Pharmaceutical Chemistry Department[1], National Institute of Public Health, Drug Analysis Department[2], Warsaw Medical University, Warsaw, Poland, and Department of Safety and Radiation Protection[3], Research Center of Juelich, Juelich, Germany

Determination of some micro- and macroelements in preparations made from peppermint and nettle leaves

Z. Fijałek[1,2], K. Sołtyk[1], A. Łozak[1], A. Kominek, P. Ostapczuk[3]

Received January 24, 2003, accepted February 19, 2003

Dr. Peter Ostapczuk, Research Center of Juelich, D-52425 Juelich, Germany
p.ostapczuk@fz-juelich.de

Pharmazie 58: 480–482 (2003)

As, Ca, Cd, Cu, Fe, Mg, Mn, Pb and Zn were determined by ICP-MS and ET-AAS in preparations made from peppermint and nettle leaves after microwave high pressure digestion with nitric acid. In preparation from nettle leaves the Ca content was more than three times higher than in preparations made from peppermint leaves. Only very small differences (less than 10%) were observed for all the other elements. In all the samples investigated the content of inorganic elements were below the WHO limit (where existing). Variation coefficients ranged from 0.68% to 10.5% for ICP-MS measurements and from 1.48% to 10.0% for ET-ASS.

1. Introduction

Herbal products used in therapy should fulfil special requirements. Most of them are given in European Pharmacopoeia or in national norms which regulate macro- and microscopic investigations, identification and determination of compounds with pharmacological action, and purity investigation [1]. The content of main and trace elements in plant preparations depends on the geological situtation, the biological activity of the plant (bioaccumulation) and contamination from anthropogenic sources [2–4].

Great variations in trace element concentrations were observed [5, 6]. For most of the elements no international limits for their content in herbal preparations are existing. Only for arsenic, cadmium and lead the WHO recommends a limit for plant materials (1.0 $\mu g\ g^{-1}$, 0.3 $\mu g\ g^{-1}$ and 10 $\mu g\ g^{-1}$, respectively) [7].

Analytical techniques usually used for trace element determination in plant materials are electro-thermal atomic absorption spectroscopy (ET-AAS) [8–10], inductively coupled plasma/mass spectrometry (ICP-MS) [11, 12], inductively coupled plasma/atomic emission spectrometry (ICP-AES) [13] and electroanalytical techniques [14].

The aim of the work was to determine As, Ca, Cd, Cu, Fe, Mg, Mn, Pb, and Zn in preparations made by different distributors from peppermint and nettle leaves by ICP-MS and ET-AAS after microwave high pressure digestion with nitric acid.

2. Investigations, results and discussion

Table 1 present the equations for the experimental calibration curves obtained by ICP-MS and ET-AAS and the detection limits for all the elements determined. The observed coefficient of variation for ICP-MS measurements ranged between 0.7% and 10.5% and for ET-AAS measurements between 1.5% and 10.0%.

Table 1: Linear calibration curves and determination limits

Element	Method	Linear equation of calibration curve	R	DL ($ng \cdot l^{-1}$)
^{75}As	ICP-MS	$y = -1.16307 \cdot 10^2 + 2.12308 \cdot 10^3 x$	0.9995	27.3
^{114}Cd		$y = 4.28287 \cdot 10^1 + 5.02233 \cdot 10^3 x$	1.0000	6.1
^{65}Cu		$y = -9.2889 \cdot 10^2 + 4.47408 \cdot 10^3 x$	0.9998	12.6
^{24}Mg		$y = -1.16435 \cdot 10^3 + 1.0185 \cdot 10^4 x$	0.9984	97.5
^{55}Mn		$y = 3.04910 \cdot 10^2 + 2.411125 \cdot 10^4 x$	0.9999	2.4
^{208}Pb		$y = -2.61776 \cdot 10^3 + 5.12838 \cdot 10^3 x$	0.9998	33.2
^{66}Zn		$y = -6.897 \cdot 10^2 + 2.97716 \cdot 10^3 x$	0.9998	22.4
Ca	ET-AAS	$y = 2.109 \cdot 10^{-6} x^2 + 3.4 \cdot 10^{-3} x$	0.9997	537
Fe		$y = 8.535 \cdot 10^{-6} x^2 + 3.5 \cdot 10^{-3} x$	0.9998	429

[a] 3 standard deviations of blank
ICP-MS: x – concentration ($\mu g \cdot l^{-1}$); y – cps
GF-AAS: x – concentration ($\mu g \cdot l^{-1}$); y – absorbance

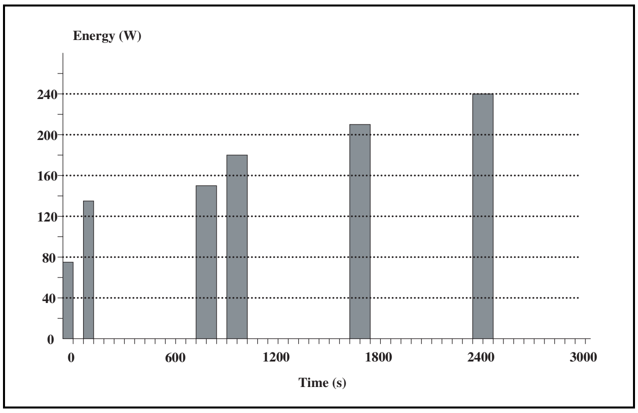

Fig. 1: Energy program for the microwave digestion

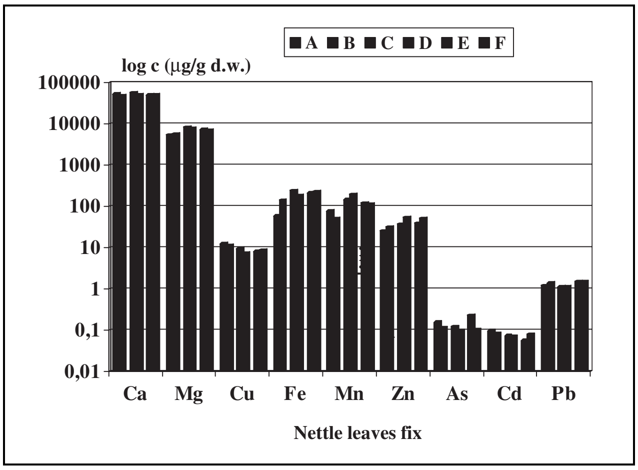

Fig. 2: Element pattern in nettle leaves fix after microwave digestion with nitric acid. Preparation made by Herbapol Lublin: A (batches 1), B (batches 2); Herbapol Pruszków: C (batches 1), D (batches 2); Phytopharm: E (batches 1). F (batches 2)

The determination procedures were validaded using a certified reference material: Oriental Tobacco Leaves (CTA-OTL-1). Table 2 presents the results obtained. A good agreement between found and certified values for 8 elements (Ca value was not certified) indicates that the described procedure can be used for element determinations in plant materials.

Results of the determination of Ca, Mg, Cu, Fe, Mn, Zn, As, Cd and Pb in preparations from nettle leaves and peppermint leaves are presented in Figs. 2.

In the nettle leaves the highest concentrations were found for Ca and Mg (about 80 g/kg and 9 g/kg, respectively). The concentration of Fe, Mn Zn and Cu was found in the range between 10 and 200 mg/kg. The lead concentration was around 1 mg/kg and for Cd and As only values of about 0.1 mg/kg were found. For Ca no significant variation between series or producers was observed. Mg content in nettle leaves samples was constant between the series but in the sample from one producer (Herbapol Lublin, sample A and B) about 10% less Mg was found than in other samples. For all other elements variations between series and producers were observed. This is probably the result of the change of element content in the row material used for production collected in different areas.

For iron and manganese the differences between the maximum and the minimum value were significantly higher than for all other elements (Table 3).

The Ca concentration found in peppermint leaves was about 4 times lower than in nettle leaves. For all other elements similar contents for both plants were found (Fig. 3). In opposite to nettle leaves, in peppermint leaves a variation between series but also between producers were observed for all the elements. Due to the small size of the peppermint plant probably some soil particles are collected with the leaves, which can not be removed from the leave surface by washing. This is a possible explana-

Table 2: Results for certified reference material: CTA-OTL-1 (Oriental Tobacco leaves) (n = 6)[a]

Element	Certified value ($\mu g \cdot g^{-1}$)	Found ($\mu g \cdot g^{-1}$)
As	0.539 ± 0.060	0.581 ± 0.027
Cd	1.12 ± 0.12	1.115 ± 0.015
Cu	14.1 ± 0.5	14.09 ± 0.31
Fe	989[b]	986.2 ± 34.6
Mg	4470 ± 210	4330 ± 114
Mn	412 ± 14	417.6 ± 2.8
Pb	4.91 ± 0.80	4.27 ± 0.04
Zn	49.9 ± 2.4	49.7 ± 1.0

[a] mean of 1 determination from 6 digestion solutions
[b] informative value

Table 4: Temperature program for the Ca and Fe determination by ET-AAS

Step	Process	Ca		Fe		Gas flow ($l \cdot min^{-1}$]
		temp. (°C)	time (s)	temp. (°C)	time (s)	
1	Drying	120	20	120	20	1.5
2	Ashing	700	20	500	20	1.5
3	Atomisation	2400	4	2300	4	0
4	Cleaning	2700	5	2500	5	1.5

Table 3: Minimum and maximum metal content in nettle and peppermint leaves (µg/g d.w.)

Element	Minimum value		Maximum value		Max/Min	
	Nettle	Peppermint	Nettle	Peppermint	Nettle	Peppermint
As	0.094	0.045	0.244	0.126	2.60	2.57
Ca	45413	11724	58954	20236	1.30	1.73
Cd	0.057	0.055	0.101	0.092	1.77	1.67
Cu	7.26	10.2	13.0	13.8	1.79	1.35
Fe	56.2	95.0	266	244	4.72	2.57
Mg	5212	4975	8647	8475	1.66	1.70
Mn	50.1	110	204	237	4.07	2.15
Pb	1.10	0.33	1.75	2.48	1.59	7.54
Zn	24.4	34.3	58.9	54.3	2.41	1.58

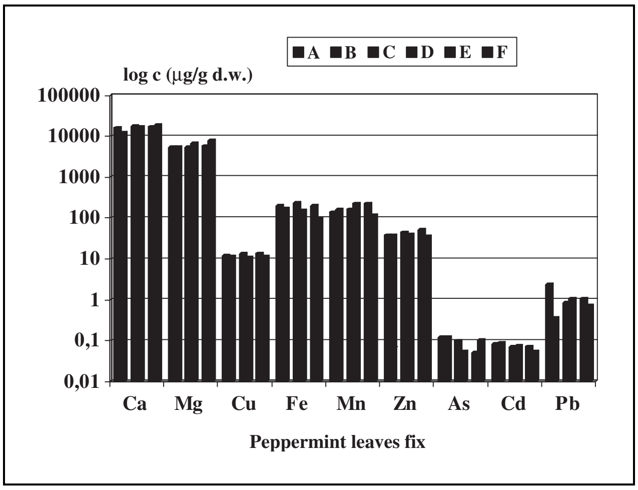

Fig. 3: Element pattern in peppermint leaves fix after microwave digestion with nitric acid. Preparation made by Herbapol Lublin: A (serie 1), B (serie 2); Herbapol Pruszków: C (serie 1), D (serie 2); Phytopharm: E (serie 1). F (serie 2)

tion, why the preparations of peppermint leaves are less homogeneous than the preparations of nettle leaves.

In all analysed samples the content found for each element of interest never cross the WHO limits for As (1 mg/kg), Cd (0.3 mg/kg) or lead (10 mg/g).

The data presented demonstrate that the element content in preparations made from plant materials change with the area in which the raw material was collected. The stability of the content of some elements in the final product can be guaranteed. Information about the element concentrations in plant collected in different areas can be helpful for the production of more homogenous preparations.

3. Experimental

3.1. Equipment

ICP-MS: VG PlasmaQuad 3 (Thermo-Elemental); ET-AAS: AA-660 (Shimadzu) with whole cathode lamp; graphite tube; microwave digestor (Plazmatronika).

3.2. Samples

Nettle leaves: Herbapol Lublin, Poland, series No. 01072000-G and 02082000-G; Herbapol Pruszków, Poland, series No. 020600 14 and 020901 16; Phytopharm series No. 001662 28 2 and 001727 08 2. Peppermint leaves: Herbapol Lublin, series No. 02082000-B and 05072000-B; Herbapol Pruszków, series No. 020500 15 and 030700 14; Phytopharm series No. 001656 25 2 and 001726 08 1.

3.3. Chemicals

Standard solutions of 1 mg ml^{-1} for As, Ca, Cd, Cu, Fe, In, Mg, Mn, Pb, Zn o (Merck); nitric acid (Merck); deionized water (Nanopure, Barnstead); argon 5N.

3.4. Sample preparation

About 1 g of dry plant material was weighted into Teflon vessels (170 ml volume) and after addition of 5 ml of concentrated nitric acid stored at room temperature for 24 h. Microwave digestion was done in 6 steps (Fig. 1) in a closed system with the maximum energy of 240 W. Digested solutions were transfered into a volumetric flask of 100 ml and diluted to the mark with deionized water.

3.5. Element determination by ICP-MS:

Determination parameters: excitation power of plasma 1380 W; flow rate for: plasma (12.5–14.0 l min^{-1}), nebulizer (0.7–0.8 l min^{-1}) and auxiliary (0.7–0.8 l min^{-1}) gases; base line of the background below 10 cps; amount of doubly charged ions 70/140 Ce^{2+}/Ce and 69/138 Ba^{2+}/Ba below 3.0%; amount of oxide ions 156/140 CeO/Ce below 3% and that of 154/138 BaO/Ba below 0.2%; aspiration time of a sample 180 s; measurement time 15 s in threefold repetitions. The concentration range for determined elements was 0.5 to 10 µg/l. As an internal standard in the determinations, indium at a concentration of 10 µg/l was used.

3.6. Ca and Fe determination by ET-AAS

The determinations of Ca and Fe were performed in a pyrolytically coated graphite tube. The background Smith-Hieftje correction was applied at a current intensity of the lamp 12 mA and 480 mA for Ca and 10 mA and 320 mA for Fe. The measurements were performed at a wavelength of 422.7 nm (Ca) and 248.3 nm (Fe), the slit width being 0.25 nm (Table 1).

References

1 Farmakopea Polska Edition V, Ed.: Polskie Towarzystwo Farmaceutyczne
2 Kabata-Pendias, A.; Pendias, H. (Eds.): Biogeochemia pierwiastków śladowych, Wydawnictwo Naukowe PWN, Warszawa 1999
3 Kohlmünzer, S. (Ed.): Farmakognozja,: Wydawnictwo Lekarskie PZWL, Warszawa 2000
4 Wierzchowska-Renke, K.; Kozyra, M.; Głowniak, K.; Zobel, A. M.: Herba Pol. **43**, 404 (1997)
5 Gawęda, M.; Capecka, E.: Herba Pol. **47**, 149 (2001)
6 Mirosławski, J.; Wiechuła, D.; Kwapuliński, J.; Rochel, R.; Loska, K.; Ciba, J.: Bromat. Chem. Toksykol. **4**, 363 (1995)
7 Walas, S.; Borowska, E.; Herda, M.; Herman, M.; Mrowiec, H.: Int. J. Environ. Anal. Chem. **72**, 217 (1998)
8 Sołtyk, K.; Łozak, A.; Fijałek, Z.: Biuletyn Instytutu Leków **3**, 579 (2001)
9 Gajewska, R.; Nabrzyski, M.; Ganowiak, Z.; Cybulski, M.; Kułakowska, D.: Roczn. PZH **51** **(3)**, 251 (2000)
10 Sołtyk, K.; Fijałek, Z.: Chem. Anal. (Warsaw) **45**, 879 (2000)
11 Łozak, A.; Sołtyk, K.; Ostapczuk, P.; Fijałek, Z.: Sci. Total Environ. **289**, 33 (2002)
12 Budic, B.; Klemence, S.: Spectrochim. Acta Part B **6**, 681 (2000)
13 Łukasiak, J.; Denejka, W.; Zobel, A.; Wierzchowska-Renke, K.: Chem. Inż. Ekol. **3**, 41 (1996)
14 Ożarowski, A.; Jaroniewski, W. (Eds): Roliny lecznicze i ich praktyczne zastosowanie, Instytut Wydawniczy Związków Zawodowych, Warszawa 1987