizates were performed. From the analysis of mass spectra obtained from the so prepared solutions it follows that the $^{53}Cr/^{52}Cr$ ratio was consistent with the value calculated for the natural abundance of these isotopes, thus indicating that there is no interference influencing the results. From the data given in Table 3 it follows that the developed analytical procedure can be used to the determination of chromium occurring in both inorganic and organic compounds as well as in complex matrices.

Diacerein was also dissolved with the use of microwave energy. Similarly as in the case of tablets, in the obtained mineralizates complete removal of carbon was not achieved. In view of the low chromium concentration in the examined sample, the obtained mineralizates could not be diluted. The effect of matrix on the chromium determination was eliminated by introducing a suitable equation, which correction factor was 0.0738. In order to confirm the results obtained by the ICP-MS method, additional determination of chromium was performed using the ET AAS method. From the results shown in Table 4 it

Table 3
Statistical evaluation of chromium determination in pharmaceutical preparations by the ICP-MS method ($n = 6$[a])

Preparation	Content of chromium		R.S.D. (%)
	Declared (μg per capsule)	Determined (μg)	
Ovulavit (chromium orotate)	62.0	61.4 ± 2.2	5.4
Witagin (chromium(III) chloride)	30.0	32.1 ± 2.0	5.3
Redusan (yeast containing chromium organically bound)	4.16	4.3 ± 0.2	4.0

[a] One measurement from six mineralizates.

Table 4
Statistical evaluation of chromium determination in the diacerein substance by the ICP-MS and ET AAS methods ($n = 6$[a])

	Method	Content Cr (μg g^{-1})	R.S.D. (%)
ICP-MS	^{52}Cr[b]	1.60 ± 0.01	1.0
	^{52}Cr (after introduction of a correction equation)	1.04 ± 0.01	0.8
	^{53}Cr	1.10 ± 0.01	0.8
ET AAS		1.09 ± 0.06	4.8

Permissible chromium content 5 μg g^{-1}.
[a] One measurement from six mineralizates.
[b] Positively false Cr result by interferences with ArC^+ ions.

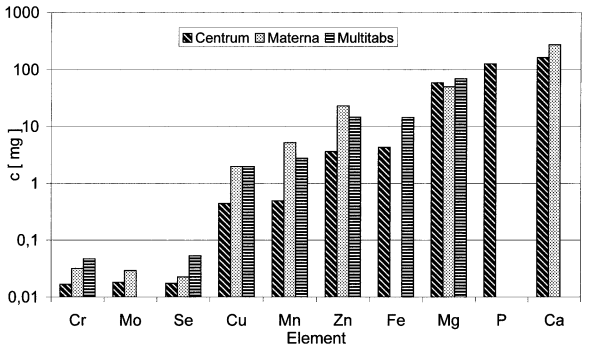

Fig. 1. Content of the selected microelements (Cr, Cu, Fe, Mn, Mo, Se, Zn) and macroelements (Ca, Mg, P) in preparation Centrum Junior A+Zinc, Materna, Multitabs Classic.

follows that the value obtained by the ET AAS method is above 47% lower than that calculated from the ^{52}Cr isotope. However, it agrees with the results obtained by the ICP-MS method from the ^{53}Cr and ^{52}Cr isotopes after introducing the correction equation. Thus, the conformity of the applied correction equation was confirmed.

The accuracy of the determination results of chromium and selected elements was confirmed by the analysis of the certified reference materials. At present, there is a lack of reference materials, which could be used to estimate the conformity of mineralization procedure and accuracy of quantitative determination of elements in multimineral and multivitamin pharmaceutical preparations. Therefore, for this study two certified reference materials of complex organic matrices were chosen, i.e. Green Algae (P-ACHK Number 12-2-02) and Oriental Tabacco Leaves (CTA-OTL-1). In Table 5, the certified values are given and those obtained in our experiments obtained during repeated analysis of the certified materials performed in order to evaluate the stability of the analytical procedure. The agreement of the results shows that both the proposed mineralization process of samples and the quantitative determination of elements are correct.

4. Conclusions

The obtained results of elements determination in the multielement and multivitamin tablets and pharmaceutical substance has demonstrated that the use of microwave mineralization to the dissolution of the analyzed samples is feasible. The determination of Ca, Cr, Cu, Fe, Mg, Mn, Mo, P, Se and Zn by the ICP-MS and ET AAS is possible without the necessity of their separation from the matrix. The effect of carbon residues in the mineralizates and the accompanied interferences during the determination of chromium nearly completely are eliminated either during consecutive dilutions of the mineralizate, or by applying an equation, which corrects the interferences. An advantage of the used ICP-MS method is the possibility of simultaneous determination of several elements of varying concentrations in the sample with a coefficient of variation below 5.4%.

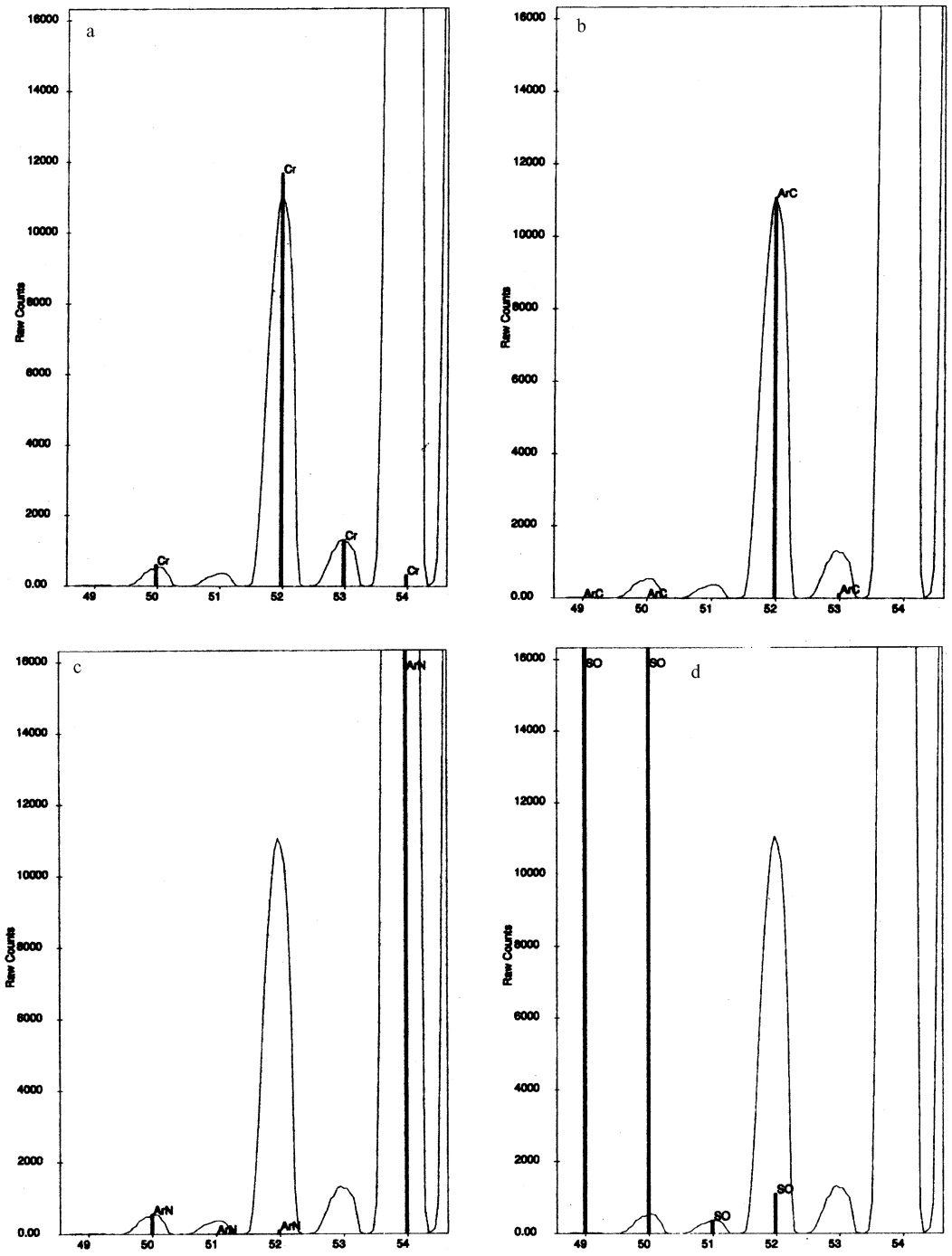

Fig. 2. Mass spectrum of chromium isotopes (a), ArC^+ ions (b), ArN^+ ions (c) and SO^+ ions (d) interfering with ^{52}Cr obtained by the ICP-MS method from preparation Witagin after microwave mineralization.

Table 5
Results of the determination of elements in the certified reference materials ($n = 12$[a])

Element	Green algae P-ACHK Number 12-2-02		Oriental tobacco leaves CTA-OTL-1	
	Certified value (μg g^{-1})	Determined value (μg g^{-1})	Certified value (μg g^{-1})	Determined value (μg g^{-1})
Cr	2.37 ± 0.42	ICP-MS, 2.41 ± 0.18; ET AAS, 2.37 ± 0.04	–	–
Cu	19.6 ± 1.1	18.5 ± 3.0	–	–
Fe	339 ± 16	343 ± 5	–	–
Mg	–	–	4470 ± 210	4417 ± 110
Mn	32.8 ± 1.0	31.7 ± 0.7	412 ± 14	409 ± 13
Mo	2.28 ± 0.54	2.27 ± 0.10	–	–
Zn	40.2 ± 0.6	40.3 ± 1.1	49.9 ± 2.4	49.7 ± 1.0

[a] One measurement from 12 mineralizates.

References

[1] G. Gałuszka, M. Cieślak-Golonka, Wiad. Chem. 53 (1999) 379.
[2] Trace elements in human nutrition and health. World Health Organization, Geneva, 1996.
[3] E. Braunwald, K.J. Isselbacher, R.G. Petersdorf, J.D. Wilson, J.B. Martin, A.S. Fauci, Harrison's Principles of Internal Medicine, Mc Graw-Hill Book Company, 1987.
[4] C. Camara, R. Cornelis, P. Quevauviller, Trends Anal. Chem. 19 (2000) 189.
[5] J. Kwapuliński, J. Mirosławski, D. Wiechuła, R. Rochel, J. Burczyk, B. Sowada, K. Iwanek, Bromat. Chem. Toksykol. 29 (1996) 243.
[6] Y.L. Huang, I.C. Chuang, C.H. Pan, C. Hsiech, T.S. Shi, T.H. Lin, At. Spectrosc. 21 (2000) 10.
[7] B.T. Yaru, D. Bainok, G.M. Day, At. Spectrosc. 20 (1999) 33.
[8] K. Ohta, H. Uegomori, S. Itoh, T. Mizuno, Microchem. J. 56 (1997) 343.
[9] E. Lendinez, M.L. Lorenzo, C. Cabrera, M.C. Lopez, Sci. Total Environ. 278 (2001) 183.
[10] M. Gonzalez, M. Gallego, M. Valcarcel, Talanta 48 (1999) 1051.
[11] H. Lachas, R. Richaud, K.E. Jarvis, A.A. Herod, D.R. Dugwell, R. Kandiyoti, Analyst 124 (1999) 177.
[12] Z. Arslan, N. Ertas, J.F. Tyson, P.C. Uden, E.R. Denoyer, Fresenius J. Anal. Chem. 366 (2000) 273.
[13] N. Daskalova, I. Boevski, Spectrochim. Acta, Part B 54B (1999) 1099.
[14] A. Lozak, K. Sołtyk, P. Ostapczuk, Z. Fijałek, Sci. Total Environ. 289 (2002) 33.
[15] M. Anke, R. Mueller, A. Truepschuch, M. Seifert, M. Jaritz, S. Holzinger, S. Anke, J. Trace Microprobe Tech. 18 (2000) 541.
[16] M. Korolczuk, Anal. Chim. Acta 414 (2000) 165.
[17] M. Korolczuk, M. Grabarczyk, Microchem. J. 62 (1999) 311.
[18] E.M. deSouza, A.D.L.R. Wagener, P. Farias, Croat. Chem. Acta 70 (1997) 259.
[19] N. Violante, F. Petrucci, P. DelleFemmine, S. Caroli, Microchem. J. 59 (1998) 269.
[20] A. Krushevska, S. Waheed, J. Nobrega, D. Amarisiriwardena, R.M. Barnes, Appl. Spectrosc. 52 (1998) 205.