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High performance liquid chromatographic determination of globularin in *Globularia alypum* L.

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Globularia alypum L. is a widespread sub-shrub found around the mediterranean littoral. The leaves of this plant have been used in traditional medicine as laxative and anti-ulcerous remedy [1–3]. Globularin and catalpol (iridoids) are the major constituents of the plant [4]. Until now, no quantitative HPLC method for the determination of constituents of this plant have been reported. In this work, we have developed and validated a sensitive and precise analytical method for the quantitative determination of globularin in the leaves of *Globularia alypum* using 4-hydroxybenzoic acid methyl ester as internal standard (I.S.). Under the chromatographic conditions used, satisfactory results were obtained within 30 min for the determination of globularin (Fig.). The chromatographic profils obtained from leaves dried at room temperature and by-micro-waves were similar. Two major peaks are detected corresponding to globularin (t_r 23.5 min) and to 4-hydroxybenzoic acid methyl ester (I.S.) (t_r 27.3 min).

The validation procedure was carried out using 4-hydroxybenzoic acid methyl ester as internal standard to quantify globularin which is the major compound in the leave extract. For calibration, globularin standard and aqueous-methanolic extract from leaves dried by micro-waves, were injected with fixed amounts of the internal standard and with five concentrations of globularin standard [range: 5–25–50–75–150 $\mu g \cdot ml^{-1}$] and five concentrations of aqueous-methanolic extract [range: 50–200–300–500–1000 $\mu g \cdot ml^{-1}$]. Calibration curves were plotted by correlating the area ratio (globularin/I.S.) with the corresponding concentration ratios. The assay was linear in the concentration range studied. The coefficients of correlation for the least squares regression lines (Y = globularin/I.S. peak area ratio, X = globularin/I.S. concentration ratio) are reported in the Table.

The precision of the method was tested by both intra-day (n = 6) and interday (3 d, n = 6) reproductibilities for the aqueous-methanolic extract at a concentration of 0.2 $mg \cdot ml^{-1}$. The coefficients of variation were below 2% (Table). The estimated limit of detection for globularin in the extract of leaves was 0.17 $\mu g \cdot ml^{-1}$ and the estimated limit of quantification was 0.61 $\mu g \cdot ml^{-1}$.

The concentration of globularin was determined in aqueous-methanolic extracts obtained from leaves dried at room temperature and by micro-waves. The concentration of globularin was 14.8% in the leaves dried by micro-waves, and 10.9% in the leaves dried at room temperature. This difference in concentration could be explained by the fact that in leaves dried at room temperature, globularin was probably degradated to catalpol by the loss of the cinnamoyl moiety, which is not detectable at 280 nm.

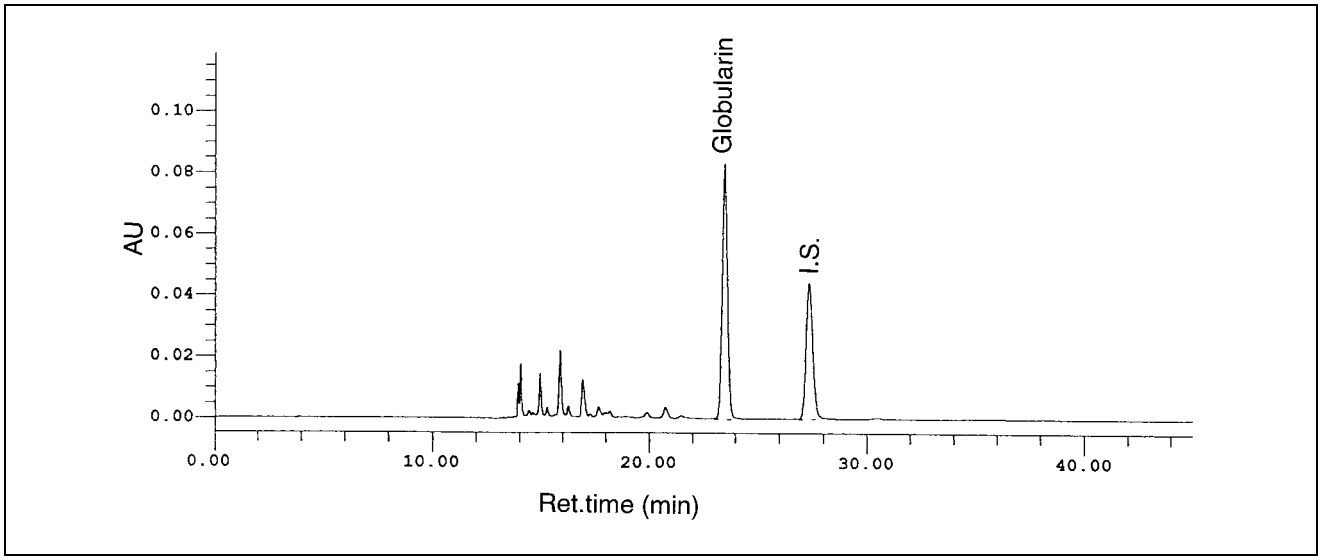

Fig.: Typical chromatogram of an extract of *Globularia alypum* L. leaves dried by micro-waves

Table: Validation data

Linearity	Concentration ($\mu g \cdot ml^{-1}$)	n	Slope	Intercept	Correlation coefficient (r)
Globularin	5–150	3	2.125	−0.025	0.999 93
Globularia extract	50–1000	3	0.297	−0.0027	0.999 98
Precision	**Concentration ($\mu g \cdot ml^{-1}$)**		**n**	**Relative standard deviation (%)**	
Intra-day					
1	200		6	1.05	
2	200		6	0.94	
3	200		6	1.64	
Interday	200		18	1.28	

Experimental

1. Apparatus and HPLC conditions

A Waters (Milford, MA, USA) high-performance liquid chromatograph was used. The HPLC system consisted of two solvent delivery systems (510), an automatic sample injector (WISP 717), and a detector (486), connected to a computer to monitor chromatographic parameters and process data (Millenium, V 2.15). Separations were performed on a reversed-phase Symmetry C-18 column (250 × 4.6 I.D. mm, 5 µm, 100 Å, Waters) and a Symmetry Guard column (20 × 3.9 I.D. mm, 5 µ, 100 Å, Waters). The mobile phase was distilled water acidified with 0.2% of orthophosphoric acid 0.1 M (solvent A) and acetonitrile (Carlo Erba, HPLC-grade) acidified with 0.2% of orthophosphoric acid 0.1 M (solvent B).
The linear gradient was: solvent A/solvent B; v/v [5 min (95 : 5); 7 min (76 : 24); 27 min (76 : 24); 29 min (95 : 5)]. The flow rate was 1 ml · min^{-1} and the injection volume was 20 µl. Detection was assessed at 280 nm. The run time was 30 min.

2. Samples

Globularia alypum L. leaves were collected in Marseille in October 1997. Half of the leaves were dried at room temperature, the others were dried by micro-waves (4 × 1 min; Panasonic 1330; 50 Hz–2680 watts),
All samples are prepared in a solution of 76% of solvent A and 24% of solvent B. 4-Hydroxybenzoic acid methyl ester (Fluka, 8 mg) was dissolved in 20 ml of solvent (I.S. solution).
Pure globularin was isolated from *Globularia alypum* L. according to a previously reported procedure [5]. A stock solution of globularin with a concentration of 300 µg · ml^{-1} was prepared by dissolving 15 mg of globularin in 50 ml of solvent.
Five standard solutions (10–300 µg · ml^{-1}) were made by further dilution of the stock solution; 1 ml of every standard solution was added to 1 ml of I.S. solution.
Extract solutions were prepared from dried and powdered leaves. Powdered leaves (0.1 g, 0.4 g, 0.6 g, 1.0 g and 2.0 g) were macerated and extracted with 200 ml methanol-water (60 : 40). The extract solutions were diluted to 1/5 with the solvent; 1 ml of every diluted solution was added to 1 ml of I.S. solution.

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Adsorption studies of sugars on calcium hydroxy apatite ceramics and haemoglobin on sugar coated ceramics

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Whole blood serves a number of important functions but the most critical function is oxygen transfer. In emergency situations like accidents, major surgery, treatment of chronic anaemias, where the supply of whole blood is low, transfusion of blood or oxygen carrying solutions may provide temporary life support. The various potential applications of the oxygen carrying solutions are reviewed by Minato et al. [1]. A number of approaches have been developed during last 5–6 decades, to transport and deliver oxygen effectively and safely using haemoglobin (Hb), crosslinked Hb, perfluorocarbons etc. but all are associated with several limitations. Various carrier systems were developed in order to overcome problems associated with Hb delivery e.g. microcapsules, liposomes, niosomes, multiple emulsions etc. [2]. Aquasomes have also been developed for the same purpose [3].
Aquasomes are colloidal ceramic-carbohydrate composites on which a variety of therapeutic proteins are attached. Basically, they consist of relatively few atoms clustered in solid crystals onto which glassy carbohydrates are adsorbed. This carbohydrate coated core serves as a non-denaturating solid surface for the subsequent attachment of a wide variety of biochemically active molecules like vaccines, therapeutic proteins etc. [4–5]. Carbohydrates (sugars) protect bioactives from degradation and dehydration effects [6]. Aquasomes are prepared by a three step method which involves: production of ceramic cores, adsorption of sugars, and immobilization/adsorption of bioactive protein.
Adsorption is a surface phenomenon which involves weak Van-der-Waal forces. Drug adsorbates for drug delivery and activated powders for detoxification have long been used in pharmaceutical field [7–8].
In the present study, Langmuir and Freundlich adsorption isotherms of various sugars like cellobiose, maltose, sucrose and trehalose were studied on calcium hydroxy apatite (HA) powder prepared by the precipitation-sintering method. The isotherms for the adsorption of haemoglobin on sugar coated ceramics were also studied. The adsorption parameters were calculated from the graphical data and explained on the basis of molecular arrangement.
Adsorption data of sugars on HA powder were found to fit satrisfactorily to both Freundlich and Langmuir isotherms. The regression coefficients of Freundlich isotherm i.e. the logarithm of the equilibrium concentration against the amount adsorbed per gram of adsorbent (Log C vs. Log x/m) and Langmuir isotherm (C vs. C/{x/m}) were in the range of 0.932 to 0.998. The Freundlich adsorption parameters (Log K, n) and the Langmuir adsorption parameters i.e. binding constant (b), and sugar adsorbed per g of HA(Ym) as determined from the graphical data are shown in the Table. The binding constants were found in the order of cellobiose > maltose > sucrose > trehalose while the amount of sugar adsorbed per g of HA followed the reverse profile. This can be explained on the basis of an epitaxial arrangement of sugars adsorbed or the packing of adjacent molecules of sugars adsorbed on HA pow-