Determination of Chromium, Cooper, Iron, Zinc, Cadmium and Led by Graphite Furnace Atomic Absorption Spectrometry in Seven Phytopharmaceutical Products

LIVIU CHIRIGIU[1]*, RAUL POPESCU[2], MARIA-VIORICA BUBULICA[1], ALEXANDRU POPESCU[3]
[1]Faculty of Pharmacy, University of Medicine and Pharmacy, 2-4 Petru Rares Str., 200638, Craiova, Romania
[2]Faculty of Dental Medicine, University of Medicine and Pharmacy, 2-4 Petru Rares Str., 200638, Craiova, Romania
[3] Faculty of Chemistry, University of Craiova, 107 Calea Bucuresti, 200515, Craiova, Romania

Control of heavy metal content in medicinal and aromatic plants is one of their quality assessment procedures. Depending on growing region, there are large differences in the uptake and concentrations of those in plant tissues. In this research, we determined the concentrations of Zn, Cr, Cd, Pb, Cu and Fe in three medicinal plants: Lonicera tatarica (flowers and fruits), Viburnum opulus (bark and fruits) and Sambucus ebulus (leaves, stems and fruits). The content of the heavy metals can be considered in normal limits, even for Pb^{2+} *and* Cr^{3+}*, markers for soil and air pollution. High levels of* $Fe^{2+/3+}$ *have been determined in all samples. The study has been realized using GF-AAS and microwave digestion.*

Keywords: GF-AAS, medicinal plants, trace elements

The use of medicinal plants for healing is ancient and universal. 61 percent of the 877 new discovered drug molecules between 1981 and 2009 are secondary metabolites [1].

Finding new therapeutic agents derived from plants (especially anticancer drugs) appear to be drawing attention again since an immense development of separation and identification technologies was held [2]. In past few years an increased number of hyphenated techniques are used for this purpose (eg. LC-MS or GC-MS).

Many heavy metals are essential as important constituents of pigments and enzymes, mainly zinc and nickel. However, all metals are toxic at high concentration.

The determination of heavy metals in phytopharmaceutical products is very important and represents one of the factors for the evaluation of their quality, control of heavy metal content in medicinal and aromatic plants beeing one of their quality assessment procedures. Depending on growing region, there are large differences in the uptake and concentrations of those in plant tissues. One of the factors that influence their assimilation is acid or alkaline soil, factor responsible for the mobility and plant availability of heavy metals [3].

High levels of heavy metals can cause morphological cell abnormalities and mutagenic effects in humans [4].

The objective of this study was to determine the levels of six heavy metals from three less known medicinal plants. Of these, four are considered essential elements (copper, zinc, iron and chromium) and two are considered markers for soil and air pollution [5].

The most employed method for determining Cu, Fe, Zn, Cd, Pb and Cr in complex matrices such as foods and plants is GF-AAS because it is a very precise and sensitive method.

Experimental part

Plant material

Samples were collected from Botanical Garden of Craiova City, Dolj County, Romania between May and June 2010. The plants were identified and voucher specimens were deposited in the institution herbarium.

Sample preparation

Sample preparation is critical in obtaining accurate data and reliable interpretation of plant analysis results. The vegetal products should be processed during decontamination, drying, particle-size reduction, storage and organic matter destruction. Plant material must be cleaned and free of extraneous substances, including soil and dust particles that may influence analytical results [6].

Decontamination procedures involving washing and rinsing with deionized water and 0.2% detergent solution (non-phosphate), should only be used for fresh, fully turgid plant samples. After decontamination, water is removed from plant tissue, at temperatures under 60°C, to stop the enzymatic reactions and to stabilize the samples.

Plant tissue samples are reduced to 0.5 to 1.0 mm particle size to ensure homogeneity and to facilitate organic matter destruction [7].

250 mg of each sample were digested by adding 5,5 mL suprapure HNO_3 65 % and microwaved in a MWS 2 - Berghof microwave using a program in three stages: T_1 = 160°C, t_1 = 15 min, p_1 = 80 %, T_2 = 210°C, t_2 = 15 min, P_2 = 90 % and decreasing T_3, t_3 = 15 min, p_3 = 0 %. Under the same conditions was prepared a blank using the same operating mode and the same reagents but replacing the sample with deionized water.

The solutions were transferred quantitatively to measuring flasks and filled up to 100 mL with deionized water.

Calibration

A novAA 400G – Analytik Jena atomic absorption spectrometer with heated graphite furnace equipped with autosampler MPE60 and software Win AAS 3.17.0 was employed. Working solutions (with concentrations ranging between 0 – 100 μL) of examined ions were prepared by dilution of standard certified solutions (1000 mg/L).

---

* *email: liviu_chirigiu@yahoo.com, Tel: 0040251533713*

Table 1
OPERATING PARAMETERS FOR GF-AAS DETERMINATION OF METALS

Step	Temperature (° C)	Ramp (s)	Hold (s)	Gas flow (ml/min)
Chromium (λ = 357,9 nm, δ = 0,8 nm, i = 25 mA)				
1.	120	10	15	300
2.	150	15	15	300
3.	1000	5	15	300
4.	1400	0	5	0
5.	2100	1	3	300
Lead (λ = 283,3, δ = 0,2 nm, i = 25 mA)				
1.	110	10	25	250
2.	140	15	15	250
3.	800	10	20	250
4.	1700	0	5	0
5.	2500	3	4	250
Copper (λ = 327,4 nm, δ = 0,5 nm, i = 5 mA)				
1.	110	25	15	250
2.	140	10	15	250
3.	900	1	15	250
4.	2100	0	5	0
5.	2500	1	3	250
Cadmium (λ = 228,8 nm, δ = 0,5 nm, i = 10 mA)				
1.	110	20	20	250
2.	140	10	15	250
3.	900	1	15	250
4.	2200	0	5	0
5.	2500	1	3	250
Zinc (λ = 213,9 nm, δ = 0,8 nm, i = 25 mA)				
1.	110	10	10	250
2.	140	10	10	250
3.	700	20	15	250
4.	2000	1	5	0
5.	2500	1	3	250
Iron (λ = 302,1 nm, δ = 0,8 nm, i = 12 mA)				
1.	110	30	15	250
2.	140	10	15	250
3.	1000	5	15	250
4.	2600	0	5	0
5.	2800	2	3	250

Table 2
PERFORMANCE PARAMETERS OBTAINED BY GF-AAS

Performance parameters	Cd	Pb	Zn	Cu	Cr	Fe
R^2	0,9989	0,9949	0,9193	0,9987	0,9981	0,9842
Slope	0,0954	0,0027	0,0335	0,0108	0,0209	1,0217
Detection limit (LOD)	0.0789^a	0.1197^a	0.3220^a	0.0892^a	0.0829^a	0.2416^b
Quantification limit (LOQ)	0.2391^a	0.3628^a	0.9759^a	0.2703^a	0.2513^a	0.0732^b

^{a}µg/mL; ^{b}mg/mL

Working parameters were optimized, calibration standards concentrations were established, an amount of matrix modifier was added ($NH_4H_2PO_4$ and $Mg(NO_3)_2$) in all samples (5 µL).

Working parameters established for each element are given in table 1.

Results and discussions

Plants are bio-indicators of environmental pollution, and for medicinal plants, a close monitoring is needed since certain species accumulate remarkable amounts of these metals. It is known that approximately half of the amount of Pb and Zn ingested by humans comes from foods of vegetal origin [8].

For medicinal plant with internal use in powder form or extract is recommended to avoid collection in areas affected by pollution. The only sure way of prevention of large accumulations of heavy metals in the body is the quality control of raw materials.

Microwave digestion

Since in vegetal samples heavy metals are found in amounts of microgram order, a special attention must be paid to the process of mineralization, whereas incomplete digestion leads to incomplete transfer of analytes into sample solution.

A new procedure of mineralization of samples was established, different that those existing in the literature [9 – 12], where samples are generally calcined, and then the ash is digested with nitric acid, hydrogen peroxide, hydrochloric acid, or a mixure of them. Thus, samples were subject to digestion with concentrated nitric acid before placing them in the oven.

Performance parameters

Performance parameters obtained by GF-AAS are given in table 2. The detection limit was establised from the calibration curve as 3.3 (SD/S) and the quantitation limit was calculated as 10 (SD/S), where SD is standard deviation and S is the slope. Since measurements were made in triplicate, the average value of the three determinations was approximated as the true one, calculating for each data set standard deviation and relative standard deviation. Accepted criterion for % RSD in the study of method precision was that RSD < 5 %.

Quantitative determinations

Maximum limits allowed for heavy metals in medicinal plants are: 10 mg % Zn^{2+}, 100 mg % $Fe^{2+/3+}$, 50 mg % Pb^{2+} şi Cr^{3+}, 10 mg % Cu^{2+} and Cd^{2+} [13].

All samples contain Pb^{2+} and Cr^{3+}, markers for soil and air pollution (table 3). The levels of Pb^{2+} (normal limits) in

Table 3
HEAVY METAL CONTENT IN *VIBURNUM OPULUS*, *LONICERA TATARICA* AND *SAMBUCUS EBULUS*

No.	Sample	Concentration (µg/g)					
		Cd	Cu	Cr	Zn	Pb	Fe
1.	*Viburnum opulus* fruits	*	1.47 ±0.06	10.08 ±0.34	26.07 ± 0.84	4.19 ±0.08	97.08 ± 0.26
2.	*Viburnum opulus* bark	*	6.23 ±0.04	12.91 ±0.23	28.61 ±1.16	6.28 ±0.05	64.92 ± 0. 72
3.	*Sambucus ebulus* fruits	*	2.34 ±0.09	9.85 ±0.29	45.92 ±0.93	9.21 ±0.07	113.40 ± 0.35
4.	*Sambucus ebulus* leaves	0.32 ±0.11	5.16 ±0.70	10.27 ±0.14	36.58 ±1.06	12.63 ±0.14	99.29 ±0.24
5.	*Sambucus ebulus* stems	*	2.16 ±0.34	*	16.58 ±0.96	*	*
6.	*Lonicera tatarica* fruits	0.75 ±0.04	1.42 ±0.26	7.13 ±0.48	51.15 ±0.70	5.46 ±0.12	66.31 ± 0.22
7.	*Lonicera tatarica* flowers	0.60 ±0.18	1.60 ±0.39	8.54 ±0.15	43.22 ±1.02	7.20 ±0.09	60.15 ±0.19

values are given as mean ± SD (n=3).

* below the detection limit;

vegetal tissues samples appear because the plants collected were relatively closely to roads (cars circulation) and where reach from fuels into the atmosphere [14] tetra alkyl derivates of this element which are photocatalytic decomposed into mono, di or tri alkyl derivates. *Sambucus ebulus* accumulate significant amounts of Fe$^{2+/3+}$ and Zn^{2+}.

However of the six determined metals, in *Sambucus ebulus* stems have been found only two, this fact indicating that this vegetative organ serves as transporter rather than storing them. Cadmium instead was found only in three of the six samples but in extremely low concentrations, below the maximum allowed limit.

Conclusions

Flame-AAS has some limitations (high sample volume, interferences from solute vaporisation, etc) while GF-AAS has sensitivity, precision and accuracy.

Because plants are highly inhomogeneous, complete mineralization during microwave digestion is needed. Adding concentrate HNO$_3$ before microwaving leads to complete transfer of analytes into solution.

The results of this study indicate that all metals monitored by atomic absorption spectrometry are within normal limits in the studied products and these can be used in pharmaceutical purposes.

References

1. SARKER S.D., NAHAR L., Chemistry for pharmacists. General, organic and natural product chemistry, Wiley and Sons, England, 2007, p.284-286.
2. CIULEI, I., ISTUDOR, V., PALADE, M., ALBULESCU, D., GÂRD, C., Analiza farmacognostică şi fitochimică a produselor vegetale, vol 1, Ed. Tehnoplast, Bucureşti, 1995, p. 409.
3. MINDAK, W. R., CHENG, J., Elemental Analysis Manual: Section 4.3B Graphite Furnace Atomic Absorption Spectrometric Determination of Cadmium and Lead in Food Using Microwave Assisted Digestion, Food and Drug Administration – FDA, 1882.
4. IONCICA R., BUBULICA M.V., CHIRIGIU L., MOGOSANU G. D., POPESCU R., POPESCU H., C.H.S.J., **36**, nr.1, 2010, p.51–54.
5. HALIK, J., SZAKOVA, J., DRABEK, O., BALIK, J., KOKOSKA, L., Food Chem., **111**, 2008, p. 520.
6. DOBRINAŞ, S., SOCEANU, A.D., STANCIU, G., BRATU, S., Ovidius University Annals of chemistry, **22**, nr. 1, 2011, p.37.
7. POPA, T., BUBULICA, M.V., CHIRIGIU L., MOGOSANU, G. D., POPESCU, R., POPESCU H., C.H.S.J., **36,** nr. 2, 2010, p. 111-114.
8. ***: Farmacopeea Română ediţia a X-a, Ed. Medicală, Bucureşti, 1993, p. 1051.
9. DUDKA, S., MILLER, W.P., J. Environ. Sci. Health B, **34**, no.4, 1999, p.681-708.
10. BAKIRDERE, S., YAMAN M., Environ. Monit. Assess., **136**, 2008, p.401–410.
11. UNTEA, A., CRISTE, R.D., VLADESCU, L., Rev. Chim.(Bucharest), **63,** no. 4, 2012, p. 341
12. MARIA, M.F., HOSSU, A.M., IOVI, A., NEGREA, P., STOICA, A., Rev. Chim.(Bucharest), **61**, no. 9, 2010, p.822
13. KALRA P.Y., Handbook of reference methods for plant analysis, Soil and Plant analysis Council, CRC Press, Boca Raton–Washington, 1998, p.56.
14.POP, A., TOADER, M., PARASCHIV, T., The first International Proficiency Testing Conference, Sinaia, Romania, 11-13 octombrie, 2007, p. 386-392.

Manuscript received: 28.05.2012