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Determination of major, minor and trace elements in Glyceric Macerates and Mother Tinctures and in the starting plant materials

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ABSTRACT

Glyceric Macerates (GMs) and Mother Tinctures (MTs) are liquid preparations obtained from plant buds (for GMs) and flowers, leaves or roots (for MT) by extraction with a mixture of solvents. Their quality depends on the quality of the plant materials and on the preparation procedures. In this work we determined the concentrations of major, minor and trace elements in buds, flowers and other plant components and in the GMs and MTs obtained from them by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) after microwave mineralization. To the best of our knowledge, this procedure has been applied for the first time here to the analysis of buds. We have taken into account spectral interferences and other causes of errors. Analogies and differences with regard to the method reported by European Pharmacopoeia for heavy metal determination in herbal drugs have been highlighted. The experimental results have been interpreted with chemometric techniques. No significant contamination was detected during the manufacturing step. Element concentrations in GMs and MTs, taking into account their daily dosages, are lower than acceptable intake levels.

1. Introduction

In the last decades the demand of natural, plant-based product has been increasing. In the past, only raw dry herbs were commercially available, which were usually consumed for infusions and decoctions, commonly referred to as tisanes. Nowadays many plant-derived products are marketed in several forms, e.g. tablets, powders and liquids. Some producers do not prepare formulations starting from raw herbs, but use semi-finished products, because they are easier to deal with, enable one to save time and are more homogeneous than the raw materials. The quality of such products is extremely important for the quality of the final formulations. Glyceric Macerates (GM) and Mother Tinctures (MT) are two examples of semi-finished stuffs; they are obtained by extraction of plant parts (Section 2.2). They are also directly consumed after dilution; in addition, MTs are used as bases for homoeopathic and cosmetic products.

Plants are sources of both secondary metabolites (some of which represent the "active principles" of plants and are the reason why plants are used as health-promoting agents) and of the so-called mineral nutrients, i.e. most alkali, alkaline earth and transition metals, some metalloids (e.g. Si and Se) and nonmetals (e.g. P and Cl). These elements are essential for plant growth and for human health [2,3], but become harmful at high concentrations [1,4]. Other elements, like Pb, Hg and Cd, do not have known physiological roles and are simply tolerated by the organism at low concentrations. The "low" and "high" concentration levels depend on the effect of each element: even common metals like Na or Ca are detrimental if present in excess in the human body.

Plants assimilate mineral nutrients primarily from the soil and partly from the atmosphere and from the water used for irrigation. Such nutrients are transferred, in part or completely, to plant-derived products [5]. Also improper manufacturing of semi-finished and end products, such as the use of contaminated solvents, unclean vessels or working places can be a source of essential and non-essential elements. Therefore it is important to determine their concentrations both in plant raw materials and in plant-derived products. Many papers report element contents in plants [e.g. [4,6–8]], but less attention was devoted to plant-derived products [e.g. [9]]. Furthermore, to the best of our knowledge no

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papers deal with the concentrations in buds or bud-derived products, and the information on MTs is scarce [10,11]. For these reasons we focused our attention on GMs obtained from buds and MTs prepared from selected plant parts, and on their starting materials; since the manufacturing steps may cause contamination, we also analysed the extracting reagents and the filters used for the preparation.

The analytical techniques commonly adopted for element determination in plants are atomic absorption (AAS), inductively coupled plasma optical emission (ICP-OES) or mass spectrometry (ICP-MS), preceded by mineralization of the samples [e.g. [3,8,9]]. The European Pharmacopoeia [12] reports a method for heavy metal determination in herbal drugs and fatty oils, based on mineralization with a mixture of nitric and hydrochloric acid and analyte determination by AAS. This combination of acids is quite aggressive, probably because the method is designed also for fatty oils. We used nitric acid alone, because it is extensively adopted for plant digestion [e.g. [13]], and chose ICP-OES instead of other instrumental techniques because it is more rapid than graphite furnace AAS (GF-AAS) and less expensive than ICP-MS. We took into account the instrumental interferences and other sources of error and treated the results with chemometric techniques. The limits of quantification (LoQs) are higher than those of GF-AAS and ICP-MS: anyway we were able to evaluate the hazards associated to the presence of potentially toxic elements in GMs and MTs using worst-scenario conditions, assuming that their concentrations were equal to the LoQs (Section 3.3). For such evaluation we compared element concentrations with reference acceptable intake values.

The outcomes of our study can have different applications. First of all, we report a protocol for the analysis of buds and highlight the sources of errors and interferences. Secondly, the concentrations found in buds and MGs, presumably being the first published data on these matrices, can be used as a basis of comparison in future studies. Finally, the results reported can be of interest to both producers and consumers of plant-derived products.

2. Experimental

2.1. Sample collection

Buds, flowers and other plant parts were provided by GEALpharma (Bricherasio, Torino, Italy), a small company manufacturing GMs and MTs. Most samples had been harvested from plants spontaneously growing in areas unaffected by local sources of vehicular traffic or industrial activities in Val Pellice, Val Chisone and Val Germanasca, Torino province, Piedmont, Italy. *Echinacea angustifolia* DC, *Passiflora incarnata* L. and *Rheum officinale* Baill., which are not spontaneous in Piedmont, had been purchased from vendors growing plants in open fields in areas with the same characteristics. Table 1 reports the list of the investigated species, the identification code used in this paper, the common name, family, order, the balsamic time, the used parts and the obtained product. For the nomenclature and taxonomy of the plants the projects "The Plant List" and "Angiosperm Phylogeny Website v.13" were taken as reference [14,15].

2.2. Extraction procedure

According to the European Pharmacopoeia [12], Glyceric Macerates are liquid preparations obtained from raw materials of botanical, zoological or human origin by using glycerol or a mixture of glycerol and either alcohol of a suitable concentration or a solution of sodium chloride of a suitable concentration. Tinctures are liquid preparations usually obtained using either one part of herbal drug or animal matter and ten parts of extraction solvent, or one part of herbal drug or animal matter and five parts of solvent.

GMs and MTs were prepared by GEALpharma according to the European Pharmacopoeia 8th edition [14], following the procedure deriving from the French Pharmacopoeia. Briefly, buds or other plant materials were transferred to glass jars and the following solvents were added: 50/20/30 (by weight) water/ethanol/glycerol for GMs; 60/40 (by weight) water/ethanol for MTs. Fresh plants were used, and their humidity was calculated on an aliquot of the material. About 1 kg of stuff was treated, and the amount of solvent was adjusted so as to obtain a weight ratio of 1/20 between (calculated) dry plant and final product for GM and 1/10 for MT. After 21 days of maceration, the suspension was filtered and the residue was pressed. The percolate was added to the filtrate, and the GM or MT so obtained was stored in stainless steel containers, from which it was transferred in glass vessel for commercialization.

2.3. Reagents and apparatus

High purity water (HPW) produced with Millipore Milli-Q system was used throughout. The reagents adopted were of analytical grade.

Standard and sample solutions were prepared and stored in high-density polyethylene (HDPE) vessels or in polypropylene Falcon tubes. All vessels were previously washed in 1 M HNO_3, rinsed with HPW and stored in 0.01 M HCl. Standard analyte solutions were prepared by dilution of concentrated stock solutions (Merck Titrisol or Sigma–Aldrich).

Sample mineralization was carried out with a Milestone MLS-1200 Mega (Milestone, Sorisole, Italy) microwave laboratory unit equipped with polytetrafluoroethylene (PTFE) bombs.

The analytes were determined with a Perkin Elmer Optima 7000 (Perkin Elmer, Norwalk, CT, USA) ICP-OES.

2.4. Mineralization and analysis procedures

Buds and other plant materials were dried and smashed with a ceramic knife. Small portions of new (i.e. not used for extract preparation) filters were cut and analysed without further pretreatments.

Aliquots of 0.5 g of solid or liquid sample were transferred into PTFE bombs and added with 5 ml of concentrated HNO_3. The bombs were heated in the microwave oven according to the scheme: 250 W (2 min), 0 W (2 min), 250 W (6 min), 400 W (5 min), 600 W (5 min), and ventilation (5 min). The resulting solutions were filtered on Whatman 5 filters and diluted to 50 ml with HPW or to 25 ml for filter and pure solvent samples. Analyte concentrations were determined by ICP-OES using an external calibration performed with standard solutions prepared in aliquots of sample blanks. The emission wavelengths are shown in Table 1S (Supplementary data).

The accuracy of the procedure was evaluated with a Certified Reference Material (CRM), namely Tomato Leaves SRM 1573a, supplied by the National Institute of Standards and Technology (NIST); analyte recoveries are reported in Table 2S (Supplementary data). Analyses were performed in duplicate and blanks were simultaneously run. The limits of quantification (LoQ) were estimated as ten times the standard deviation of the blank ($10s_b$).

2.5. Data processing

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) [16] were carried out with the aid of XLSTAT 4.4 software package, used as a Microsoft Excel plug-in, whereas Unscrambler X 10:2 was used for Linear Discriminant Analysis (LDA). Unscrambler X 10:2 was also employed for data standardization, obtained by mean-centering (for each variable) and dividing