Determination of Heavy Metal Contents in Samples of Medicinal Herbs

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Abstract

The heavy metals Cd, Pb, Zn, Ni and Mo were determined in 27 samples of medicinal herbs taken from various places in Bielsko Biata and the neighbouring area. Samples were digested by the wet method in a microwave oven. Flame AAS and differential pulse polarography (anodic and stripping voltammetry) were used for determination of these metals. Oriental tobacco (CTA-ATL-1) as the certified reference material was applied for calibration and validation of analytical procedures.

Keywords: Heavy metals, microwave mineralisation, differential pulse voltammetry, herbs

Introduction

A practical application of living organisms as bio-indicators for monitoring of environmental pollution has been observed for many years in various countries. For permanent observation of diverse kinds of transformations in the environment different vegetable species, able to absorb and cumulate potentially toxic substances are in use. Usually these include moss, lichen, bark, needles of pine or some species of herb. Among medicinal raw materials dandelion *(Taraxacum officincale)* [1,2] and black poplar *(Populus nigra)* [3] are worth mentioning. Using herbs in medical treatment of various illnesses one should be aware that apart from the pharmacological effect they could turn out to be toxic because of the presence of heavy metals like Pb, Cd, Zn, Ni and other impurities. For these reasons it is essential to control the level of contaminants in medicinal raw materials.

Because of the low concentration range, determination of Zn, Cd, Pb, Ni and Mo needs attention during sample preparation, especially mineralisation process, and the determination itself. Mineralisation of samples containing organic matter can be carried out by wet, dry, high-pressure methods as well as in the presence of oxygen [4]. Among many methods described in the literature for trace determination of aforementioned metals in plant material, most often AAS [5, 6] and electro-chemical methods like differential pulse voltammetry (DPASV) [7, 8] are applied.

This work is a continuation of previous research [9, 10] on the determination of trace amounts of heavy metals in plant materials (e.g. moss).

Experimental

Reagents and Solutions

0.1 M ammonium buffer, pH = 9.3; 0.2 M acetate buffer, pH = 4.7 and pH = 3.5; 0.1 M solution of dimethylglyoxime in 96% ethanol; 0.2 M solution of 8-hydroxyquinoline in CCl_4; tin chloride (II) - 10% in HC1; 4% potassium peroxydisulphate(VI). All reagents produced by POCH-Gliwice.

Nitric acid(V) sp.pure, 68% (Zaktady Azotowe, Tarnow-Mościce).

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Working solutions of examined ions (Pb, Zn, Cd, Ni and Mo) were prepared by dilution of standard solutions 1 mg/ml (Backer).

Certified reference material (CRM): oriental tobacco (CTTA-OTL-1, Poland).

Apparatus

Flame AAS:
- AAS-30 Carl Zeiss Jena (Germany) spectrometer with pneumatic nebulisation and mono-element lamps with hollow cathode made by Photron and Narva.

    Differential pulse voltammetry (anodic and adsorptive stripping voltammetry):
- EKO-TRYBO POLAROGRAPH (Praha, R. Czech) with ETP software working in MS-DOS and IM-PmAutoLab system with software GPSE4 (Ecochemie, Praha).

Electrodes: - hanging mercury working microelectrode, silver-silver chloride reference electrode.

Mineralisation:
    Milestone 1200 ML MEGA (Italy) microwave oven.

Calculations:
    Statistica 5.5 PL, StatSoft for Windows 98 software

Collecting and Preparation of Samples

Samples of herbs were collected from various places in Bielsko-Biala and its neighbouring area between April and September. The following herbs were gathered: camomile, mint, briar, dandelion, nettle and St. John's wort.

Samples were collected and prepared for experiments in a standard way described in the literature for these kinds of materials. The specification of collecting stands is depicted in Table 1.

Samples were gathered from different places starting from motorway shoulders, trunk roads to the other roads in mountain regions. One sample of mint was taken from the swamps near a motorway in Slovakia.

Samples of herbs were thoroughly segregated and purified but not washed. Then they were dried on a cotton cloth in a shadow in a warm room. Weighed amounts of dried herbs of about 1.0 g were weighed on the analytical balance with accuracy of 0.0001 g.

Mineralisation of Samples

1 g samples of herbs were placed in teflon vessels suitable for pressure mineralisation in microwave oven. Next 5 ml of conc. HNO_3 sp. pure were added to each vessel. The following program of mineralisation was established: (1) 6 min. 350 W; (2) 1 min. without heating; (3) 6 min. 400W; (4) 1 min. without heating; (5) 7 min. 450 W; (6) 6 min. 650 W; (7) 10 min. ventilation. After mineralisation samples were transferred quantitatively to 10 and 25 ml measuring flasks and filled with water to the mark.

Electrochemical Determination of Zn, Cd, Pb, Ni and Mo by DPASV Method

Anodic and adsorption stripping voltammetry were applied for the determination of trace amounts of metals in examined samples of herbs [10].

Basic electrolyte and solutions of samples were introduced to the polarographic vessel. Before each measurement the sample solution was bubbled with nitrogen for about 10 min. and then blank was recorded. Concentration of analytes in samples was found by three-five times standard addition method. After each addition of standard to a polarographic vessel, the solution was deoxidised for 30 sec.

To the solutions obtained in the mineralisation process acetate buffer of pH = 4.7 was added. For the determination of Zn, Cd and Pb electrolytic concentration at the potential E = -1.10 V was carried out on a hanging mercury electrode against Ag/AgCl electrode during 40 s.

Analysis of nickel was carried out in 0.1 M ammonium buffer medium of pH = 9.3, containing 1% dimethylglyoxime (30 µl) in ethanol and potassium peroxydisulphate (VI). Enrichment was continued for 140 s. In the case of molybdenum 0.2 M hydroxyquinoline (20 µl) and $SnCl_2$ (II) as a reducing agent (10 µl) were applied. Measurements were recorded at a speed of potential change 20 mV/s, impulse height 50 mV, impulse width 100 ms, sensitivity 0.1 nA.

For calibration and validation of analytical procedure CRM - oriental tobacco was applied. Solutions of CRM used in the standard addition method were obtained by successive dilution of stock solution.

Evaluation of obtained results was done by the use of EKO TRYBO POLAROGRAPH software.

Determination of Heavy Metals by FAAS Method

Optimum parameters like absorbance maximum and working stability of apparatus were established separately for each element, based on solutions of known concentration: CRM (Table 2) and model solutions.

Investigations were done in two stages:
1. In the first stage the solutions of CRM - oriental tobacco and model solutions of a wide range of concentrations were applied for calibration of the apparatus.
2. In the second stage the particular metals were determined in examined samples under optimum conditions found for CRM and model solutions.

The basic way to interpret a measured signal is an empirical establishing the dependence between this signal value and concentration of the component. On this basis an analytical curve was prepared in a graphical form or in a form of mathematical dependence, based on 5 solutions: either certified reference materials or model solutions, within the concentration range embracing an amount of a particular element in a tested sample.

Table 1. Specification of collecting stands, kind of herbs gathered for analyses and collecting period.

Sample	Month of collecting	Description of collecting stand	Name of herb
P 1	VI	Urban road	St. John's wort
P 2	VI	Express way	
P 3	VIII	Allotment	
P 4	VIII	Estate access road	
P 5	V	Allotment	mint
P 6	VII	Water dam in Porąbka	
P 7	VIII	Railway station Komorowice	
P 8	VIII	Express way Slovakia	
P 9	VIII	Urban road	
P 10	IX	Beskidy Mountains	
P 11	IV	Beskidy Mountains	dandelion
P 12	IV	Valley of Biała river, Komorowice	
P 13	IV	Railway station Komorowice	
P 14	IV	Express way	
P 15	IV	Beskidy Mountains	nettle
P 16	IV	Railway station Komorowice	
P 17	IV	Express way	
P 18	VIII	Estate access road	
P 19	VI	Urban road	camomila
P 20	VI	Express way	
P 21	VII	Clean area (meadows)	
P 22	VIII	Estate access road	
P 23	VIII	Railway station Komorowice	briar
P 24	VIII	Express way	
P 25	VIII	Urban road	
P 26	IX	Estate access road	
P 27	IX	Beskidy Mountains	

Table 2. Spectral parameters for FAAS determination of metals by the use of AAS-30 spectrometer, worked out based on CRM-oriental tobacco.

Parameter	Pb	Zn	Cd	Ni	Mo*
Wavelength [nm]	283.3	213.9	228.8	232.0	313.3
Lamp feed current [mA]	9	13	11	12	15
Band width [nm]	0.42	0.31	0.38	0.22	0.30
Air flow [dm³/h]	590	620	615	710	
Acetylene flow [dm³/h]	65	65	75	69	150
N_2O flow [dm³/h]	–	–	–		620
Kind of flame	ox.	ox.	ox.	ox.	red.
Height of burner [mm]	12.0	11.0	14.0	13.0	12.0
Integration time [s]	5	6	5	7	5

* - element, for which certified value is not given for CRM.

Table 3. Results of heavy metals determination in samples of herbs by FAAS method,

n = 4, α = 0.05

Herbs	Sample	Element mg/kg x ± μ				
		Pb	Cd	Ni	Zn	Mo
St. John's wort	P 1	8.75 ± 0.65	0.78 ± 0.02	1.29 ± 0.15	41.30 ± 0.95	1.49 ± 0.23
	P 2	21.60 ± 1.60	1.20 ± 0.03	3.55 ± 0.40	94.16 ± 4.66	1.56 ± 0.16
	P 3	8.12 ± 0.44	0.30 ± 0.04	0.95 ± 0.05	32.15 ± 0.80	0.49 ± 0.02
	P 4	15.26 ± 1.85	0.60 ± 0.02	2.38 ± 0.22	38.16 ± 0.96	1.32 ± 0.11
mint	P 5	7.14 ± 0.78	0.28 ± 0.04	0.74 ± 0.08	32.44 ± 1.14	0.55 ± 0.05
	P 6	5.00 ± 0.28	0.24 ± 0.04	0.84 ± 0.06	15.65 ± 0.55	1.01 ± 0.07
	P 7	32.75 ± 1.25	1.03 ± 0.37	3.33 ± 0.31	100.50 ± 8.50	2.42 ± 0.35
	P 8	46.15 ± 2.85	1.28 ± 0.42	4.63 ± 0.53	89.16 ± 4.20	1.01 ± 0.07
	P 9	26.15 ± 1.15	0.97 ± 0.07	4.28 ± 0.42	90.12 ± 5.52	0.99 ± 0.03
	P 10	7.13 ± 0.11	0.20 ± 0.02	1.47 ± 0.15	21.60 ± 1.10	0.21 ± 0.02
dandelion	P 11	4.12 ± 0.12	0.05 ± 0.01	1.29 ± 0.05	19.16 ± 0.46	0.97 ± 0.09
	P 12	6.43 ± 0.17	0.34 ± 0.04	1.51 ± 0.07	28.32 ± 0.46	1.01 ± 0.12
	P 13	73.56 ± 8.72	1.16 ± 0.12	3.81 ± 0.45	232.14 ± 7.12	1.38 ± 0.22
	P 14	51.18 ± 2.14	0.88 ± 0.12	3.52 ± 0.30	273.24 ± 8.14	0.71 ± 0.05
nettle	P 15	7.23 ± 0.13	0.27 ± 0.02	1.49 ± 0.07	43.16 ± 0.96	1.18 ± 0.07
	P 16	39.96 ± 4.46	1.42 ± 0.08	3.69 ± 0.09	120.76 ± 8.46	0.58 ± 0.05
	P 17	51.00 ± 5.50	1.14 ± 0.08	3.34 ± 0.12	285.20 ± 6.10	0.21 ± 0.02
	P 18	32.00 ± 0.50	0.40 ± 0.04	2.56 ± 0.25	92.30 ± 4.25	1.57 ± 0.07
camomile	P 19	16.18 ± 0.14	0.82 ± 0.08	3.63 ± 0.17	72.36 ± 0.82	1.54 ± 0.07
	P 20	66.42 ± 7.82	0.73 ± 0.06	2.47 ± 0.37	212.05 ± 8.45	1.74 ± 0.21
	P 21	8.08 ± 0.11	0.30 ± 0.04	1.17 ± 0.07	38.44 ± 1.22	0.93 ± 0.06
	P 22	26.14 ± 0.44	0.74 ± 0.09	2.41 ± 0.07	115.00 ± 6.00	1.54 ± 0.12
	P 23	40.16 ± 3.86	1.07 ± 0.12	3.65 ± 0.40	169.27 ± 8.13	3.62 ± 0.50
briar	P 24	68.13 ± 8.83	1.00 ± 0.14	2.67 ± 0.17	63.15 ± 1.115	2.55 ± 0.15
	P 25	15.21 ± 0.13	0.75 ± 0.05	2.22 ± 0.12	90.26 ± 1.20	0.95 ± 0.10
	P 26	28.62 ± 0.87	0.82 ± 0.20	2.79 ± 0.37	65.72 ± 0.72	1.63 ± 0.16
	P 27	6.45 ± 0.11	0.22 ± 0.02	1.11 ± 0.10	10.34 ± 0.82	1.23 ± 0.11

Results and Discussion

Biological materials like herbs are highly in-homogeneous. They contain a number of organic substances of different steps of stability and impurities of sparingly soluble mineral components. Incomplete mineralisation of samples during the microwave digestion process may cause difficulty in transferring analytes into solution and, on the other hand, disturb electrochemical measurements.

The aim of these investigations was to assess (validation) worked out procedures and conditions for mineralisation of samples, as well as measuring parameters in SV and FAAS methods, based on CRM CTA-OTL-1 oriental tobacco.

Application of concentrated HNO_3 for mineralisation of herbs leads to the complete digestion of samples, which is proved by determined values of metals concentration, placed within the confidence interval of CRM. Statistical evaluation of results using Student's t-test did not reveal statistical essential differences between the results gained for CRM ($p > 0.005$).

However, various mineralisation procedures are recommended for diverse plant materials in our opinion, based on the obtained results, satisfactory results for examined plants can be obtained by microwave mineralisation only in the presence of HNO_3 at the given above working parameters of the equipment (power, time).

Results of heavy metals determination in herbs (Table 2) revealed expected dependence - lower level of concentration of investigated metals was found in herbs growings in clean regions than in those in polluted areas. A relatively great amount of heavy metals was found in
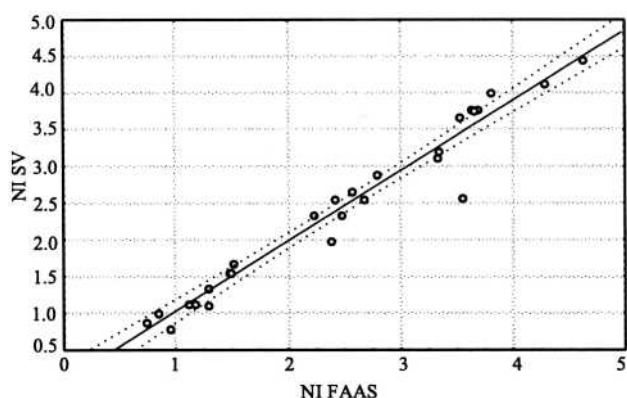
Fig. 1. Linear correlation dependence for Ni determination in herbs by DPASV and FAAS method with the 95% confidence interval (dotted line). R = 0.9770.
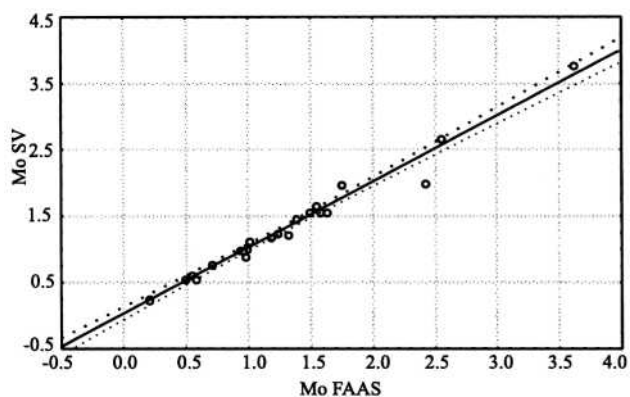

Fig. 2. Linear correlation dependence for Mo determination in herbs by DPASV and FAAS method with the 95% confidence interval (dotted line). R = 0.98770.

herbs living near a railway station (almost equal to those obtained for samples collected near communication routes and motorways). This might be caused by the existence of a factory in this area which buys up heavy metals scrap, as is clearly seen in the case of molybdenum. Its content in samples taken from the railway station is much higher than in samples coming from the vicinity of motorways.

It was found that the percentage of particular elements in samples of herbs emphasises some predisposition of plants to accumulate the same amounts of the particular metal in various circumstances. This concerns mainly lead and zinc.

Using a few kinds of plants it was possible to compare their cumulating properties. All of them react to changes of heavy metals concentration in the environment. (Table 3).

For validation of DPASV analytical procedure and comparison of obtained results with those obtained by FAAS method the certified reference material - oriental tobacco CTA-OTL was analysed. (Table 4). Good

Table 4. Comparison of results obtained by FAAS and DPASV in CTA-OTL-1 oriental tobacco.

Element	FASS $\bar{x} \pm \mu$	DPASV $\bar{x} \pm \mu$
	ppm	
Ni	6.21 ± 0.10	6.14 ± 0.13
Pb	4.92 ± 0.11	5.11 ± 0.12
Zn	48.32 ± 0.61	50.11 ± 0.79
Cd	1.19 ± 0.03	1.20 ± 0.05

accordance of results proves that the worked out procedure is sufficient for this purpose.

The DPASV method was applied for analysis of samples of herbs: camomile, mint, St. John's wort, dandelion, nettle and briar. The results were compared with those obtained by FAAS method. Good linear correlation is illustrated in Figs. 1 and 2. Correlation coefficients for Pb, Cd and Zn equal respectively: R = 0.96451, R = 0.97737 and R = 0.97631.

Summarising the obtained results shows that examined herbs are significantly subjected to contamination of the environment by heavy metals. Awareness of this phenomenon should be disseminated to prevent collecting medicinal herbs near communication routes.

The main aim of this work was also recommending DPASV as an alternative method to the commonly used AAS for controlling herb purity. Obtained results encourage us to recommend working out a procedure to this kind of investigation.

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