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Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 569–576
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Metal content monitoring in *Hypericum perforatum* pharmaceutical derivatives by atomic absorption and emission spectrometry

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Received 16 July 2003; received in revised form 6 November 2003; accepted 9 November 2003

Abstract

Metals have been investigated in different plant materials in order to establish their normal concentration range and consider their role in plants as part of human medicinal treatment. Metal monitoring as a pattern recognition method is a promising tool in the characterization and/or standardization of phytomedicines. In the present work measurable amounts of Ca, Cu, K, Li, Mg, Mn, Na, Ni, and Zn were detected in phytopharmaceutical derivatives of *Hypericum perforatum* by atomic techniques. Atomic methodologies like flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ETAAS) allow reliable determination of mineral content in pharmaceutical quality control of medicinal plants. Additionally, capillary electrophoresis (CE) patterns of characteristic components (fingerprints) have been performed for the search of adulterants in phytopharmaceutical products.

Keywords: Herbal medicines; Phytopharmaceuticals; St. John's wort; Metal content; Atomic spectrometry

1. Introduction

Medicinal plants are important for pharmacological research and drug development, and are widely consumed as home remedies and raw materials for the pharmaceutical industries. There are different ways by which countries define medicinal plants, herbs or products derived from them, and they have adopted various approaches to licensing, dispensing, manufacturing and trading them to ensure their safety, quality and efficacy [1–4]. In our country, herbal medicines including vegetable drugs, their mixtures and their preparations and chemical drugs are controlled by the same Drug Law [5].

The use of medicinal plants in both crude and prepared forms has greatly increased. The World Health Organization (WHO) has estimated that 80% of the global population relies mainly on traditional medicine for primary care and there are reports indicating that about 51% of all drug preparations in industrialized

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doi:10.1016/S0731-7085(03)00643-5

countries derived from plants. Taking into account the complexity of these drugs and their inherent biological variation, it turns imperative to evaluate their safety, efficacy and quality [6]. Furthermore, considering the great variability in chemical composition that results from factors like exposition during their growth, storage and the different stages of manipulation; the characterization and/or standardization of phytopharmaceuticals is necessary [7].

Standardized phytopharmaceuticals must have a known content of active or characteristic substances. External substances must be below specified limits recommended by regulatory agencies [8]. Adulterations with synthetic drugs and toxic heavy metals are major problems with herbal medicine [3,9,10]. Microbiological contamination and foreign materials are important quality criteria at testing medicinal plants. Organic or inorganic substances of natural or synthetic origin can contaminate any product of agricultural or wild sources.

While many investigations of the quality values of medicinal plants are being reported in current literature [11], less emphasis has been made on the metal content of herbal products. Metallic elements are constitutive plant compounds with biological activity as essential or toxic agents in metabolism. Environment in developing countries, pollution in irrigation water, atmosphere, soil, sterilization methods and storage conditions all play an important role in contamination of medicinal plants by metals. Metals may contaminate different plants causing serious health hazards such as injury of kidney, symptoms of chronic toxicity, renal failure and liver damage [12–14].

Metals have been investigated in different plant materials in order to establish their normal concentration range and evaluate their role in plants as part of human medicinal treatment [15–17]. Standardization of herbal preparations should allow the knowledge of their composition and prevent, or at least do unprobable, the consumption of drugs of questionable quality [18,19].

Fingerprint analysis has been introduced and accepted by WHO as a strategy for the assessment of herbal medicines [20]. Marker compounds and chromatographic and/or electrophoretic profiles may be used with the aim to evaluate herbal growers and suppliers, to standardize raw materials and control formulation and tablet content uniformity [21–24]. For many herbals, compendial analytical methods are not available. Natural variation of the raw material and the influence of processing, make standardization more difficult than for synthetic compounds. Preparations with known and with unknown active constituents have to be treated differently. Analytical reference standards are unavailable for many herbal materials. Only some of them are commercially available, others could be obtained by purification or synthesis.

The use of fingerprinting in herbs trends to focus on identifying and checking for an appropriate procedure to obtain a well-characterized product. Thus, the application of metal monitoring as a pattern recognition method in medicinal herbs is a promising tool for their characterization [25,26]. Characterization of plant material requires the following steps: identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes and ideally provide additional information by means of separation methodologies to obtain stability profiles for marker compounds. Metal monitoring can be useful because minerals are good candidates for a characterization system due to their stability.

Atomic absorption spectrometry is probably the most widely used instrumental methodology for determining a variety of metals due to its excellent analytical performance.

Medicinal plant extracts or herbal teas are used for the treatment of chronic diseases like depression. Herbal extracts and phytopharmaceuticals derivatives of *Hypericum perforatum* commonly known as St. John's wort are now successfully competing for status as a standard antidepressant therapy [27,28]. St. John's wort derivatives are some of the most commercialized phytomedicines in the world, their samples were analyzed by different CE modes in order to obtain CE fingerprints, and search for traditional adulterants. The aim of this study is to develop a methodology for simple, direct, fast, and precise way for monitoring the metal content in phytopharmaceuticals and obtain a characteristic fingerprint of metallic constituents, helpful at characterizing *Hypericum perforatum* derivatives of Argentine's market. Furthermore, a simple and sensitive CE method was performed to monitoring the extracts' genuinity and to analyze caffeine, theobromine, theophylline and ephedrine present as possible adulterants in antidepressive herbal products.

2. Experimental

2.1. Reagents and chemicals

The water used in all studies was ultra-high-quality water (18 MΩ cm) obtained from a Barnstead Easy pure rf compact ultrapure water system. Metal standard solutions were prepared by appropriate dilutions of 1000 mg l^{-1} stock solutions immediately before use.

Sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$) was supplied by Mallinckrodt (Saint Louis, USA), sodium dodecylsulphate (SDS) and β-cyclodextrin by Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan). Caffeine, theobromine, theophylline and ephedrine were purchased from Sigma (St. Louis, MO). Capillary electrophoresis solutions were degassed by ultrasonication (Testlab, Argentina), and running electrolytes and samples were filtered through a 0.45 μm Titan Syringe filters (Sri, Inc., Eaton Town, NJ. USA).

2.2. Instrumentation

The measurements were performed with a Shimadzu Model AA-6800 atomic absorption spectrometer (Tokio, 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.

For the capillary electrophoresis analysis a Beckman P/ACE MDQ instrument (Beckman Instruments, Inc., Fullerton, CA) equipped with a diode array detector and a data handling system comprising and IBM personal computer and P/ACE System MDQ Software was utilized. Detection was performed at 274 and 588 nm. The fused-silica capillaries were obtained from MicroSolv Technology Corporation and had the following dimensions: 67 cm total length, 50 cm effective length, 75 μm i.d., 375 μm o.d. The temperature of the capillary and the samples was maintained at 25 °C. Samples were pressure-injected at the anodic side at 0.5 Psi for 5 s.

2.3. Phytopharmaceutical samples

Samples analyzed (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).

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 mineralized tablet
4	Commercial tincture
5	Commercial tincture
6	Recent prepared tea of sample 1
7	Recent prepared tea of sample 2

2.4. Sample preparation and analysis

To mineralize the solid samples (dried powered St. John's wort leaves and flowers), a nitric-perchloric digestion was used. The digestion was performed in a Teflon vessel. Ten milliliter of nitric acid were added to 2 g of sample. The solution was evaporated to dryness, later 5 ml of $HClO_4$ and five drops of HF were added, until white fumes were observed. The tablet sample was digested with 5 ml of nitric acid. After digestion, the samples were diluted up to 50 ml with ultrapure water in a volumetric flask.

Diluted samples 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 the teas preparation was: 200 ml 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.

For monitoring organic constituents, fingerprints of *Hypericum perforatum* extract and its derivatives commercial products were performed in different modes of capillary electrophoresis. Various dilutions of a recent prepared tea, diluted samples of tinctures and a

tablet finely powered were analyzed to obtain CE fingerprints.

3. Results and discussion

3.1. Determination of metal content

The flame atomic absorption spectrometry (FAAS) instrumental and operating conditions that provided the best sensitivity are detailed in Table 2. Li, Na and K were determined by atomic emission spectroscopy and the remaining elements (Ca, Cu, Mg, Mn and Zn) were determined by atomic absorption spectroscopy. The Ni concentration was measured by electrothermal atomic absorption spectrometry (ETAAS). The operating conditions of ETAAS are listed in Table 3.

The metals selected for these determinations were chosen considering their nutritional and medicinal interest. However, is important remark that some metals are toxic if excessive amounts of them are ingested.

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 recommended by the World Health Organization [29]. From the results obtained for solid samples, it can be seen that Li, K, Cu, Mg and Ni, were found at higher concentrations for sample 3, fact that could be explained considering that such formulation was obtained from the vegetal extract. On the other hand, in the case of liquid samples, the higher metal contents for tinctures samples 4 and 5 compared to samples 6 and 7 could be ascribable to the manufacturing process.

3.1.1. Macrocomponents

Macronutrient elements are needed in relatively large amounts in the human diet such as calcium, sodium, potassium and magnesium. The obtained values for Ca were within the range 100–500 $\mu g\,g^{-1}$ for dried herb and the table and 80–5200 $\mu g\,l^{-1}$ for the teas and tinctures. The highest concentration was observed for K. The amount found was 820–1100 $\mu g\,g^{-1}$ for solid samples and 44,000–70,000 $\mu g\,l^{-1}$ for the liquid samples. Content of Na ranged from 400 to 640 $\mu g\,g^{-1}$ for the tablet and dried herb and from 30,000 to 45,000 $\mu g\,l^{-1}$. Mg content was 30–200 $\mu g\,g^{-1}$ for solid samples and 15,000–53,000 $\mu g\,l^{-1}$ for the liquid samples.

3.1.2. Copper

Copper is an abundant trace element, which is considered as an essential micronutrient and as a toxic element, depending on its concentration level [29]. Recently, it has been reported that some diseases, are believed to be closely linked to the chronic intake of elemental copper, even at low concentration [30,31]. In the USA, the estimated safe and adequate dietary intake for copper is 1.5–3 mg per day for adults, 0.4–0.6 mg per day for infants and 0.7–2 mg per day for children [32]. The copper concentrations detected in the present study are in the range of 0.013–0.017 $mg\,g^{-1}$ of the solid samples, and 0.039–0.045 $mg\,l^{-1}$ for the liquid samples. In the teas samples the copper level was undetectable.

3.1.3. Nickel

Nickel is one of the more mobile and bioavailable heavy metals ions that may be present in both industrially contaminated and pristine soils [33]. In

Table 2
FAAS instrumental parameters employed to metals determination

Element	Flame type	Burner height (mm)	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Lamp mode	Fuel gas ($l\,min^{-1}$)
Na	Air–C_2H_2	7	589.0	0.1	–	Emission	1.8
K	Air–C_2H_2	7	766.5	0.1	–	Emission	2.0
Li	Air–C_2H_2	7	670.8	0.1	–	Emission	1.8
Ca	Air–C_2H_2	7	422.7	0.5	10	BGC-D_2	2.0
Cu	Air–C_2H_2	7	324.8	0.5	6	BGC-D_2	1.8
Zn	Air–C_2H_2	7	213.9	0.5	8	BGC-D_2	2.0
Mg	Air–C_2H_2	7	285.2	0.5	8	BGC-D_2	1.8
Mn	Air–C_2H_2	7	279.5	0.2	10	BGC-D_2	2.0

Table 3
Main instrument parameters and furnace temperature program for Ni determination

Parameters				
Wavelength (nm)	232.0			
Slit width (nm)	0.2			
Lamp current (mA)	12			
Calibration mode	Absorbance, peak height			
Background correction	Deuterium lamp			
Stage	Temperature (°C)	Time (s)		Argon gas flow ($l\,min^{-1}$)
		Ramp	Hold	
Furnace program				
Drying	120	20	–	0.10
	250	10	–	0.10
Pyrolisis	800	10	–	1.0
	800	–	10	1.0
	800	–	3	0.0
Atomization	2500	–	2	0.0 (read)
Cleaning	2500	–	2	1.0

a recent study, Ni was shown to the Negev desert [34]. Among the known health related effects of Ni are skin allergies, lung fibrosis, variables degrees of kidney and cardiovascular system poisoning [35]. A few samples in this study contained very small quantities of nickel below $0.5\,\mu g\,g^{-1}$ in the case of solid samples and below $0.96\,\mu g\,l^{-1}$ in the case of liquid samples.

3.1.4. Zinc

Several trace metals, zinc included, are chemical elements that plays an important role as oligoelements in several biological processes. Nevertheless, these metals may also be the origin of adverse effects on living organisms if the dosages exceed certain levels [36]. The uptake of zinc above certain concentration levels for a period may lead to bioaccumulation

Table 4
Element concentrations of *Hypericum perforatum* derivatives

Elements	Element concentration						
	Solid samples			Liquid samples			
	Sample 1 ($\mu g\,l^{-1}$)	Sample 2 ($\mu g\,l^{-1}$)	Sample 3 ($\mu g\,l^{-1}$)	Sample 4 ($\mu g\,l^{-1}$)	Sample 5 ($\mu g\,l^{-1}$)	Sample 6 ($\mu g\,l^{-1}$)	Sample 7 ($\mu g\,l^{-1}$)
Li[a]	0.05	0.03	0.07	0.14	0.19	0.29	0.09
Na[a]	639	516	400	42576	44218	30717	40358
K[a]	927	818	1090	65467	69332	55600	44145
Ca[b]	460	105	111	2105	5210	89	81
Cu[b]	13.5	12.8	16.9	39.2	42.3	<5	<5
Mg[b]	112	34.5	192	41882	52890	46476	15476
Mn[b]	80.9	91.6	9.7	240	322	13.5	15.1
Zn[b]	42.8	46.3	40.4	88.5	121.4	11	14.2
Ni[c]	0.04	0.12	0.49	0.96	0.76	0.74	0.51

[a] Flame atomic emission spectrometry (FAES).
[b] Flame atomic absorption spectrometry (FAAS).
[c] Electrothermal atomic absorption spectrometry (ETAAS).

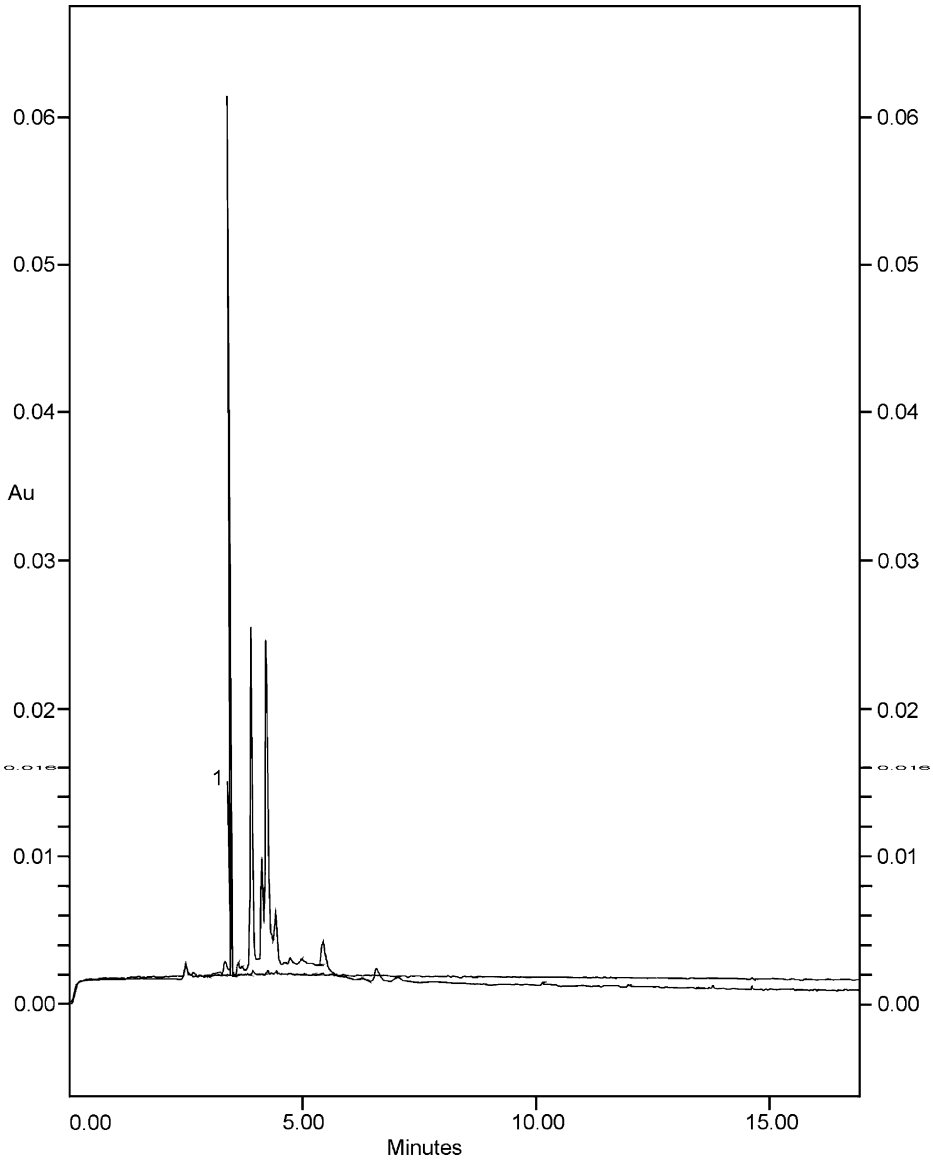

Fig. 1. Fingerprint in CZE mode of a recent prepared tea. Conditions: 20 mM sodium tetraborate buffer, pH 9.22; capillary, 67 cm full lengh, 50 cm effective length, 75 μm i.d., 375 μm o.d.; hydrodynamic injection at 0.5 Psi, 5 s; 20 kV constant voltage; 25 °C, detection by UV-Vis absorbance at 274 and 588 nm (superimposed). Peak identification: 1, hypericin.

processes that could produce adverse effects or even mortality. Therefore, the control of zinc concentrations is necessary to assure good quality standards in medicinal herbs and its infusions. The zinc levels found were within the range 44–47 $\mu g\,g^{-1}$ for the solid samples and 11–125 $\mu g\,l^{-1}$ for the liquid samples.

3.1.5. Lithium

Lithium is used in the prophylaxis of manic-depressive (bipolar) and recurrent unipolar depressive disorders [37]. Lithium has a number of adverse effects including weight gain, edema, and thyroid problems. One of the most serious adverse effects however, is diabetes insipidus leading to polydipsia

[38]. Lithium toxicity is a problem, and can lead to problems such as confusion, sleepiness, convulsions and ultimately coma. The obtained values for Li are below of 0.07 $\mu g\,g^{-1}$ for solid samples and below of 0.29 $\mu g\,l^{-1}$ for liquid samples.

3.1.6. Manganese

Manganese is an essential metal of low toxicity and trace amounts occur in biological materials [39]. Any excess is, however, toxic to plants and animals [40,41] and might be hazardous [42]. Estimated safe and adequate daily dietary intake levels for manganese are 1–2 mg per day for children 4–10 years old and 2–5 mg per day for people over 10 years old [43]. The Mn level in the dried herb was ranged from 9 to 92 $\mu g\,g^{-1}$ and from 15,000 to 53,000 $\mu g\,l^{-1}$ for the teas and tinctures.

3.1.7. Capillary electrophoresis (CE)

Marker compounds like hypericins and hyperforins may be used as potency standards in the control of *Hypericum perforatum* samples [44,45] because they are characteristic markers for this genus and allow genuine products to be differentiated from adulterants. Hypericins absorb visible light with a maximum absorption at 588 nm and are highly fluorescent in methanol when exposed to UV light.

Fingerprints of a recent prepared tea and a commercially available tincture were obtained by three different modes of CE. First, the ordinary capillary zone electrophoresis (CZE) separation mode was investigated. Sodium tetraborate solution (20 mM), pH 9.22 adjusted by 0.1 mol l^{-1} NaOH and 0.1 mol l^{-1} HCl was used like BGE. For micellar electrokinetic chromatography (MEKC) analysis a background electrolyte (BGE) of sodium tetraborate solution (20 mM), pH 9.22, containing 10 mM sodium dodecylsulphate (SDS) was used. Finally, a buffer containing sodium tetraborate (20 mM), pH 9.22, β-cyclodextrin (20 mM) was tested.

Results obtained with MEKC and a buffer containing 20 mM sodium tetraborate, 20 mM β-cyclodextrin showed no significant differences from those obtained with the CZE mode. The samples under investigation were initially screened for synthetic drugs commonly used as stimulants. Neither caffeine, nor theophylline or theobromine or ephedrine were detected. Fig. 1 shows the CZE fingerprint for a recent prepared tea performed at 274 and 588 nm. As can be seem only the hypericin peak is present in the electropherogram at 588 nm.

4. Conclusions

One of the challenges in the quality assurance of medicinal plants is their standardization and authentication, a topic of growing interest. If metal species are correlated to the geographical origin or manufacture of phytopharmaceuticals, they could be important for addressing quality assurance of medicinal plants. The classification of biological species in relation to their chemical composition, which of course includes metal species, is also important in the area of chemotaxonomy.

The present work shows a good performance with respect to selectivity, linearity and accuracy in regard to the samples under investigation. The results of this study clearly demonstrate the potentiality and versatility of these methodologies, which could be applied to routine monitoring metal content in phytopharmaceutical formulations.

Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) (PICT-BID) and Universidad Nacional de San Luis (Argentina).

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