

THE CONTENT OF SOME TRACE ELEMENTS IN SELECTED MEDICINAL PLANTS COLLECTED IN THE PROVINCE OF LUBLIN

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Abstract. Nowadays, the increasing tendency to use of herbs and herbal preparation is observed; however, one of the main problem is accumulation of hazardous contaminations in living organisms. In view of these facts, the analysis of toxic components including heavy metals in plants is particular importance. The contents of trace elements: Fe, Zn, Cu, Mn and Ni in medicinal plants collected in the region of Lublin were determined by use of ion chromatography method. The presence of these metals in various amounts was observed in all investigated herbs, however, nickel was found only in *Vitis idaeae folium* (0.0410 mg g⁻¹) and *Polygonii herba* (0.0137 mg g⁻¹). Fe and Mn occurred in the highest amount (1.5378 mg g⁻¹ in *Polygonii herba* and 1.1040 mg g⁻¹ in *Vitis idaeae folium*, respectively). The content of zinc ranged from 0.2541 mg g⁻¹ (*Euphrasiae herba*) to 0.0264 mg g⁻¹ (*Equiseti herba*). The smallest amount of copper was noted in *Urticae folium* (0.0046 mg g⁻¹) and the highest in *Crataegi inflorescentia* (0.0155 mg g⁻¹). The extraction of selected ions to water infusion depending on time and temperature was also determined however, only iron passed into water infusion in significant concentration. The highest percentage of extraction was obtained after 10 min. at 95°C.

Key words: trace elements, herbs, SPE, ion chromatography

INTRODUCTION

Recently, the trend to use of herbs and herbal preparations has increased significantly. According to the World Health Organization above 70% of population use plant or plant-based medicines to treatment of various diseases. Nowadays, one of the main problem in many developing countries is environmental pollution by toxic metals related to industrial activity and the use of chemical fertilizers, pesticides, and herbicides in agriculture. Living organisms have a tendency to accumulate hazardous contaminations [Baker et al 1994, Porębska and Ostrowska 1999, Tyksiński and Kurdubska 2005, Barthwal et al 2008]. The use of plants to removal, reduction and for detoxification of

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heavy metals from soil, water and air (phytoextraction) is regarded as an alternative for remediation of polluted regions [Bosiacki 2009, Lin et al 2010, Bosiacki and Zieleziński 2011]. However, the excessive assimilation of trace elements by plants increases the possibility of including them into human food chain. Bioaccumulation of toxic metals such as Pb, Cd, Hg, As may cause harmful gastrointestinal, neurological, and immunological symptoms, on the other hand, there are some micro-minerals such as Fe, Zn, Cu, Mn essential in low doses for human health [Kabata-Pendias and Pendias 1999]. In view of these facts, according to the WHO recommendations the analysis of both biologically active compounds and toxic components including heavy metals, pesticides, fungi and microorganisms in medicinal plants is particular importance [WHO 2003, Monographs... 2007].

Herbal preparations such as infusions, extracts and syrups, in addition to their therapeutic properties can be a valuable source of microelements. The objective of our research was to examine the potential use of herbs in order to supplement the trace elements deficiency in diet. The analysis was performed with use of ion chromatography (IC) method. IC due to its many advantages such as: possibility of simultaneous determination of several ions in complex matrix and high sensitivity can be an alternative to the most frequently used AAS [Hussain and Khan 2010, Porębska and Ostrowska 1999, Tyksiński and Kurdubska 2005].

MATERIAL AND METHOD

All experiments were carried out in 2010 during spring –autumn season. The plant material were collected from different natural habitats and local herb gardens in the province of Lublin (51°13' N; 22°34' E) and authenticated by Department of Pharmaceutical Botany, Medical University in Lublin. *Crataegus monogyna* Jacq. (flos), *Urtica dioica* L.(folium), *Polygonum hydropiper* L. (herba), *Equisetum arvense* L. (herba), *Euphrasia rostkoviana* Hayne (herba), *Salvia officinalis* L. (folium) were harvested in June, while *Vaccinium vitis idaea* L. (folium), *Linum usitatissimum* L. (semen), at the end of September. The sites of harvest were localized at least 20 km from the city center and at least 1 km from road. Plant material was stored in paper bags and dried at temperature to 30°C in a ventilated place to constant weigh. The dried plants were micronized with use laboratory vibratory mill (TESTCHEM, Pszów, Poland).

Mineralization: 1.0 g of each plant material was placed in a closed polytetrafluoroethylene (PTFE) beaker and mixed with 6 mL of 65% nitric (V) acid and 4 mL of deionized water. The mineralization was conducted with use of microwave-assisted high-pressure digestion system (UniClever BM-1, Plazmotronika, Poznań, Poland) according to program given in Table1. After the removal of nitrogen oxides by drying for 30 min., the residues were diluted in deionized water to 100 mL volume. Before IC analysis the samples were diluted 1:10. The infusions from selected herbs (2 g of dry weight) were prepared at various temperatures (80, 90, 95 and 100°C) and at various time of extraction (2, 4, 6, 8 and 10 min). Each infusion was purified from organic compounds on SPE column with sorbent based on silica gel covered polyaniline film.

Table 1. The conditions of mineralization process with use of microwave-assisted high-pressure digestion system UniClever BM-1

Tabela 1. Warunki procesu mineralizacji przy użyciu systemu UniClever BM-1

Parameter – Parametr	Step I Krok I	Step II Krok II	Step III Krok III
Pressure – Ciśnienie (atm)	17–20	27–30	43–45
Time – Czas (min)	2	3	10
Power of microwave generator Moc generator mikrofalowego (%)	60%	80%	100%

IC analysis: The samples were analyzed using an ion chromatograph DX-500 IC (Dionex, USA) with a spectrophotometric detector. The aqueous mixture of 0.5 mM PAR (4-(2-pyridylazo)resorcinol), 1.0 M 2-dimethylaminoethanol, 0.50 M ammonium hydroxide, 0.3 M sodium bicarbonate was used as the post column derivatisation reagent. As eluent, aqueous solution containing 7.0 mM PDCA (pyridine-2,6-dicarboxylic acid), 66 mM potassium hydroxide, 5.6 mM potassium sulphate, 74 mM formic acid was used. The flow rate was 0.3 mL min⁻¹, the flow-rate of post-column reagent was 0.15 mL min⁻¹. All analyses were made at 30° C. The analytical wavelength was 530 nm.

RESULTS AND DISCUSSION

Trace elements are essential for living organisms, although they become toxic at high concentrations. Thus, both their deficiency and excess can be harmful for human health. The valuable sources of microelements are plants and vegetables. In our experiments, the contents of iron, zinc, manganese, nickel and copper ions in some frequently used herbs were estimated. The presence of these metals was established in all investigated plants, however, nickel was found only in *Vitis idaeae folium* and *Polygonii herba* (0.0410 mg g⁻¹ and 0.0137 mg g⁻¹, respectively). The content of iron ranged from 0.1705 mg g⁻¹ (*Lini Semen*) to 1.5378 mg g⁻¹ (*Polygonii herba*). The highest concentration of manganese (1.1040 mg g⁻¹) was observed in *Vitis idaeae folium*. The amounts of zinc oscillated between 0.2541 mg g⁻¹ (*Euphrasiae herba*) and 0.0264 mg g⁻¹ (*Equiseti herba*). The smallest amount of copper was noted in *Urticae folium* (0.0046 mg g⁻¹) and the highest in *Crataegi inflorescentia* (0.0155 mg g⁻¹). All these values did not exceed Polish standards [Rozp. Min. Zdrowia 2003]. The obtained data shows that the assimilation of trace elements depends not only on the metal content in the soil or a harvest date [Sembratowicz et al 2009] but also on plant species which is consistent with reports in other publications [Porębska and Ostrowska 1999, Barthwal et al 2008]. The difference in contents of heavy metals in investigated plants is presented in Table 2.

Table 2. The content of investigated ions in plant material
Tabela 2. Zawartość badanych jonów w surowcach roślinnych

Plant material Materiał roślinny	The content of element (mg g ⁻¹ of dried plant material) Zawartość pierwiastka (mg g ⁻¹ suchego materiału roślinnego)				
	Fe ³⁺	Mn ²⁺	Zn ²⁺	Ni ²⁺	Cu ²⁺
<i>Vitis idaeae folium</i> Liść borówki brusznicy	0.2107	1.1040	0.0325	0.0410	0.0047
<i>Crataegi inflorescentia</i> Kwiatostan głogu	0.7216	0.0308	0.0318	–	0.0155
<i>Lini Semen</i> Nasiona lnu	0.1705	0.0139	0.0306	–	0.0090
<i>Urticae folium</i> Liść pokrzywy	0.3534	0.0171	0.0866	–	0.0046
<i>Polygonii herba</i> Ziele rdestu	1.5378	0.012	0.1006	0.0137	0.0602
<i>Euphrasiae herba</i> Ziele świetlika	0.2687	0.0288	0.2541	–	0.0077
<i>Equiseti herba</i> Ziele skrzypu	0.5277	0.0268	0.0264	–	0.0115
<i>Salviae officinalis folium</i> Liść szalwi lekarskiej	1.1186	0.2869	0.0509	–	0.0147

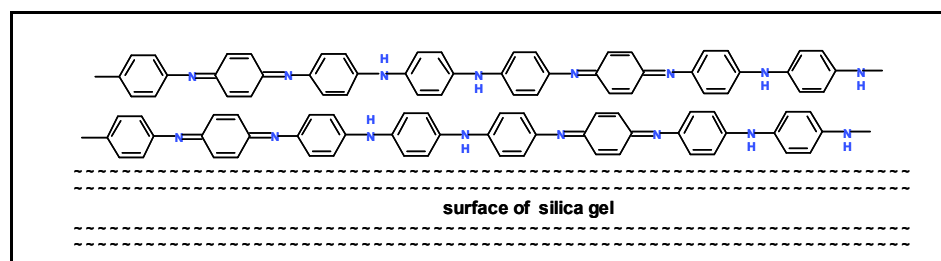


Fig. 1. The structure of silica gel modified with polyaniline
Ryc. 1. Struktura żelu krzemionkowego modyfikowanego polianiliną

Infusions are the most frequently used form of herbal preparations. Water extracts of commercially available herbal mixes were investigated with use of voltamperometry by Suchacz and Wesołowski [2010] however the authors didn't analyze content of trace elements in herbs and didn't show the correlation between concentration of heavy metal ion in dried material and in aqueous extraction. In the second part of our experiments, percentage of extraction of selected ions from *Polygonii herba* was determined depending on time and temperature of infusion. This herb was chosen due to the highest total content of determined microelements. IC technique required removing of organic matrix. Dilution of water samples after mineralization with concentrated acid was too high

thus, the new sorbent based on silica covered polyaniline (fig. 1) was used to purification from organic residues. Due to hydrophobic properties of polyaniline film all macromolecular organic compounds was successfully retained.

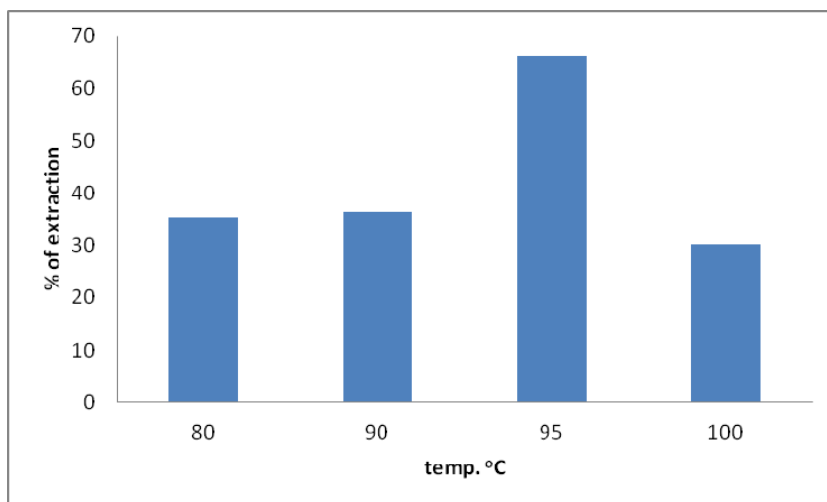


Fig. 2. The percentage of iron extraction in sample depending on temperature of infusion
Ryc. 2. Procent ekstrakcji jonów żelaza w naparze w zależności od temperatury naparzania

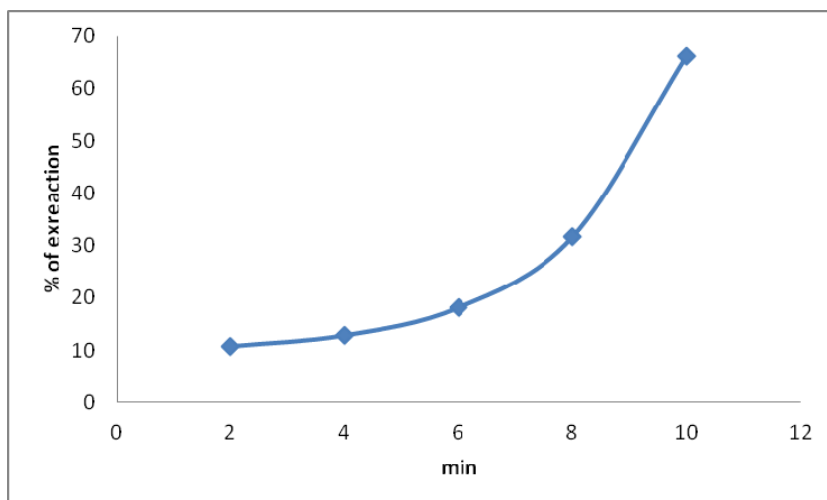


Fig. 3. The percentage of iron extraction in sample depending on time of infusion
Ryc. 3. Procent ekstrakcji jonów żelaza w naparach w zależności od czasu naparzania

Table 3. Validation parameters for determined elements
Tabela 3. Parametry walidacyjne oznaczanych pierwiastków

Elements Pierwiastek	Regression curve Równanie regresji	R ²	LOD (ppm)	LOQ (ppm)	RSD (%) (average)
Fe	$y = -0.000158 + 0.02581x$	0.9973	0.003	0.009	3.98
Cu	$y = -0.000236 + 0.02421x$	0.9783	0.002	0.004	6.55
Ni	$y = 0.00733x - 0.000337x^2$	0.9772	0.003	0.008	4.56
Zn	$y = 0.000559 + 0.02761x - 0.00082x^2$	0.9909	0.009	0.025	6.23
Cd	$y = 0.0004760x - 0.000094x^2$	0.999	0.008	0.023	5.45
Mn	$y = 0.01362x - 0.000073x^2$	0.9957	0.004	0.011	5.01

Our experiments show that only iron passed into water infusions in amount which enable their quantification. The concentration of these ions was different depending on the temperature of water, however, the highest was obtained at temperature of 95°C (fig. 2). The content of iron after 2, 4, 6, 8, 10 and 12 min. was also determined and it was observed nonlinear relationship between time of infusion and amount of extracted ion (fig. 3). The highest amount of iron was found after 10 minutes. The longer time of infusion did not influence the percentage of extraction. The obtained results show that concentration of trace element in infusion is too low to complement deficiency of microelements in human diet. The analysis of certified material of mixed polish herbs (INCT-MPH-2, Institute of Nuclear Chemistry and Technology, Warsaw, Poland) and obtained validation parameters such as correlation coefficients, RSD values, limits detection and quantification (tab. 3) show that ion chromatography method can be successfully employed to separation and determination of heavy metal ions in plants. This method is routine used in environmental analysis of drinking, surface, ground, rain water or wastewater [Michalski and Kurzyca 2006]. Ion chromatography enables simultaneous determination of a few ions in a short time thus the time and costs of analysis are reduced and sample throughput is higher in comparison to frequently used AAS [Jiang et al 2009, Lu et al 2000, Tyrrell et al 2009].

CONCLUSION

The ion chromatography method due to its high sensitivity enables the determination of trace element on ppb level and can be employed as alternative to AAS. Its advantage is the possibility of simultaneous analysis of several ions in a single chromatographic run. The average content of iron, zinc, manganese, nickel and copper ions in all investigated medicinal plants collected from natural habitants of the province of Lublin did not exceed Polish standards. The sorbent based on silica modified with polyaniline can be successfully used to purification of water samples from organic matrix. The efficiency of extraction of ions depends on time and temperature of water infusion, although only

iron ions passed into water infusion in amounts which enable their quantification. Medicinal plants contain essential amount of trace element however their concentration in infusion is too low to complement deficiency of microelements in human diet.

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ZAWARTOŚĆ PIERWIASTKÓW ŚLADOWYCH W WYBRANYCH ROŚLINACH LECZNICZYCH ZBIERANYCH NA TERENIE WOJEWÓDZTWA LUBELSKIEGO

Streszczenie. Obecnie obserwuje się wzrastającą tendencję do stosowania ziół i preparatów ziołowych, jednak jednym z głównych problemów jest kumulacja niebezpiecznych zanieczyszczeń w organizmach żywych. W świetle tych faktów, analiza składników toksycznych, w tym metali ciężkich, w roślinach jest szczególnie ważna. W wybranych roślinach leczniczych pozyskiwanych z regionu Lubelszczyzny oznaczono metodą chromatografii jonowej zawartość pierwiastków śladowych: Fe, Zn, Cu, Mn i Ni. Obecność tych metali zaobserwowano w zróżnicowanych ilościach we wszystkich badanych roślinach z wyjątkiem niklu, który występował tylko w liściu borówki brusznicy ($0,041 \text{ mg g}^{-1}$) oraz w ziele rdestu ostrogorzkiego ($0,0137 \text{ mg g}^{-1}$). W największych ilościach w ziołach występowały żelazo i mangan, odpowiednio $1,5378 \text{ mg g}^{-1}$ w ziele rdestu ostrogorzkiego i $1,1040 \text{ mg g}^{-1}$ w liściu borówki brusznicy. Zawartość cynku wahała się od $0,2541 \text{ mg g}^{-1}$ (ziele świetlika) do $0,0264 \text{ mg g}^{-1}$ (ziele skrzypu polnego). Najmniejsze ilości miedzi zanotowano w liściu pokrzywy ($0,0046 \text{ mg g}^{-1}$), a największe w kwiatostanie głogu ($0,0155 \text{ mg g}^{-1}$). Badano również ekstrakcję wybranych jonów jednakże tylko żelazo przechodziło do naparu wodnego w znaczącym stężeniu. Najwyższy procent ekstrakcji uzyskano w temperaturze 95°C i po 10 minutach zaparzania.

Słowa kluczowe: pierwiastki śladowe, zioła, SPE, chromatografia jonowa

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