TECHNICAL COMMUNICATIONS

Determination of Metal Content in Valerian Root Phytopharmaceutical Derivatives by Atomic Spectrometry

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Phytopharmaceuticals containing Valerian are used as mild sleep-inducing agents. The elemental composition of 3 different marks of *Valeriana officinalis* roots commercially available in the Argentinian market, their teas, and a commercial tincture have been studied. The content of Al, Ca, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, V, and Zn was determined in phytopharmaceuticals by flame atomic emission/absorption spectrometry, electrothermal atomic absorption spectrometry, and ultrasonic nebulization coupled to inductively coupled plasma-optical emission spectrometry. Prior to analyses of the samples, a digestion procedure was optimized. The analytical results obtained for Fe, Al, Ca, and V in the solid sample study were within the range 100–1000 mg/kg, and for Mn, Zn, and Pb within the range 10–100 mg/kg. Cadmium was found at levels up to 0.0125 mg/kg.

Valerian (*Valeriana officinalis*) is an herb that has long been advocated for promoting sleep (1). Valerian root derivatives are widely consumed as home remedies and raw material for the pharmaceutical industries. There are different ways by which countries define medicinal plants, herbs, or products derived from them. In Argentina, herbal medicines, including vegetable drugs, their mixtures, and their preparations, are considered to be medicinal products.

Trace elements have both a curative and a preventive role in combating diseases. It is, therefore, of major interest to establish the levels of some metallic elements in common herbal plants because, at elevated levels, these metals can also be toxic (2, 3).

The World Health Organization (WHO; 4), in a number of resolutions, has emphasized the need to ensure the quality control of plant products by using modern techniques and applying suitable standards. The analytical determination of metals in medicinal plants is part of quality control in order to establish their purity, safety, and efficacy. Among different target analytes for quality control of herbal drugs, heavy metals play an important role. Recently, a monograph in the *European Pharmacopoeia* (5) concerning the analysis of heavy metals in herbal drugs and fatty oils by absorption spectrometry has been reported.

Many studies have documented the health problems associated with heavy metal exposure (6–8). Chronic exposure to heavy metals such as Cu, Pb, and Zn is associated with Parkinson's disease, and the metals might act alone or together over time to cause the disease (9).

The most widely used techniques for trace determination are, among others, electrothermal atomic absorption spectrometry (ETAAS; 10), and inductively coupled plasma-optical emission spectrometry (ICP-OES; 11, 12) and -mass spectrometry (ICP-MS; 13, 14). These methodologies are suitable for the determination of trace amounts of metals in different matrixes.

The objective of this study was to quantify the content of toxic metals in valerian phytopharmaceutical derivatives. Furthermore, the quantification of various elements that might be responsible for some properties of *Valeriana*, such as its sedative and hypnotic activities, was achieved. Investigated elements were chosen (Ca, Li, Zn, Cu, Fe, Mn, Ni, Co, Cd, Pb, Al, Cr, and V) according to their role and importance in many biological systems.

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Experimental

Reagents and Chemicals

The water used in all studies was ultrapure (18 MW cm), obtained from a Barnstead Easy Pure RF compact ultrapure water system (Newton, MA). HNO_3, $HClO_4$, and HF were of ultrapure quality (Merck, Buenos Aires, Argentina). Metal standard solutions were prepared by appropriate dilutions of 1000 mg/L stock solutions immediately before use. Matrix modifier [$(NH_4)H_2PO_4$] was supplied by Merck.

Instrumentation

The AAS measurements were performed with a Shimadzu Model AA-6800 atomic absorption spectrometer (Tokyo, Japan) equipped with a deuterium background corrector, and the measurements were based on peak height. For Li (FAES), the wavelength used was 670.8 nm, the slit width was 0.2 nm, the fuel gas flow rate was 1.8 L/min (air–C_2H_2), and the sample flow rate was 3 mL/min. For the Ca (FAAS), the wavelength used was 422.7 nm, the slit width was 0.5 nm, the fuel gas flow rate was 2.0 L/min (air–C_2H_2), and the sample flow rate was 3 mL/min. The graphite furnace and the atomization used were pyrolytic and from the wall, respectively. Metal hollow-cathode lamps (Hamamatsu Photonics K.K., Hamamatsu City, Japan) were employed as radiation sources; wavelengths used were 228.8, 240.7, 232, and 283.3 nm for Cd, Co, Ni, and Pb, respectively. The temperature program was the following: drying 120°, 150°C, pyrolysis 500°C, atomization 2500°C for Cd, and drying 120°, 250°C, pyrolysis 400°C, atomization 2500°C for Co. The temperature program for Ni was drying 120°, 250°C, pyrolysis 800°C, atomization 2500°C, and for Pb was drying 120°, 150°C, pyrolysis 800°C, atomization 2400°C.

The ICP-OES measurements were performed with a sequential ICP spectrometer (ICP 2070, Baird, Bedford, MA). The 1 m Czerny-Turner monochromator is based on a holographic grating with 1800 grooves/mm. Tygon pump tubing (Ismatec, Cole-Parmer, Vernon Hills, IL) was employed to carry the sample.

Nebulization was performed with an ultrasonic nebulizer with a desolvation system (U-5000 AT, CETAC Technologies, Omaha, NE).

Table 1. ICP-OES instrumental parameters employed for Al, Cr, Co, Fe, Zn, Mn, and V determination

RF generator	1.0 kW
Forward power	40.68 MHz
Nebulizer	Pneumatic[1] and ultrasonic[2]
Plasma gas flow rate	8.5 L/min
Auxiliary gas flow rate	1.0 L/min
Sample gas flow rate	0.5 L/min
Solution uptake rate	1.5 mL/min
Observation height	15 mm
Analytical wavelengths, nm	
Mn[a]	257.610
Zn[a]	213.856
V[a]	309.311
Al[b]	308.215
Cr[b]	267.716
Co[b]	228.616
Cu[b]	324.754
Fe[b]	240.488

[a,b] Nebulizer type used for each element is designated.

Phytopharmaceutical Samples

Samples analyzed consisted of both solid (dried herbs of different marks) and liquid (tea, tincture) formulations. Three different commercial packed samples of *Valeriana officinalis* dried herbs, and their water extracts (teas), and a commercial tincture were investigated. The herbal samples were collected randomly from the Argentinian market and were slightly washed with doubly distilled water in order to remove soil. The commercial tincture was purchased from a local pharmacy (San Luis, Argentina).

Sample Preparation and Analysis

Herbal medicines mainly contain organic materials and require a large amount of HNO_3 to be digested, and this reaction is difficult to control. A digestion method with an acid mixture (including HNO_3, $HClO_4$, and HF) was used to destroy the organic material. The digestion was performed in a Teflon vessel on 2.0 g sample by treatment with 10 mL HNO_3. The solution was evaporated to dryness. Then, the solid samples (dried powdered valerian roots) were treated by strong oxidation with $HClO_4$. Subsequently, the silica salts, which could occlude the analytes, were digested with HF until white fumes were observed. The residue was diluted to 50 mL with ultrapure water in a plastic volumetric flask and filtered. The tablet samples were digested with 5 mL HNO_3 and were diluted to 50 mL with ultrapure water in a plastic volumetric flask.

Dilutions of commercial liquid formulations were prepared as follows: 10 mL valerian tincture was carefully measured into a volumetric flask and diluted to 100 mL with ultrapure water. The procedure adopted for tea sample preparation was as follows: 200 mL boiling ultrapure water was poured onto 5 g dried preparation, covered, left to infuse for 30 min, filtered, the moisture squeezed out, and the volume made up to 200 mL.

Results and Discussion

Determination of Metal Content

AAS is the most common analytical method adopted for measuring trace metals in biological materials (15). However, conventional flame techniques show low sensitivity for the determination of trace amounts of heavy metals in

Table 2. Comparison among reported data from literature and found values in *V. officinalis*

Medicinal plants	Cu, mg/kg	Mn, mg/kg	Fe, mg/kg	Zn, mg/kg	Ni, mg/kg	Cd, mg/kg	Pb, mg/kg
Melissa officinalis (20)	10.6 ± 3.3	36.1 ± 18.1	282 ± 174	33.7 ± 11.1	3.4 ± 5.3	0.022 ± 0.012	1.3 ± 1.5
Mentha piperita (20)	11.5 ± 2.4	49.0 ± 23.0	264 ± 151	31.0 ± 14.7	3.9 ± 4.6	0.028 ± 0.014	0.7 ± 0.4
Salvia officinalis (20)	11.9 ± 6.5	57.0 ± 34.4	446 ± 477	45.5 ± 21.8	3.8 ± 4.1	0.061 ± 0.054	2.1 ± 2.4
Hypericum perforatum (20)	11.3 ± 3.1	45.6 ± 41.1	85.9 ± 53.7	37.9 ± 8.9	3.2 ± 4.4	0.179 ± 0.158	0.3 ± 0.2
Papaver somniferum (20)	18.0 ± 3.3	60.3 ± 23.1	70.3 ± 21.6	65.5 ± 11.2	0.8 ± 0.4	0.230 ± 0.210	0.1 ± 0.1
Valeriana officinalis	4.97 ± 0.15	83.47 ± 2.5	2293 ± 45.86	27.3 ± 0.82	4.5 ± 0.18	0.0125 ± 0.0006	17.04 ± 0.68

phytomedicines. Therefore, the ETAAS technique was selected for Cd, Co, Ni, and Pb determination at trace levels.

For Cd, Ni, and Pb determination, $(NH_4)H_2PO_4$ was the matrix modifier used at a concentration level of 0.05 mg/mL (5 mL, 1% w/v). In the case of Co, the use of the matrix modifier was not necessary, in agreement to the previously reported approach by Carlosena et al. (16). However, Al, Cr, Fe, and V were determined by ICP-OES coupled with an ultrasonic nebulization system (USN-ICP-OES). The operating conditions are summarized in Table 1.

Table 2 shows some metal concentrations in plant material previously reported in the literature. No significant differences were observed for the metals under study between the previously reported values and the Valerian-based samples.

Our study was performed on 3 different commercial marks, and similar results were obtained for all the samples. All the measurements were corrected against a reagent blank. The metal content found in one of the samples under investigation is shown in Table 3. Also, Ca, Li, Ni, Cd, and Pb were investigated in a sample of Valerian-based tincture. The concentrations of Li, Ni, and Cd were 0.01 mg/L, 0.01 mg/L, and 0.368 μg/L respectively. Ca and Pb were not found in the tincture sample. Although in our country medicinal herbs are considered pharmaceutical products and are consequently

Table 3. Element concentrations of *Valeriana officinalis*-based phytopharmaceuticals

	Contents		
Elements	Element concentration in dried herb, mg/kg	Soluble element concentration in hot water, tea, mg/kg	Element concentration in tea[a], μg/200 mL
Li[b]	2.30 ± 0.07	0.05 ± 0.002	0.25
Ca[c]	1223 ± 15.70	292.10 ± 8.76	1460.50
Cu[d]	4.97 ± 0.15	1.32 ± 0.04	6.60
Mn[d]	83.47 ± 2.50	49.10 ± 1.47	245.50
V[d]	227.25 ± 6.82	1.90 ± 0.06	9.50
Zn[d]	27.30 ± 0.82	8.80 ± 0.26	44
Al[e]	1240 ± 10.20	112.50 ± 4.50	562.50
Co[e]	1.66 ± 0.07	ND[f]	ND
Cr[e]	5.40 ± 0.22	ND	ND
Fe[e]	2293 ± 18.86	74.60 ± 2.24	373
Ni[e]	4.55 ± 0.18	0.98 ± 0.05	4.90
Cd[g]	0.0125 ± 0.0006	0.0036 ± 0.0002	0.018
Pb[g]	17.04 ± 0.68	0.37 ± 0.02	1.85

[a] Values were calculated from the amount of sample and procedure described.
[b] Flame atomic emission spectrometry (FAES).
[c] Flame atomic absorption spectrometry (FAAS).
[d] Inductively coupled plasma-optical emission spectrometry (ICP-OES).
[e] Ultrasonic nebulizer-inductively coupled plasma-optical emission spectrometry (USN-ICP-OES).
[f] ND = Not determined.
[g] Electrothermal atomic absorption spectrometry (ETAAS).

Table 4. Results of validation recovery test

Solid sample	Base value, mg/kg	Concentration added, mg/kg	Concentration found[a], mg/kg	Recovery, %[b]
Li[c]	2.33	5	7.325	99.90
Ca[d]	1223	100	1322.8	99.80
Cu[e]	4.97	5	9.96	99.80
Mn[e]	83.47	10	93.44	99.70
V[e]	227.25	100	327.35	100.10
Zn[e]	27.0	10	36.85	98.50
Al[f]	1240	100	1339.5	99.50
Co[f]	1.66	1	2.65	99.00
Cr[f]	5.40	1	6.388	98.80
Fe[f]	2293	100	2392.7	99.70
Ni[f]	4.55	1	5.54	99.00
Cd[g]	0.0125	0.05	0.0626	100.20
Pb[g]	17.04	10	27.02	99.80

[a] Mean value (n = 6).
[b] 100 × [(Concentration found – base value)/concentration added].
[c] Flame atomic emission spectrometry (FAES).
[d] Flame atomic absorption spectrometry (FAAS).
[e] Inductively coupled plasma-optical emission spectrometry (ICP-OES).
[f] Ultrasonic nebulizer-inductively coupled plasma-optical emission spectrometry (USN-ICP-OES).
[g] Electrothermal atomic absorption spectrometry (ETAAS).

controlled by the same regulatory authorities, the total elemental content is not regulated (17).

Method Validation Recovery Test

The proposed method of digestion following FAES, FAAS, ETAAS, and USN-ICP-OES determination was applied to the quantification of trace amounts of heavy metals in *Valeriana officinalis* derivative samples.

The method of standard addition is considered as a validation method (18, 19). In order to demonstrate the validity of our method, we performed a recovery study. A synthetic solution containing Al, Ca, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, V, and Zn was prepared for the performance of the recovery test (Table 4). Portions of 2.0 g dried commercial herb (Sample 1) were spiked with the synthetic solution, and then the elements were determined following the recommended procedure after dilution of the samples to 50 mL. As can be seen in Table 4, the results are considered satisfactory; recoveries ranged between 98.5 and 100.2%.

Conclusions

Monitoring metals in plant tissues is important in order to assess excessive buildup of toxic metals in medicinal plants, and it is a useful tool to investigate their role as part of human medicinal treatment.

Based on the results obtained in the present work (Tables 3 and 4), it can be concluded that the proposed technique is suitable for the sensitive and reliable determination of the metal content in medicinal plants. Adequate spike recoveries were achieved within the range 98.5–100.2%. The precision is acceptable for the determination of low quantities of metals. The method might be suitable for the metal trace determination in routine pharmaceutical quality control of medicinal plants.

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