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Using microwave mineralization in order to determine heavy metal concentration in samples of herbs used for pharmaceutical purposes

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Abstract. The purpose of the recent study was to determine the heavy metals (Pb, Zn, Cd and Ni) content in samples of medicinal plants collected from Batak Municipality by the application of microwave system Mileston 1200 MEGA for sample preparation. ICP - OES was used for the quantity determination of the heavy metals. Certified Virginia Tobacco CTA-VTL-2 material was applied to test the analyses accuracy. The analytical results obtained displayed that the concentrations of the metals determined were below the permissible levels regulated by the World Health Organization. It was established that due to the considerably reduced acid consumption and analyses time, microwave mineralization could be accepted as the most economically profitable sample preparation method.

Keywords: microwave mineralization, heavy metals, St John's wort, nettle.

Abbreviation: ICP-OES - inductively coupled plasma-optical emission spectrometry, ICP-MS - inductively coupled plasma-mass spectrometry, ETAAS - electrothermal atomic absorption spectrometry, CTA-VTL-2 - certified Virginia tobacco leaves.

Introduction

Medicinal plants and the galecinals derived from them have been widely used as raw materials in the pharmaceutical industry (Eisenberg et al. 1998). Recent years have seen a significant increase in the use of herbs in medicine. Doctors also prescribe herbal teas and herbal extracts as an additional type of treatment in daily practice. Although herbal medicines are often perceived as natural and therefore safe, they are not exempt from adverse effects (Ernst, 2000; Ernst, 2002). Given the complexity of medicinal plants and their inherent biological functions, it is necessary their safety, effectiveness, and quality control to be evaluated (Environment Health Criteria, 1995). Although many studies on the quality of medicinal plants have been reported in contemporary literature (Brantner and Males, 1999), very little research has been done on their heavy metal content. Heavy metals infect plants and cause serious risks to human health, such as kidney failure, symptoms of chronic toxicity, liver damage, etc. (Andrew et al., 2003). According to the World Health Organization (Environment Health Criteria, 1995), heavy metals in the body definitely need to be controlled by medicines for safety reasons. One of the main reasons for monitoring levels of toxic metals in medicinal plants is that environmental pollution in recent years has increased dramatically. The sources of this pollution are quite diverse and range from agricultural to industrial (Gossim et al., 2007). Thus, the monitoring of the content of heavy metals in medicinal plants is a promising tool for their characterization (Brown et al., 1992; Latorre et al., 1999). The monitoring is usually includes a variety of plant species that are able to take in and absorb toxic substances, such as: moss, pine needles, dandelion, nettle, St. John's wort, black poplar and others. (Ingermann et al., 1997; Simon et al., 2004).

Overall, the most widely used techniques for the determination of trace elements in plant samples are ETAAS (Chuang et al., 1999),

ICP-OES (Farias et al., 2002) and ICP-MS (Matsuura et al., 2001). These methods are rapid and sensitive in determining the amount of trace elements in samples. Low levels of certain trace elements are not compatible with conventional ICP-OES detection limits, thus the use of ultrasound nebulizer system is necessary. By ebullizer ultrasound system attached to the ICP-OES, the detection limit can be improved from 5 to 50 times. The purpose of this study is to analyze the content of heavy metals in St John's wort and nettle, using microwave mineralization in the samples in order to good commercial quality of these products (Farias et al., 2002).

Material and methods

Facilities

Measurements for the content of heavy metals in the plant samples was done with a ICP - OES. In sample preparation for analysis a microwave system Mileston 1200 MEGA with a rotor 10 MRD 300 (10 positions) was used. The maximum power was 1000 W, and the maximum pressure in the Teflon pots was 30 bar.

Reagents

Reagents are qualified "AR" (pa Merck i Fluka). The starting standard solutions for ICP determination of Ni, Zn, Pb, Cd with conc. 1000 mg / l were supplied by Merck, Darmstadt Germany. In all procedures doubly distilled water was used.

Samples

In order to check the accuracy of the method a reference material CTA-VTL-2 was used. To assess the accuracy of assignment in conducting the experiment and the accuracy of the results obtained by different methods, three generally accepted criteria were used, as follows:

$D = X - X_{CRM}$, where X is the measured value and X_{CRM} is the certified value. When D is within the borders of $\pm 2\sigma$, where σ is the

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standard deviation from the certified value, the result is considered to be good; when it is $-3\sigma \leq D \leq 3\sigma$ - satisfactory, and beyond these limits the result is unsatisfactory (ISO/DIS 13528, 2002).

$D \% = D / XCRM \cdot 100$ – percentage difference. When the values of $D \%$ are in the limits $\pm 200\sigma / XCRM$ the result is considered to be good, when the value is in the limits $\pm 200\sigma / XCRM$ and $\pm 300\sigma / XCRM$ - satisfactory, and when it is out of the limits $\pm 300\sigma / XCRM$ the result is unsatisfactory.

$Z = X - XCRM / \sigma$. When $Z \leq 2$ the result is considered to be good, when $2 \leq Z \leq 3$ - satisfactory, when $Z > 3$ - unsatisfactory.

In presenting the results in the tables, the good results are marked with 2 stars (**), and the satisfactory with 1 star (*).

For easier evaluation of the effectiveness of the different methods for sample preparation we have used R criterion showing the extent of extraction of the element in percents from the certified value. Samples of St. John's wort and nettles were collected near Tsigov Chark- Batak road (from 100 m to 500 m distance from the road). The samples were prepared for the experiment in a standard way; they were dried at 65° C in a fan oven and stored in dark

polyethylene bottles.

Sample mineralization

0.5 g. of dried and well homogenized sample material is weighed and then moved into Teflon pots and adding to each of them 10 ml. nitric acid. Program mineralization includes 3 stages: (I) 5 min nonpulse irradiation - 250 W; (II) 5 min pulse irradiation - 400 W and (III) 5 min pulse irradiation - 600 W. After ventilation for one minute the sample is cooled, transferred quantitatively and some water is added up to 50 ml.

Results and discussion

Heavy metal content values in Tobacco-CTA-VTA-2 certified reference material are presented in Table 1. The results of the effectiveness of microwave mineralization in the determination of Pb, Zn, Cd and Ni in Virginia Tobacco-CTA-VTA-2 certified reference material are presented in Table 2. From the results presented it can be concluded that the Ni and Cd extraction degree to the certified

Table 1. Heavy metal content in Virginia Tobacco (Nicotiana tabacum)-CTA-VTA-2 certified reference material.

Certifying marerial Tobacco-CTA-VTA-2	Element, $X \pm \sigma$ mg/kg			
	Pb	Cd	Ni	Zn
	22.1 $\pm$ 1.2	1.52 $\pm$ 0.17	198 $\pm$ 0.21	43.3 $\pm$ 2.1

Table 2. Effectiveness of microwave mineralization in the determination of Pb, Zn, Cd and Ni in Virginia Tobacco -CTA-VTA-2 certified reference material

Method	X , mg/kg	σX , mg/kg	D	D, %	Z	R
Ni-Microwave mineralization, HNO3	192.3	11.2	- 5.7	- 2.87	- 0.51**	97.1
Zn-Microwave mineralized, HNO3	36.5	2.7	- 6.8	- 15.7	- 3.24	84.3
Pb-Microwave mineralization. HNO3	18.0	0.7	- 4.1	18.6	- 3.42	81.4
Cd-Microwave mineralization HNO3	1.65	0.1	0.13	8.6	0.76**	108.5

value is good, but that for Pb and Zn is less than satisfactory. Analyses of the results of the laboratories participated in FAPAS (Food Analysis Performance Assessment Scheme) in the period 1991 to 2000 Rose et al. (2001) show that the satisfactory result percentage ranges of 34 to 100 percent depending on the material, method of mineralization and measurement techniques. The comparison of 5 sample decomposition methods shows that the use of closed pressure pots leads to good analytical results. The same is Poykio et al. (2000) conclusion based on comparison of methods for the dissolution of several certified plant multi analysis materials, but according to the author, microwave dissolution combined with ICP - OES and ICP-MS is the fastest method for botanical material analysis. There are also studies concerning heavy metal content in tobacco leaves (Vaisanen et al., 2002).However, only a few of them

concern the techniques for preparing analysis samples (Dospatljev et al., 2007). It can be concluded that the analysis sample dissolution is a critical step in obtaining accurate data on heavy metal quantity . This is particularly important in the study of plant material, because most often they are not sufficiently homogeneous and usually contain variable matrices. The heavy metal content results in St John's wort and nettle are presented in Table 3 and 4. The results establish the following relationship: Samples taken from 000-100 m contain the highest concentration of heavy metals, and sample taken from 300-500 m have the lowest.

The analytical results obtained for heavy metals in plant sample show that they have lower concentrations than those recommended by the World Health Organization.

Table 3. Heavy metal content in St. John's Wort (*Hypericum perforatum* L.).

Herb	Samples, m	Element, $X \pm \sigma$ mg/kg			
		Pb	Cd	Ni	Zn
St John's wort	0-100	49.87 $\pm$ 1.73	2.23 $\pm$ 0.173	0.73 $\pm$ 0.057	3.87 $\pm$ 0.173
	100-200	41.87 $\pm$ 1.73	1.8 $\pm$ 0.104	0.6 $\pm$ 0.17	3.43 $\pm$ 0.173
	200-300	27.17 $\pm$ 4.45	1.17 $\pm$ 0.288	0.33 $\pm$ 0.057	3.03 $\pm$ 0.130
	300-500	19.70 $\pm$ 1.73	0.77 $\pm$ 0.173	0.13 $\pm$ 0.057	2.30 $\pm$ 0.217

Table 4. Heavy metal content in nettle (*Urtica dioica* L.).

Herb	Samples, m	Element, $X \pm \sigma$ mg/kg			
		Pb	Cd	Ni	Zn
Nettle	0-100	134.77 $\pm$ 1.83	2.4 $\pm$ 0.16	0.86 $\pm$ 0.057	6.31 $\pm$ 0.42
	100-200	102.55 $\pm$ 2.72	1.87 $\pm$ 0.173	0.74 $\pm$ 0.173	5.47 $\pm$ 0.54
	200-300	87.19 $\pm$ 3.79	1.50 $\pm$ 0.16	0.55 $\pm$ 0.057	4.50 $\pm$ 0.24
	300-500	52.35 $\pm$ 5.11	1.10 $\pm$ 0.16	0.25 $\pm$ 0.057	3.57 $\pm$ 0.21

Conclusion

Microwave mineralization is the most appropriate sample preparation method for the determination of heavy metals concentrations in a plant material used in the pharmaceutical industry.

Microwave mineralization could be recommended due to the rapidity of samples decomposition and service simplicity.

The application of microwave mineralization could be accepted as the most economically profitable sample preparation method due to the considerably reduced acid consumption and analyses time.

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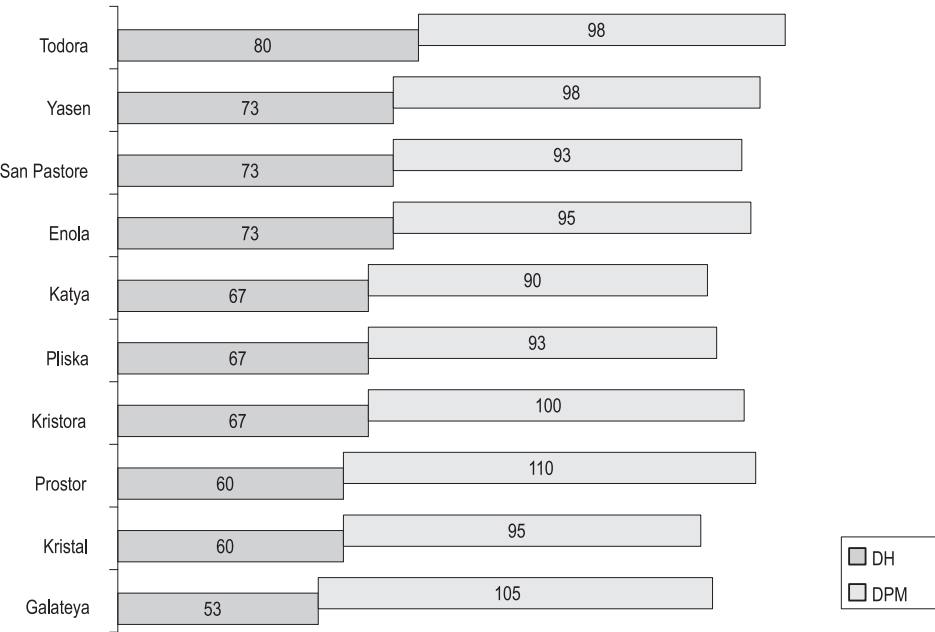
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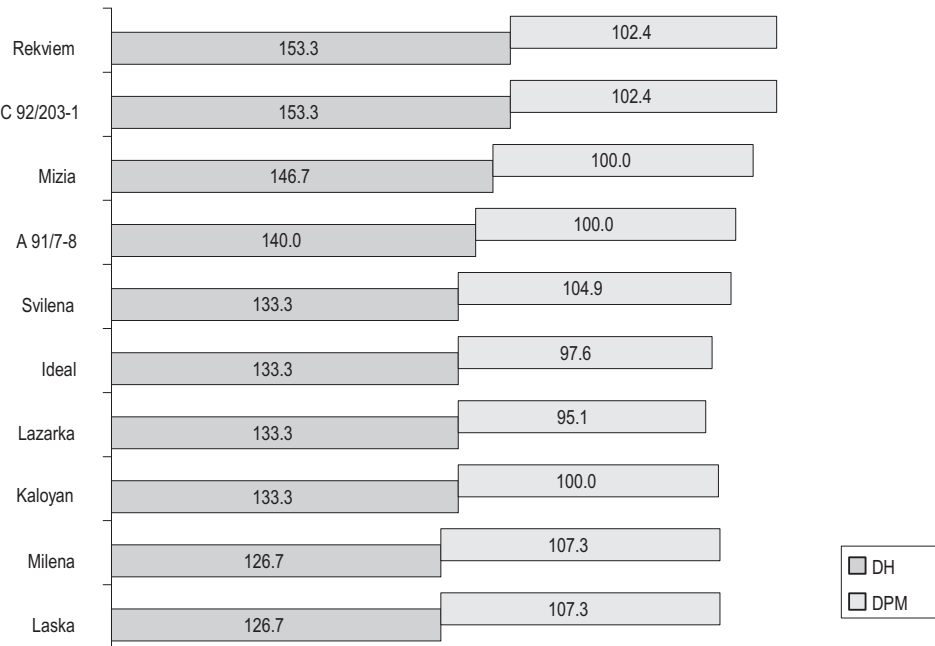


Figure 4. Duration of the period to heading and maturity in the group of late varieties expressed as relative value from the standard

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