Inorganic analysis of herbal drugs. Part I. Metal determination in herbal drugs originating from medicinal plants of the family *Lamiacae*

SLAVICA RAŽIĆ[*#1], SVETLANA ĐOGO,[1] LATINKA SLAVKOVIĆ[2#] and ALEKSANDAR POPOVIĆ,[3#]

[1]*Institute of Analytical Chemistry, Faculty of Pharmacy, University of Belgrade, P. O. Box 146, 11211 Belgrade (e-mail: snsn@sezampro.yu),* [2]*Vinča Institute of Nuclear Sciences, P. O. Box 522, 11522 Belgrade and* [3]*Faculty of Chemistry, University of Belgrade, P. O. Box 158, 11000 Belgrade, Serbia and Montenegro*

Abstract: Elemental profiles of the total analyte content of major, minor and trace elements (Cu, Zn, Mn, Fe, K, Ca, Mg, Al, Ba and B) in 8 herbal drugs, originating from medicinal plants of the family *Lamiacae*, were determined. Flame atomic absorption/emission spectroscopy (FAAS/FAES), inductively coupled plasma atomic emission spectroscopy (ICP-AES) and energy dispersive X-ray fluorescence (EDXRF) were applied, and the advantages and limitations of these techniques are also discussed. The whole procedure, from sample preparation *via* dissolution to the actual measurements, was validated by using CRM (NIST 1573a – Tomato leaves). The recovery values obtained were in the range 90.64 – 101.58 %. A high degree of similarity in their elemental profiles was noticed from the results of qualitative analysis, while quantitative analysis shows significant diversity due to the variety of the influencing sources. The medicinal plants investigated in this work contained Cu (5.92–14.79 mg kg^{-1}), Zn (15.0 – 43.0 mg kg^{-1}), Mn (25 – 111 mg kg^{-1}), Fe (74 – 546 mg kg^{-1}), K (1.80 – 6.24 %), Ca (0.90 – 1.43 %), Mg (0.17 – 0.67 %), Al (49 – 378 mg kg^{-1}), Ba (15.53 – 69.84 mg kg^{-1}) and B (34.7 – 56.5 mg kg^{-1}).

Keywords: major, minor and trace elements, FAAS, FAES, ICP–AES, EDXRF, herbal drugs, *Lamiacae*.

INTRODUCTION

Some elements are essential for plants but have harmful effects at excessive concentrations[1] hence their different roles in the physiological processes of plants strongly depend on their concentrations[2–4] in a particular plant tissue. The herbal drugs analyzed in this work are known in herbal medicine in Serbia for their different pharmacological ac-

---

tivities[5] and, thus, have a very important place in phytotherapy. The beneficial effects of the analyzed drugs are mainly atributed to the presence of constituents of essential oils, flavonoides, tannins, *etc.*[5] but, they are also influenced by inorganic components, as the macro and micro elements are. Currently, there are 17 elements known to be required by all higher plants. Nine of them are macronutritients (C, N, O, H, K, Ca, Mg, P and S). An additional eight elements are defined as micronutritients (B, Cl, Cu, Fe, Mn, Mo, Ni and Zn). Other elements may be essential for certain plant species grown under specific environmental conditions.[6] Zinc, Mn and Fe are important as co-enzimes, Cu is bound to amino acids,[7] while some elements, such as Mn, Nd and Ce, are bound to some biomacromolecules forming coordination compounds.[8] Iron, Cu, Mn, Co, *etc.*, are important components of many antioxidant processes, a deficiency of any of these essential elements may impair the function of the overall oxidant system.[9] In addition, plants in response to environmental stress, change the elemental distribution and metal accumulation to a large extent without toxic effects,[10] or to phytotoxic levels.[6] Hence, accurate quantitative analysis of the elemental content of plants is very important both in basic and applied plant studies, as a contribution to increasing concern about their potential effects on human health.[11] Taking into account the relatively low concentrations of the investigated elements and the complexity of the plant matrix, it was necessary to apply various analytical techniques (EDXRF, FAAS/FAES and ICP-AES), which are the most reliable in qualitative and quantitative analysis of plant samples,[12–15] in order to satisfy important analytical criteria, such as accuracy and sensitivity and to provide less time consuming and cost beneficial analysis as well.

EXPERIMENTAL

Solutions and reagents

All reagents used were of analytical grade. Calibration was performed using single and multi-element calibration solutions (2.5 % HNO_3) prepared from 1 g/L Merck p.a. stock solutions. Ionization in FAAS was controlled by the addition of 5.00 mL of a (10 g/L CsCl + 100 g/L La, Merck, p.a.) buffer solution to all samples and standards and dilution to a final volume of 50.00 mL.

All solutions were prepared with deionized water.

Sample preparation procedure

The different plant samples listed in a Table I were analyzed.

TABLE I. The list of herbal drugs samples from plants originating from the family *Lamiacae*

Herbal drug	Plant
1.Salviae folium	*Salvia officinalis* L.
2.Mentae pip. folium	*Mentha piperita* L.
3.Melissae folium	*Melissa officinalis* L.
4.Rosmarini folium	*Rosmarinus officinalis* L.
5.Lavandulae flos	*Lavandula angustifolia* Mill.
6.Basilici herba	*Ocimum basilicum* L.
7.Marubii herba	*Marrubium vulgare* L.
8. Origani herba	*Origanum vulgare* L.

Accurately weighed ($\pm$0.0001 g) samples ($\approx$1 g each) were prepared according to the procedure for the analysis of liquid samples described elsewhere.[16]

For solid samples analysis by EDXRF, the inorganic residue (ash), obtained after the first stage of the procedure for liquid sample analysis was used before the dissolution. The ash samples were homogenized and pressed to obtain pellets.

Instrumental and operating conditions in EDXRF

Elemental analysis of the samples was carried out with a Canbera EDXRF spectrometer. Radioactive [109]Cd was used as the excitation source. The characteristic X-rays were detected by a semiconductive Si(Li) detector with a Be window, coupled to a multichannel analyzer. The used detection system had a FWHM (full width at half-maximum) energy resolution of 156 eV at 5.9 keV. The integration time was 3600 s.

Instrumental and operating conditions in FAAS/FAES

The determination of Cu, Zn, Mn, Fe, K, Ca and Mg was performed with a Perkin–Elmer 5000 atomic absorption spectrophotometer under optimal measurement conditions. The signals were measured with background correction (deuterium lamp) at the optimal flame heights.

Instrumental and operating conditions in ICP-AES

A Perkin–Elmer 6500, ICP–AES instrument, equipped with a Perkin–Elmer cross flow nebulizer was used, with an input power of 1.2 kW and a frequency of 27 MHz, the argon flow rates were: 15 L/min (outer gas), 0.5 L/min (intermediate gas) and 1.0 L/min (carrier gas). The observation height above the load coil was in the analytical region from 2.5 to 25 mm. The sample uptake rate, in the range 0.6–1.5 mL/min, was controlled by a peristaltic pump.

TABLE II. Charactristic of the elemental spectral lines

Element line	(E_i/E_{exc})/eV	λ/nm
Cu I	7.72/3.82	324.754[a]
Zn I	9.39/5.80	213.86[a]
Mn I	7.43/4.44	279.48[a]
Fe I	7.87/4.99	248.327[a]
K I	4.39/1.61	769.898[a]
Ca I	6.11/2.93	422.673[a]
Mg I	7.64/4.34	285.213[a]
Al I	5.98/4.02	309.284[b]
Ba II	5.21/2.72	455.403[b]
B II	8.30/4.96	249.773[b]

[a]FAAS, FAES; [b] ICP-AES

The characteristics lines of the elements used for measuring by FAAS/FAES and ICP-AES are given in Table II.

RESULTS AND DISCUSSION

Taking into account some starting data of preliminary analysis,[17,18] EDXRF spectroscopy as a non-destructive analytical technique which allows the simultaneous identification of elements in different matrices,[6, 14] was applied in the first part of the investigation. The composition of the matrix in the pellets prepared

from plant powder obtained from leeaves, flowers or herbs varries, resulting in a different penetration of the X-rays. In addition, a second problem was encountered. Bulk plant material is transparent to X-rays and the absorbance of the pellets might be influenced by minor concentrations of S, Cl, K, Ca and Fe.[19] In samples prepared as pressed tablets, the enhancement effect and/or the absorption of characteristic X-rays emitted by the sample constituents make quantification of the detected elements difficult. Ashing of the plant material was performed in order to, at least partly, overcome the mentioned limitations.

The concentration of most of the elements in the crude samples was too low to be directly determined by EDXRF, so there was no possibility to detect any of the trace elements. Therefore, ashing was also carried out to enable the detection of numerous elements.

However, the biggest advantage is the possibility of multi-elemental analysis. Furhermore, a minimal sample preparation is required and the sample pellet can be achieved and re-run at a later date. This technique requires only a single run with a sample and does not require any knowledge of the incoming X-ray flux of geometry of the experimental arrangement. Enough data to proceed with further analysis can be obtained from a single spectrum. Therefore, in this work, EDXRF was also used to obtain the relative concentrations or ratios of the different elements present.

EDXRF Spectra of herbal drugs were recorded and are presented in Fig. 1.

In most cases, the only similarity observed was in the sense that the same elements were detected. Light elements, such as N, Mg and Na, emit X-rays with a low energy level, thus they are difficult to detect by XRF. In addition, the detection limit of EDXRF might limit the analysis of Cu, Fe, and Zn.[7] Therefore, the concentrations of these elements in the samples have to be relatively high in order to be detected. Therefore, taking into account the preliminary investigations,[17,18] the obtained results and the well known inorganic compositions of plants,[6, 20–22] further analysis was conducted by applying FAAS and ICP-AES.

The preparation procedure must ensure simultaneously quantitative mineralization and dissolution by the use of appropriate chemical reagents and physical means. Incomplete dissolution, as a consequence of a high content of silicates, may result in poor recoveries for many elements, especially those relatively abundant in plant media and partly associated with silicates. Aluminum is an example but, in addition, according to literature data, poor recoveries can be expected for Fe and B.[12] After the preparation procedure applied in this work, the sample matrix was largely simplified and the resulting solutions were clear, colorless and odorless with no observed residue – complete dissolution was achieved in most cases.

Concerning atomic spectroscopic analysis, no particular precaution have to be taken if the measured concentrations satisfy the principal criteria (sensitivity, detection limits, working range) and if possible interfering substances are under con-
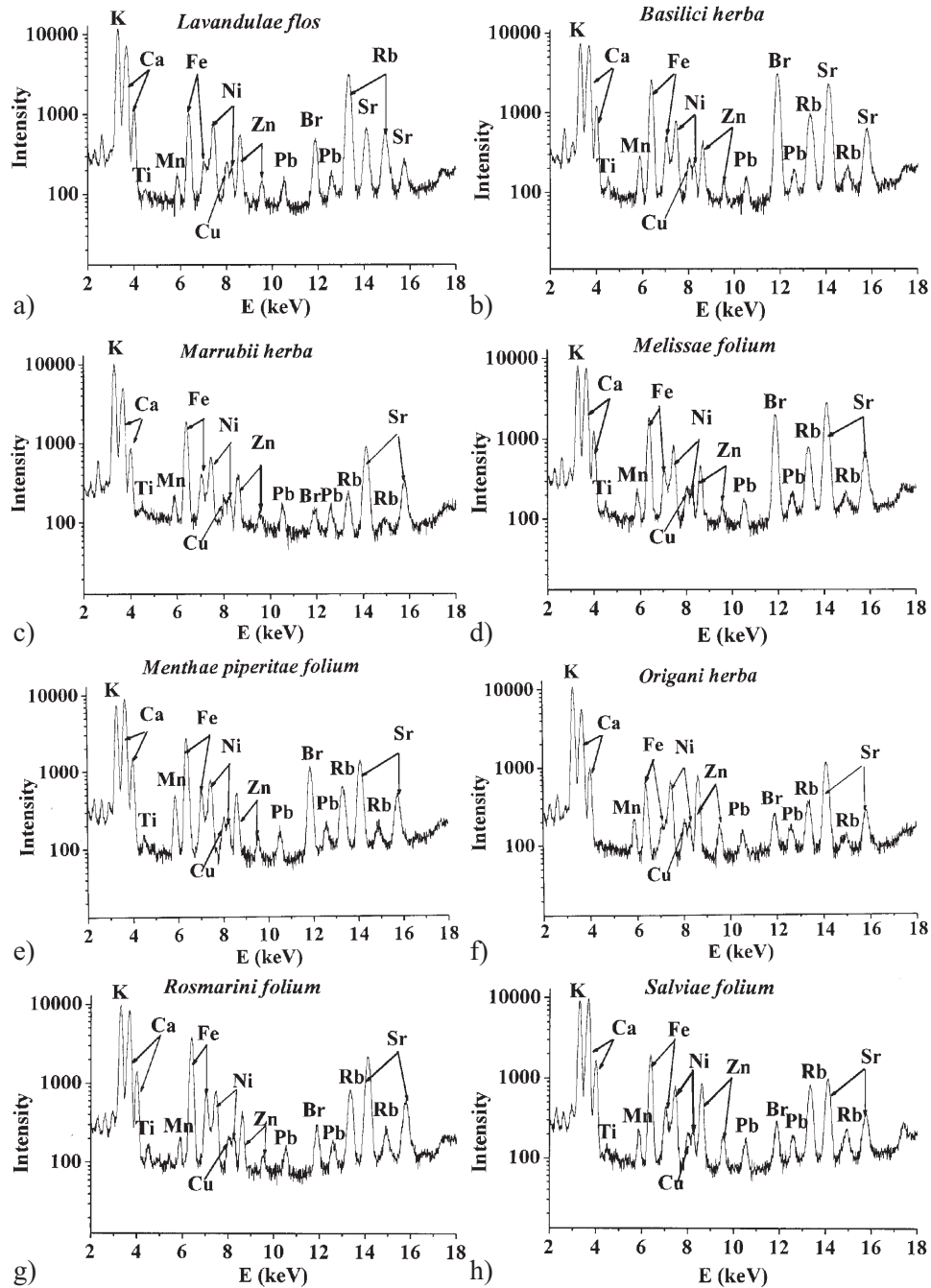
Fig. 1. EDXRF Spectra of ashed herbs samples obtained from plants of the family *Lamiacae;* a) *Lavandulae flos*; b) *Basilici herba*; c) *Merrubii herba*; d) *Melissae folium*; e) *Menthae piperitae folium,* f) *Origani herba;* g) *Rosmarini folium*; h) *Salviae folium.*

trol.[12,13] Measurements were performed under the optimal flame and plasma conditions. FAAS offers detection possibilites in the μg/L and mg/L ranges, while ICP-AES at μg/L level depending on the element considered and thus enable quantification.

The whole procedure (from mineralization, *via* dissolution, to measurements) was validated by applying the methods to CRM (NIST 1573 – Tomato leavels). Both FAAS and ICP-AES analysis give concentration values close to the certified values. Recovery values are presented in Table III.

TABLE III. Results in mg/kg (% for K, Mg and Ca) for the certified reference material NIST 1573a (Tomato leaves)

Element	Found[a]	Certified value[a]	Recovery values/%
Cu	4.26 ± 0.29	4.70 ± 0.14	90.64
Zn	29.4 ± 0.6	30.9 ± 0.7	95.15
Mn	246 ± 2	246 ± 8	100
Fe	339 ± 1	368 ± 7	92.12
Al	577 ± 11	598 ± 12	96.49
B	30.4 ± 1.9	33.3 ± 0.7	91.29
Ba	51.60 ± 12	Not specified	/
K	2.63 ± 0.17	2.70 ± 0.05	97.41
Mg	1.82 ± 0.10	Not specified	/
Ca	5.13 ± 0.11	5.05 ± 0.09	101.58

An external calibration was also applied and regression and correlation analyses were done as well. The correlation coeficients (*r*) were in the range from 0.9981 – 0.9999. The results of elemental analysis of 8 herbal drugs obtained from plants originating from the family *Lamiacae* are presented in Table IV. In addition to the standard deviation value for a particular elements, descriptive statistics of all the data presented are given. The arithmetic mean and standard deviation of the concentrations of the elements in all the samples were used to describe the trends and variations of the data.

TABLE IV. Contents of the elements in herbal drugs, based on the dry mass (mean ± SD)

| Sample | Content in herbal drugs | | | | | | | | | |
	Cu mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg	K %	Ca %	Mg %	Al mg/kg	Ba mg/kg	B mg/kg
1	6.48	43.0	35	331	2.16	0.90	0.28	198	26.7	55.6
	± 0.31	± 0.7	± 1	± 3	± 0.30	± 0.13	± 0.03	± 21	± 0.,62	± 4.1
2	10.94	25.7	111	405	1.80	1.43	0.53	331	15.53	55.4
	± 0.60	± 0.6	± 2	± 3	± 0.14	± 0.14	± 0.02	± 13	± 1.12	± 1.4

TABLE IV. Continued

Sample	Content in herbal drugs									
	Cu mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg	K %	Ca %	Mg %	Al mg/kg	Ba mg/kg	B mg/kg
3	10.37	21.4	38	285	1.88	1.32	0.67	225	44.62	55.5
	± 0.035	± 0.5	± 1	± 3	± 0.23	± 0.21	± 0.04	± 8	± 1.7	± 18.1
4	5.92	15.0	29	546	2.11	0.95	0.23	360	47.16	47.1
	± 0.35	± 0.8	± 3	± 10	± 0.24	± 0.02	± 0.01	± 7	± 1.81	± 2.2
5	9.14	25.7	25	152	2.45	1.06	0.34	139	67.37	56.5
	± 0.63	± 0.6	± 1	± 13	± 0.14	± 0.04	± 0.02	± 4	± 1.14	± 0.8
6	14.79	24.5	68	438	3.48	1.33	1.09	378	61.05	36.2
	± 0.33	± 1.1	± 7	± 9	± 0.07	± 0.01	± 0.17	± 14	± 4.04	± 13.4
7	8.37	24.4	52	390	6.24	1.10	0.23	344	69.84	40.1
	± 0.32	± 1.0	± 1	± 3	± 0.70	± 0.22	± 0.05	± 8	± 4.10	± 5.5
8	7.27	35.7	32	74	2.38	0.90	0.17	49	16.96	34.7
	± 0.60	± 0.8	± 2	± 4	± 0.10	± 0.17	± 0.02	± 1	± 0.25	± 1.4
Mean	9.16	26.9	97.5	328	2.81	1.12	0.44	253	43.7	47.6
SD	2.88	8.65	28.8	154	1.48	0.21	0.31	119	21.89	9.41
Max	14.79	43.0	111	546	6.24	1.43	0.67	378	69.84	56.5
Min	5.92	15.0	25	74	1.80	0.90	0.17	49	15.53	34.7
Max/min	2.50	.287	4.44	7.38	3.47	11.59	3.94	7.71	4.50	1.63

Number of measurements for each sample, $n = 5$

It can be seen that the spectra of 8 herbal drugs samples (Fig. 1) show ratios of Zn, Mn, Fe, K and Ca which are in good correlation with the results obtained by FAAS and ICP-AES. There is also a significant difference in the profiles of the elements amongst herbal drugs, attributed to the differences in the botanical structure of the leaves, flowers and herbs.[5] The different mobilities of elements within the plant, as well as absorption from the soil and atmosphere can also influence and change the elemental profiles within a plant.[1] Investigations in this direction are ongoing.

CONCLUSION

Determination of 10 elements in 8 herbal drugs of special importance in phytopharmacy was performed using the EDXRF, FAAS/FAES and ICP-AES methods. The complete procedures were validated using a certified reference material (NIST 1573 – Tomato leaves) and good recovery values were obtained in all cases, thus confirming the methodology. A high degree of similarity of the elemental profiles was noticed from the results of the qualitative analysis. Furthermore, there is a good correlation between the EDXRF spectra and the results obtained by flame and plasma atomic spectroscopy techniques, showing certain regularities of the ratios of some of the elements. Generaly, the diversity observed among herbal

drugs originating from plants of the same family are attributed to differences in their botanical structures, element mobility within the plant parts and other internal and extrrual sources.

И З В О Д

НЕОРГАНСКА АНАЛИЗА БИЉНИХ ДРОГА. I ДЕО. ОДРЕЂИВАЊЕ МЕТАЛА У БИЉНИМ ДРОГАМА ДОБИЈЕНИХ ИЗ БИЉАКА ФАМИЛИЈЕ *LAMIACAE*

СЛАВИЦА РАЖИЋ,[1] СВЕТЛАНА ЂОГО,[1] ЛАТИНКА СЛАВКОВИЋ[2] и АЛЕКСАНДАР ПОПОВИЋ[3]

[1]*Инс�_ии�у� за анали�ичку хемију, Фармацеу�ски факул�е�, Универзи�е� у Бео�раду, �. �р. 146, 11211 Бео�рад,* [2]*Инс�и�у� за нуклеарне науке "Винча", �. �р. 522, 11522 Бео�рад и* [3]*Хемијски факул�е�, Универзи�е� у Бео�раду, �. �р. 158, 11000 Бео�рад*

Одређени су профили елемената, изражени као укупан садржај главних компоненти и трагова (Cu, Zn,Mn, Fe, K, Ca, Mg, Al, Ba и B) у 8 биљних дрога, добијених из биљака фамилије *Lamiacae*. У анализи су примењене методе: пламена атомска апсорпциона/емисиона спектроскопија, атомска емисиона спектроскопија са индуктивно спрегнутом плазмом и флуоресцентна рендгенска анализа са дисперзијом енергије. Дискутоване су предности и ограничења примењених метода анализе. Методолошки поступак од припреме узорака, до мерења проверен је и потврђен применом сертификованог стандардног материјала (NIST 1573a – Tomato leaves). Статистичком обрадом резултата добијени су проценти приноса у опсегу 90.64 – 101.58 %. Резултати квалитативне анализе указују на велики степен сличности у саставу неорганских аналита. Квантитативном анализом одређен је садржај 10 елемената и уочена различитост концентрационих профила. Анализиране биљне дроге садрже: Cu (5.92 – 14.79 mg kg^{-1}), Zn (15.0 – 43.0 mg kg^{-1}), Mn (25 – 111 mg kg^{-1}), Fe (74 – 546 mg kg^{-1}), K (1.80 – 6.24 %), Ca (0.90 – 1.43 %), Mg (0.17 – 0.67 %), Al (49 – 378 mg kg^{-1}), Ba (15.53 – 69.84 mg kg^{-1}) and B (34.7 – 56.5 mg kg^{-1}).

(Примљено 2. децембра 2004, ревидирано 9. марта 2005)

REFERENCES

1. Z. En, A. Vasidov, V. Tsipin, T. Tillaev, G. Jumaniyazova, *Nucl. Instrum. Methods Phys. Res. Sect. A* **505** (2003) 462
2. J. Bennett, E. Chriboga, J. Coleman, D. Waller, *Sci. Tot. Envir.* **246** (2000) 261
3. F. Ennever, In *Principles and Methods of Toxicology*, W. A. Hayes, Raven Press, New York, 1994, p. 417
4. D. Schuschke, J. Saari, F. Miller, D. Lominadze, *J. Trace Elem. Exp. Med.* **9** (1996) 63
5. Max Wichtl, *Teedrogen und Phytopharmaka,* Wissenschaftiche Verlag GmbH, Stuttgart, 4 Auflage, 2002, p. 84, 330, 364, 382, 390, 523, 538
6. J. Mesjasz-Przubulowicz, W. J. Przybylowicz, *Nucl. Instrum. Method Phys. Res.* Sect. B **189** (2002) 470
7. J. Shi, Y. Chen, Y. Huang, W. He, *Micron ,* **35(7)** (2004) 557
8. H. Shen, Q. Ren, Y. MI, X. Shi, H. Yao, C. Jin, Y. Huang, W. He, J. Zhang, B. Liu, *Nucl. Instrum. Methods Phys. Res. Sect. B* **189** (2002) 506
9. E. Lemberkovics, E. Czinner, K. Szentmihalyi, A. Balazs, E. Szoke, *Food Chem.* **78** (2002) 119
10. M. Toapsern, M. Lasat, L. Kochian, K. Smolenski, D. Biderback, E. Fontes, K. Finkelstein, *CHESS Newsletter* (2000) 44

11. A. Jacob, in *Textbook of Clinical Chemistry* N. W. Tietz Ed., Counders WB Co., Philadelphia, Pensilvania, 1995, p. 965
12. L. Lajunen in *Spectrochemical Analysis by Atomic Absorption and Emission*, Royal Society of Chemistry, Cambridge, 1992, p. 230
13. C. Vandecasteele, C. Block, *Modern Methods for Trace Elemental Determination,* Wilez and Sons, 1995, p. 118
14. E. Lindgren, in *X-ray Fluorescence Analysis Energy Dispersive in Encyclopedia of Analytical Chemistry*, Ed. R. A. Meyers, John Wilely & Sons Ltd, Chichester, 2000
15. I. Szaloki, J. Osan, R. E. Van Grieken, *Anal. Chem.* **76** (2004) 3445
16. S. Ražić, A. Onjia, B. Potkonjak, *J. Pharm. Biomed. Anal.* **33** (2003) 845
17. S. Ražić, S. Đogo, A. Onjia, L. Slavković, ICOSECS 4, Book of Abstract, Vol. II, B-P 52, Beograd, 2004.
18. S. Ražić, A. Onja, L. Savković, V. Andrić, Proc. IMA 3rd, 385, Thessaloniki, 2003
19. D. Richardson, M. Shore, R. Hertree, R. M. Richardson, *Sci. Tot. Environ.* **176** (1995) 97
20. M. Hoenig, A. de Kersabiec, *Spectrochim. Acta, Part. B* **51** (1996) 1297
21. M. Hoenig, H. Baeten, S. Vanhenetenrijk, E. Vasileva, Ph. Quevauviller, *Anal. Chim. Acta* **358** (1998) 85
22. M. Hoenig, *Talanta* **54** (2001) 1021
23. L. Zaijun Zh. Zhu, T. Jan, Ch. Hsu, P. Jiaomai, *Anal. Chim. Acta* **402** (1999) 253.