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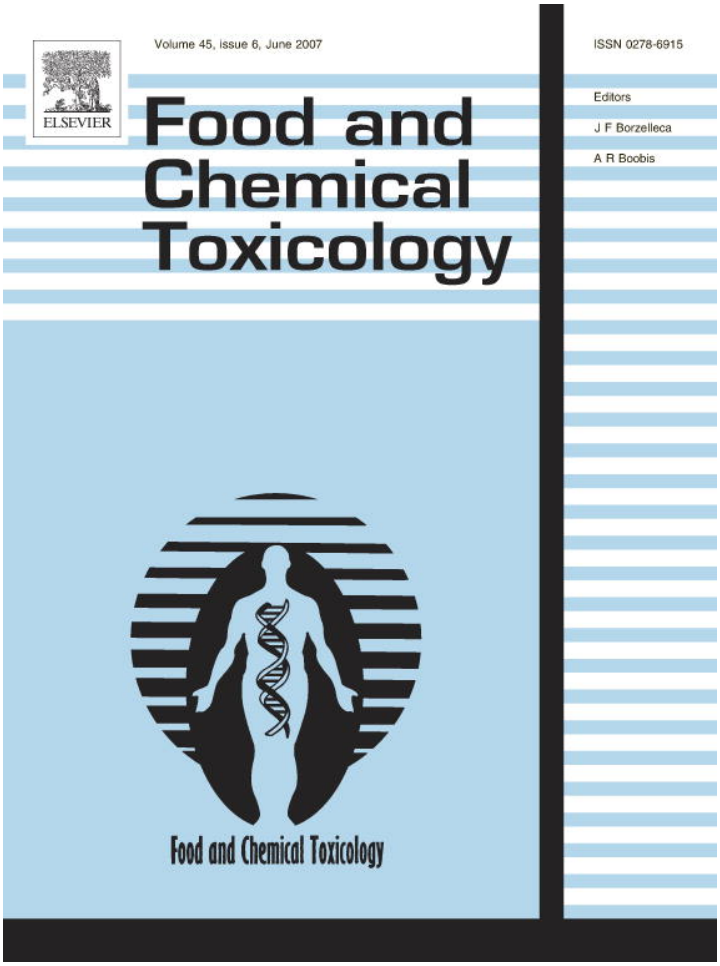

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Determination of heavy metals for the quality control in argentinian herbal medicines by ETAAS and ICP-OES

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Abstract

The determination of trace elements in *Hypericum perforatum* leaves and flowers, their teas, tinctures and tablets was carried out by Electrothermal Atomic Absorption Spectrometry (ETAAS) and Ultrasonic Nebulization System coupled to Inductively Coupled Plasma Optical Emission Spectrometry (USN-ICP-OES). *Hypericum perforatum* (St. John's wort), is a phytomedicine used for the treatment of depression. Samples were collected from different sources in the argentinian market. Heavy metals contents in the investigated samples were found at different levels. Chromium and cobalt were undetectable above their limits of detection in both liquid and solid samples; while aluminum, cadmium, lead, iron and vanadium were present in the majority of samples.

The analytical results obtained for all metals indicate that they were present at concentration well below the acceptable daily intake recommended by the World Health Organization. Based on the results obtained in the present work, it is concluded that the present techniques are suitable for the routine determination of heavy metals concentration in phytopharmaceuticals.
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1. Introduction

Medicinal plants, and galenic forms (*decocta* or *extracta*) obtained from these natural drugs are widely consumed as home remedies and raw materials for the pharmaceutical industry. The last years have seen a significant increase in the use of herbal medicine. Medical doctors are also prescribing herbal teas and herbal extracts as a supplementary type of treatment in everyday problems caused by our modern civilization, for instance against stress or insomnia. The use of medicinal plants in both crude and prepared forms has greatly increased (Eisenberg et al., 1998; Yeh et al., 2002), and although herbal remedies are often perceived as being natural and therefore safe, they are not free from adverse effects (Ernst, 2000; Ernst, 2002). Considering the complexity of these drugs and their inherent biological variation, it becomes necessary to evaluate their safety, efficacy and quality (World Health Organization, 1991).

Hypericum perforatum, commonly known as St. John's wort, is used in many countries for the treatment of mild to moderate forms of depression. Several clinical studies provide evidence that this herb is as effective as conventional synthetic antidepressants (Brenner et al., 2000; Harrer and Schulz, 1994; Philipp et al., 1999; Schrader, 2000; Volz, 1997; Woelk, 2000).

While many investigations of the quality values of medicinal plants are being reported in the current literature

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(Brantner and Males, 1999), less emphasis has been made on the metal content of herbal products. Metallic elements are constituent plant compounds with biological activity as essential or toxic agents in metabolism. Thus, the application of metal monitoring as a pattern recognition method in medicinal herbs is a promising tool for their characterization (Brown et al., 1992; Latorre et al., 1999).

One of the major reasons to monitor levels of toxic metals in medicinal plants is that the contamination of the general environment has increased. The sources of this environmental pollution are quite varied, ranging from industrial and traffic emissions to the use of purification mud and agricultural expedients, such as cadmium-containing dung, organic mercury fungicides, and the insecticide lead arsenate (Gosslim et al., 1984; Schilcher, 1987). Heavy metals, may contaminate different plants causing serious health hazards such as renal failure, symptoms of chronic toxicity, and liver damage (Andrew et al., 2003; Shaw et al., 1997). According to the stipulation of the World Health Organization (World Health Organization, 1995), lead, cadmium, chromium, and other heavy metals must definitely be controlled in medicines in order to assure their safety.

In general, the most widely used techniques for trace determination are Electrothermal Atomic Absorption Spectrometry (ETAAS) (Chuang et al., 1999), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Farias et al., 2002; Wuilloud et al., 2001) and Mass Spectrometry (ICP-MS) (Matsuura et al., 2001; Menegário and Giné, 1997). These methodologies are rapid and sensitive for the determination of trace amounts of metals in different matrices.

The low levels of some heavy metals in phytomedicines are not compatible with the conventional ICP-OES detection limits. For this reason the use of an ultrasonic nebulization system is necessary. The ultrasonic nebulization coupled to ICP-OES determination can provide a 5–50 fold improvement in detection limits (Wuilloud et al., 2001).

In our country, herbal medicines including vegetable drugs, their mixtures, preparations and chemical drugs are controlled by the same Drug Law (Farmacopea Argentina, 1978). Considerations of the potential risk for human health, the economical value of the medicinal plant exportation, as well as the frequent use of some herbal preparations in infant, elderly and even normal persons motivated the authors to develop this work. The aim of the present research is to study the metal content to assure safety and quality of *Hypericum perforatum* phytopharmaceutical products commercially available in the Argentine's market.

2. Materials and methods

2.1. Reagents and chemicals

The water used in all studies was ultrapure water (18 MΩ cm) obtained from a Barnstead Easy pure RF compact ultrapure water system. HNO_3, $HClO_4$ and HF were of ultrapure quality (Merck). Metal standard solutions were prepared by appropriate dilutions of 1000 mg l^{-1} stock solutions immediately before use. Matrix modifier ($(NH_4)H_2PO_4$) used was supplied by Merck (Buenos Aires, Argentina).

2.2. Instrumentation

The AA 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. Metals hollow-cathode lamps (Hamamatsu Photonics K.K., Japan) were employed as radiation source.

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

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

2.3. Phytopharmaceutical samples

Samples analized (Table 1) consisted of both solid (dried herb, tablet) and liquid (tea, tincture) formulations. Two different commercial packed samples of *Hypericum perforatum* dried herb, and its water extracts (teas) were used for examinations. The herbal samples were collected randomly from the argentinian market. Tablets were supplied from a local pharmacy and manufactured by Phoenix Laboratories (Buenos Aires, Argentina). Two different tinctures were purchased from a local pharmacy and an herbal shop (San Luis, Argentina).

2.4. Sample preparation and analysis

Herbal medicines mainly contain organic materials and require a large amount of HNO_3 to be digested, being this reaction 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 carried out in a teflon vessel on 2.0 g of sample by treatment with 10 ml of HNO_3. The solution was evaporated to dryness. Then the solid samples (dried powered St. John's wort leaves and flowers) were treated by strong oxidation with fuming $HClO_4$. Subsequently, the silica salts which could occlude the analites 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 of HNO_3 and diluted to 50 ml with ultrapure water in a plastic volumetric flask.

Dilutions of commercial liquid formulations were prepared as follows: 10 ml of St. John's wort's tinctures were 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 of boiling ultrapure water was poured onto 5 g of dried preparation, covered and left to infuse for 30 min, then filtered, the moisture squeezed out, and the volume made up to 200 ml.

The detection limits of Al, Cr, Fe and V using ICP-OES-USN are 0.3 μg l^{-1}; 0.2 μg l^{-1}; 0.2 μg l^{-1}; 0.05 μg l^{-1} respectively. For Cd, Co and Pb using ETAAS are 0.008 μg l^{-1}; 0.15 μg l^{-1}; 0.06 μg l^{-1} respectively.

Table 1
Origin of *Hypericum perforatum* derivatives samples

Sample no.	Formulation
1	Commercial dried powered leaves and flowers
2	Commercial dried powered leaves and flowers
3	Commercial tablet (mineralized)
4	Commercial tincture
5	Commercial tincture
6	Recently prepared tea from sample 1
7	Recently prepared tea from sample 2

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3. Results and discussion

3.1. Determination of metal content

Atomic absorption spectrometry (AAS) is the most common analytical method adopted for measuring trace metals in biological materials. However, conventional flame techniques show low sensitivity for the determination of trace amounts of heavy metals in medicines. In consequence, ETAAS methodology for Cd, Co and Pb determination at trace level was selected. The operating conditions that provided the best sensitivity are detailed in Table 2.

For Cd and Pb determination $(NH_4)H_2PO_4$ was the matrix modifier used at a concentration level of 0.05 mg (5 μl, 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. (1997).

On the other hand, Al, Cr, and V were determined by Inductively Coupled Plasma Optical Emission Spectrometry coupled with an ultrasonic nebulization system (USN-ICP-OES). The operating conditions are summarized in Table 3. Fe is an essential micronutrient necessary for utilization by the body to ensure good health so it was also determined.

Table 2
Main instrument parameters and furnace temperature program for Cd, Co and Pb determination

Parameters			
Element	Wavelength (nm)	Slit width (nm)	Lamp current (mA)
Cd	228.8	1.0	8
Co	240.7	0.2	12
Pb	283.3	1.0	10
Calibration mode		Absorbance, Peak height	
Background correction		Deuterium lamp	

Stage	Temperature (°C)	Time (s)		Argon gas flow ($l\ min^{-1}$)
		Ramp	Hold	
Furnace program Cd				
Drying	150	20	–	0.10
	250	10	–	0.10
Pyrolysis	500	10	–	1.0
	500	–	10	1.0
	500	–	3	0.0
Atomization	2200	–	2	0.0 (Read)
Cleaning	2400	–	2	1.0
Furnace program Co				
Drying	120	20	–	0.10
	250	10	–	1.0
Pyrolysis	400	–	10	1.0
	400	–	3	0.0
Atomization	2500	–	2	0.0 (Read)
Cleaning	2700	–	2	1.0
Furnace program Pb				
Drying	150	20	–	0.10
	250	10	–	0.10
Pyrolysis	800	10	–	1.0
	800	–	10	1.0
	800	–	3	0.0
Atomization	2400	–	2	0.0 (Read)
Cleaning	2500	–	2	1.0

Table 3
ICP-OES instrumental parameters employed for Al, Cr, Fe and V determination

Forward power	1.0 kW
RF generator	40.68 MHz
Nebulizer	Ultrasonic
Plasma gas flow rate	$8.5\ l\ min^{-1}$
Auxiliary gas flow rate	$1.0\ l\ min^{-1}$
Sample gas flow rate	$0.5\ l\ min^{-1}$
Solution uptake rate	$1.5\ ml\ min^{-1}$
Observation height	15 mm
Wavelength (Al)	308.215 nm
Wavelength (Cr)	267.716 nm
Wavelength (Fe)	240.488 nm
Wavelength (V)	309.311 nm

The metal content found in the samples under investigation is shown in Table 4. The analytical results obtained for all metals indicate that they were present at concentration well below the acceptable daily intake recommended by the World Health Organization (Seiler et al., 1994). Although in our country medicinal herbs are considered pharmaceutical products and they are consequently controlled by the same regulatory authorities, there is no regulation of the total elemental content in medicinal herbs (Farmacopea Argentina, 1978).

Chromium was undetectable above their limits of detection in all the samples while cobalt was found in some of solid samples. Aluminum, cadmium, lead, iron and vanadium were present in most of the samples.

3.2. Method validation. Recovery test

The proposed method of digestion following ETAAS and ICP-OES-USN determination was applied to the quantification of trace amounts of heavy metals in *Hypericum perforatum* derivatives samples.

The method of standard addition is considered as a validation method (Prichard et al., 1996; ICH Guideline, 1994). In order to demonstrate the validity of our method, a recovery study was carried out. A synthetic solution containing Al, Cd, Co, Cr, Fe, Pb and V was prepared for the performance of the recovery test (Table 5). Portions of 2.0 g of the dried commercial herb (sample 1) were spiked with the synthetic solution, then the elements were determined following the recommended procedure, after dilution of the samples to 50 ml. As can be seen in Table 5 the results are considered satisfactory; recoveries being within the range: 99.6–100.0.

4. Conclusions

Based on the results obtained in the present work (Table 4), it can be concluded that the proposed digestion technique is suitable for the determination of heavy metals concentration in phytopharmaceuticals. The simplicity and

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Table 4
Element concentrations of *Hypericum perforatum* derivatives

Samples		Elements concentration						
		ETAAS[a]			ICP-OES-USN[b]			
		Cd	Co	Pb	Al	Cr	Fe	V
Solid samples	Sample 1 ($\mu g\ g^{-1}$)	0.08	0.33	0.36	3.20	<0.005	8.79	1.63
	Sample 2 ($\mu g\ g^{-1}$)	0.05	0.09	0.21	1.23	<0.005	7.43	2.23
	Sample 3 ($\mu g\ g^{-1}$)	0.26	<0.03	<0.012	<0.06	<0.04	25.62	<0.01
Liquid samples	Sample 4 ($\mu g\ l^{-1}$)	<0.008	<0.15	16.89	6.39	<0.2	45.09	10.42
	Sample 5 ($\mu g\ l^{-1}$)	<0.008	<0.15	<0.06	5.27	<0.2	31.20	5.16
	Sample 6 ($\mu g\ l^{-1}$)	<0.008	<0.15	1.53	17.65	<0.2	20.06	8.14
	Sample 7 ($\mu g\ l^{-1}$)	0.24	<0.15	3.07	8.16	<0.2	<0.2	30.01

[a] Electrothermal atomic absorption spectrometry (ETAAS).
[b] Inductively coupled plasma-optical emission spectrometry-ultrasonic nebulizer (USN-ICP-OES).

Table 5
Method validation

	Base value ($\mu g\ l^{-1}$)	Quantity added ($\mu g\ l^{-1}$)	Quantity found[a] ($\mu g\ l^{-1}$)	Recovery (%)[b]
Sample I				
Al	128.00 ± 2.1	25.0	152.986	99.94
Cd	3.20 ± 0.2	1.0	4.197	99.70
Co	13.20 ± 0.5	5.0	18.201	100.00
Cr	–	2.0	1.992	99.60
Fe	351.6 ± 3.1	50.0	401.594	99.98
Pb	14.4 ± 0.9	5.0	19.397	99.94
V	65.2 ± 1.3	2.0	67.196	99.80

Recovery test.
[a] Mean value ($n = 6$).
[b] $100 \times$ [(Found-base)/added].

versatility of the procedure makes it attractive for its use in the pharmaceutical quality control of medicinal plants.

This study also showed that the doses of heavy metals associated with argentinian *Hypericum perforatum* products which could be ingested under normal conditions, should not drastically affect human health. However, risk assessment paradigms might underestimate the effects on children and elderly people who may be more susceptible to adverse effects of ingested low doses of heavy metals.

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