Analysis of Metal Content in Herbal Medicines

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In many European countries, including Romania, the statutory regulation of herbal products as medicines has led pharmaceutical companies to orient themselves towards the production of herbal medicines. In this paper we determined the metal content of certain phytochemical products produced by a Romanian company. The medicinal plants are cultivated by the company producing the phytochemicals on large areas and even in organic farming system. An acid-assisted microwave digestion procedure is optimized for the determination of metals in the products analyzed by the use of Inductively Coupled Plasma Optical Emission Spectrometry techniques. Thus, the analysis of the content of six types of capsules determined that they contain a lot of chemicals, metals like calcium, potassium, sodium, magnesium, zinc, strontium, manganese. The analysis also determined that the capsules contain toxic elements (Cd, As, Cr, Mo), but which are present in small quantities, within the toxicity limits stipulated by the law.

Keywords: herbal products, microwave digestion, ICP-OES

Herbal medicine was among the first medical methods practiced by humans. Owing to the therapeutic effects some plants have proved to have, people have chosen to use them to heal or at least to alleviate certain conditions. Nature is changing continuously, some species disappear, and some new species are born, while other species pass through time.

Many times herbal remedies are very helpful, both by their direct effect on various diseases and by their ability to stimulate the body's immune system in its fight against the aggression of internal or external factors. Herbal remedies can be associated in any phase of the disease, regardless of the site of the disease or the age of the patient.

Nowadays, due to the development of chemistry and pharmaceutical industry, synthetic medicines have been introduced and used in therapy with the belief that they would replace vegetal products in time. But that didn't happen. One of the causes that lead to people getting ill is the consumption of chemical substances, this being an additional reason for our attention to turn more and more towards the use of medicines offered by nature.

Therefore, a great number of plant-based products have been developed. Factories producing these kinds of products monitor in their own quality control laboratories the active substance content from the reception of the product, either dried or fresh, and then all the way through the technological process in order to guarantee the therapeutic effect of the finished products.

Experimental part

Materials and methods

Of all the pharmaceutical dosage forms containing vegetal products, solid forms have been used in this study, pharmaceutical capsules, respectively. Six types of capsules containing medicinal plants from the spontaneous flora of Romania, Bihor, Hunedoara and Arad areas, have been subject to analysis.

Description of the content of the six types of capsules, their activities and recommendations:

Sample 1 contains: *Bursae Pastoris herba*, *Betulae folium*, *Meliloti herba*, *Polygoni avicularis herba*, *Graminis rhizoma*; activity: diuretic effect, stimulating water and metabolic waste excretion from the body. It also contributes to normalizing blood pressure values.

Sample 2 contains: *Visci folium cum stipites*, *Leonuri herba, Allii sativi bulbus, Vincae minoris herba, Olivae folium*; activity: hypotensive effect, reducing cardiac frequency, coronary and peripheral vasodilators.

Sample 3 contains: *Calendulae flos, Plantaginis folium, Stachys herba, Hyperici herba, Hyssopi herba, Liquiritiae radix*; activity: reduces the inflammation of the gastric mucosa, favouring the healing of mucosal erosions and increasing the secretion of gastric mucus, having a protective effect, astringent effect, antihemorrhagic effect, antispasmodic effects, relieving stomach pains.

Sample 4 contains: *Filipendula folium, Origanum vulgarae herba, Salicis cortex, Allii sativi bulbus*; activity: complex anti-inflammatory, anti-infective, antiviral, analgesic, antipyretic, decongestant, antitussive effects.

Sample 5 contains: *Alchemillae herba, Bursae Pastoris herba, Melissae folium, Agni casti fructus, Salicis cortex*; activity: anti-inflammator - analgesic, antispasmodic, diuretic, astringent, antihemorrhagic.

Sample 6 contains: *Chelidonii herba, Taraxaci radix, Plantaginis folium, Olivae folium, Cynarae folium*; activity: effect on cholesterol metabolism in the liver, lowers the absorption of fats in the bowel, dissolves cholesterol and lipid deposits, lowers blood pressure.

Sample preparations: the content of the capsules was powdered with ceramic instruments, avoiding metal contact.

The nitric acid and the hydrogen peroxide employed in all the studies are of the required purity for the analyses, as they are purchased form Merck, Germany, and bidistilled water was used (18 MΩcm).

For the decomposition of the organic material a Milestone MLS-1200 microwave oven was used.

The first stage consists in obtaining the residuum that will be used for the detection of heavy metals in the vegetal products from the capsules.

Plant-based medicines contain mainly organic materials and require a large quantity of HNO_3 to be digested, this

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Sample/ Me(μg/g)	Al	Ba	Cu	Ca	Fe	K	Mg	Mn	Na	Sr	Zn
Sample 1	373.5	182.2	5.9	5339.7	390.2	11157.0	1647	227.6	147.9	46.1	66.5
Sample 2	98.6	29.1	4.9	3693.6	90,.	12881.0	1795	13.1	1680.6	48.3	59.4
Sample 3	373.5	94.6	8.5	6732.0	366.1	21058.0	2001	40.9	2513.5	61.7	36.1
Sample 4	42.9	22.5	3.7	9095.0	47.5	8109.0	1239	77.5	1467.5	54.7	86.4
Sample 5	216.4	32.0	72.9	6257.4	251.0	14572.0	2347	50.1	608.3	54.7	35.6
Sample 6	212.1	40.2	4.2	7495.5	176.6	17925.0	2043	55.8	436.2	79.7	19.1

Table 1
ELEMENT CONTENT OF CAPSULES CONTAINING VEGETAL PRODUCTS

reaction being very difficult to control. A digestion method, with an acid mixture (including HNO_3 and H_2O_2) was used to decompose the organic material.

Prior to digestion, on 0.1500-0.2500 g of each sample, 4.5 mL of concentrated nitric acid and 0.5 mL of hydrogen peroxide is added. After a 10 min waiting time to avoid the first vigorous chemical reaction, the digestion is carried out according to the existing programs. The vessel with the valve is closed with the ECM-30 device, inserted into the safety shield and introduced into the microwave unit.

Operating conditions for microwave digestion: step 1 – power 300 W, time 5 min, step 2 – power 600 W, time 5 min.

The second stage: Inductively coupled plasma optical emission spectrometric measurements (ICP-OES) were carried out employing a sequential spectrometer ICP Intrepid IRIS II. Low levels of certain heavy metals in herbal drugs are not compatible with the conventional detection limits of ICP- OES. Therefore, the use of an ultrasonic nebulising system was necessary. The coupling of ICP-OES with an ultrasonic nebuliser provides the determination an improvement by 5-50 times of the detection limits.

Inductively coupled plasma optical emission spectrometry is an analytical technique used for the detection of trace metals. It is a type of emission spectrometry that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiations at wavelengths characteristic of a certain element. The intensity of this emission indicates the concentration of the element within the sample. The sensitivity of this technique is quite high, varying by element, from 1ppm to 10 ppb [1 - 5].

The systems used in ICP-OES are OY Analytical (Texas, USA) and Berghoff (Germany), with twelve teflon vessels and pressure control device.

In determining the microelement, the digestion of the sample affects directly the detection limit, the accuracy and the precision. The method detection limit (MDL) was calculated using blank samples employing the microwave digestion system by preparing a clean sample. The detection limit is adequate for all the elements in order to determine the elements in the analyzed samples [6-10].

Results and discussions

In this work analyzing six phytopharmaceutical products it was determining concentration of twenty one elements, the main components and trace essential and toxic products.

The advantages of microwave digestion on classical digestion are: reduced analysis time, reduced sample and reagent volumes, volatile elements are retained in the system, reduced equipment corrosion, process safety enhancement, lower detection limits and sensitivity. ICP-OES is generally superior in accuracy, detection limit, and dynamic range and free from interferences, as compared to other classical spectrometric techniques. A multi-elemental solution can be analyzed in one minute and therefore a large number of samples can be analyzed in a very short time [11-14]. After cooling, the vessels were opened again, and the amount of residua was diluted to 50 mL with deionised water in a volumetric plastic beaker. The resulting solutions were clear, colorless, odorless, free of residua, the dissolution being complete in most of the cases. The results obtained are presented in table 1.

Calcium is the most abundant mineral in the human body, having such important structural functions that if its supplies were entirely lost the human body would become a tissular mass without form and consistency. Therefore, any additional source of calcium is welcome.

Potassium occurs in nature only as ionic salt. It can be found dissolved in seawater, and as part of many minerals. Potassium is necessary for all living cells, and is thus present in all living plant and animal tissues. It is found in high concentrations especially in plants and fruits. Potassium, in association with sodium regulates the fluid and electrolyte balance of the human body, normalizes heart rate and contributes to waste metabolites disposal.

Magnesium is important for both animal and plant development. Chlorophyll consists of porphyrins based on

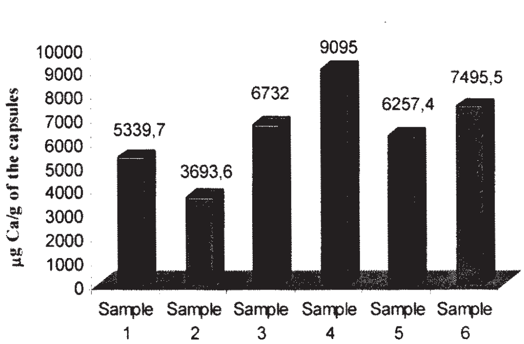

Fig. 1. Calcium content (μg/g) of capsules

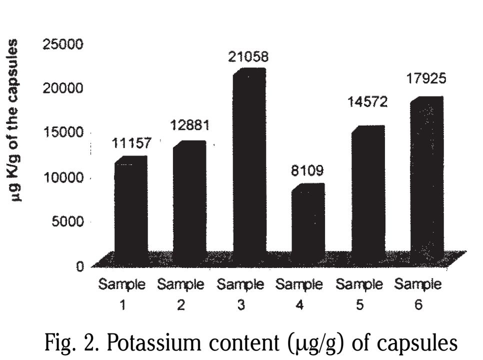

Fig. 2. Potassium content (μg/g) of capsules

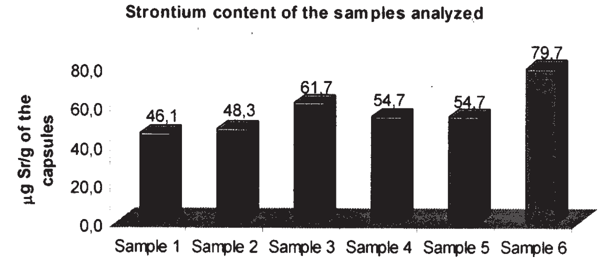

Fig. 3. Sodium content (µg/g) of capsules

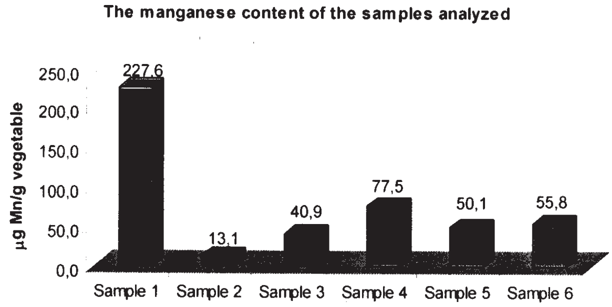

Fig. 4. Magnesium content (µg/g) of capsules

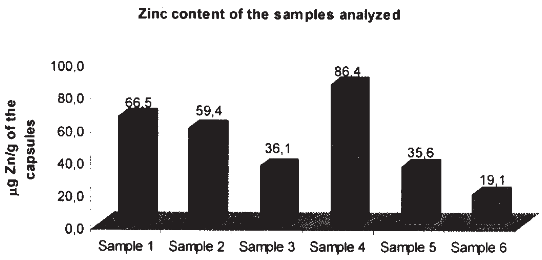

Fig. 5. Zinc content (µg/g) of capsules

magnesium combinations. Magnesium is essential for the activity of the central nervous system and the proper muscle function, playing an important role in the metabolic process by which glucose is converted into energy. In the absence of chromium, glucide metabolism is not efficient.

High aluminium content has been found in four samples.

For four samples out of six, the zinc content exceeds the zinc average in medicinal plants (40 mg/kg), but its concentration is situated within the range of non-polluting plants (15-100 mg/kg) [15-18]. Zinc content in medicinal plants is very low, as compared to normative requirements for humans (4.6 mg/day) [19].

Manganese supports the proper functioning of certain digestive enzymes and it is of great importance in the elimination of toxins.

It has also been established that these capsules contain toxic elements (Cd, Co, Cr, Mo), but which are in small quantities, below the statutory toxicity limits (Table II).

Conclusions

More and more, our attention turns towards the medicines offered by nature. Therefore, many plant based medicines have been developed.

In this paper we aimed to determine the metals contained in six phytopharmaceutical products manufactured by a Romanian company.

In order to determine the metals we employed inductively coupled plasma optical emission spectroscopy, a technique that is superior in accuracy, detection limit, dynamic range, and free from interferences, as compared to other classical spectrometric techniques, and by which a multi-elemental solution can be analyzed in a very short time.

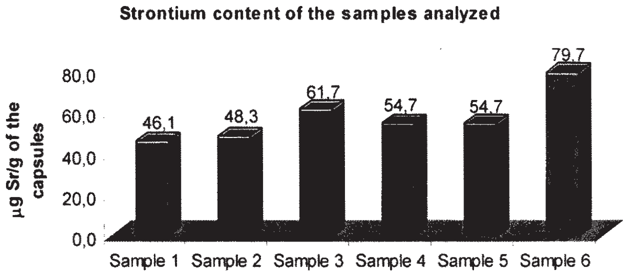

Fig. 6. Strontium content (µg/g) of capsules

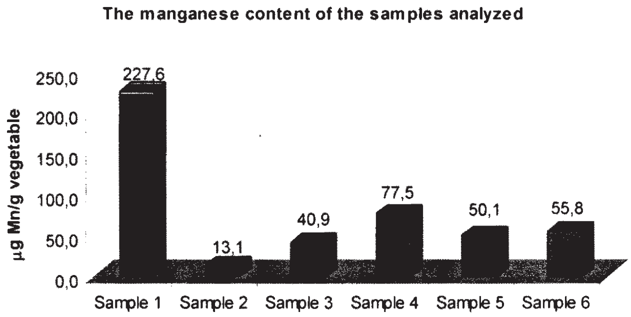

Fig. 7. Manganese content (µg/g) of capsules

Table2

TOXIC ELEMENTS CONTENT OF CAPSULES

No. sample/ metal (µg/g)	Cd	Co	Cr	Mo
Sample 1	0.3	0.4	<0.1	0.3
Sample 2	0.1	0.0	<0.1	<0.1
Sample 3	0.2	0.3	<0.1	0,5
Sample 4	0.5	0.3	<0.1	0.6
Sample 5	0.1	0.1	<0.1	<0.1
Sample 6	0.1	0.2	<0.1	<0.1

Therefore, analyzing the content of the six types of capsules we have found that these contain a multitude of metals such as calcium, potassium, magnesium, zinc, strontium and manganese in concentrations that are useful for the desired therapeutic effect of the products. It has also been established that these capsules contain toxic elements (Cd, Co, Cr, Mo), but which are in small quantities, below the statutory toxicity limits.

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