HPLC DETERMINATION OF D-PANTHENOL AND GLYCYRRHETINIC ACID FROM A HERBAL ANTI-HERPETIC SUPPLEMENT

DETERMINAREA PRIN METODA HPLC A D-PANTENOLULUI SI A ACIDULUI GLICIRETINIC DINTR-UN SUPLIMENT ANTIHERPETIC PE BAZA DE PLANTE

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Key words: panthenol, glycyrrhetinic acid, UV-VIS RP-HPLC reversed phase high performance liquid chromatography, Glycyrrhiza glabra, herpes virus.
Cuvinte cheie: pantenol, acid gliciretinic, UV-VIS cromatografie de lichide de performanţă ridicată de fază inversă, Glycyrrhiza glabra, virus herpetic.

Abstract

HPLC was used for the quantitative determination of panthenol and glycyrrhetinic acid in an herbal anti-herpetic supplement containing panthenol (B_5 provitamin) and alcoholic extract of *Glycyrrhiza glabra*. For the determination of panthenol, the separation was carried out on a Kromasil 60-S Hilic D C_{18} column (250x4.6 mm, 5 µm), with methanol – 20 mM potassium phosphate dibasic, pH 6 (10:90, volume ratio) as the mobile phase. The detection wavelength was set at 210 nm. Flow was 1 $mL \cdot min^{-1}$ and the column temperature was maintained at (30±0.5) °C. For glycyrrhetinic acid, a Hypersil C_{18} BDS column (250x4.6 mm, 5 µm) with acetonitrile – 0.1% phosphoric acid in water (75:25, volume ratio) isocratic elution. The detection wavelength was set at 250 nm. Flow was 1 $mL \cdot min^{-1}$ and the column temperature was maintained at (30±0.5) °C.

Rezumat

Metoda HPLC a fost utilizata pentru determinarea cantitativa a pantenol si a acidului gliciretinic dintr-un supliment antiherpes pe baza de plante, constituit din pantenol (provitamina B5) si un extract alcoolic de Glycyrrhiza glabra (lemn dulce). Pentru determinarea pantenolului separarea s-a realizat pe o coloana Kromasil 60D Hilic C18, 250 x 4.6 mm, 5 µ, utilizand ca si faza mobila un amestec format din metanol si o solutie tampon de fosfat dipotasic, 20 mM, pH 6, in raport volumetric de 10/90. Lungimea de unda a fost setata la 210 nm. Fluxul a fost de 1 ml/min, iar temperatura coloanei a fost mentinuta la (30±5) °C. Pentru acidul gliciretinic a fost folosita elutia izocratica pe o coloana Hypersil C18 BDS, 250x4.6 mm, 5 µ, cu un amestec acetonitril – solutie apoasa de acid fosforic 0.1%, in raport volumetric de 75/25. Lungimea de unda a fost stabilita la 250 nm. Fluxul a fost de 1 ml/min, iar temperatura coloanei a fost mentinuta la (30±5) °C.

Introduction

Licorice *(Glycyrrhiza glabra)* is an herbaceous perennial leguminous plant, growing in Southern Europe and in some parts of Asia (India). The useful part is the root, rich in glycyrrhetinic acid (glycyrrhizin). Glycyrrhetinic acid is also used as sweetener, being 30-50 times sweeter than sugar.

The antiviral, antimicrobial, anti-inflammatory and blood pressure enhancing effects of glycyrrhizin were studied both *in vitro* and *in vivo*.

The intravenous and oral administration of glycyrrhizin slows the evolution of viral and autoimmune hepatitis. The topical application is effective against atopic dermatitis.

The anti-hyperlipidemia effect was also demonstrated in the treatment of swelling-induced hyperpigmentation of skin, as well as in the prevention of neurodegenerative disorders and dental cavities.

Anti-ulcer, laxative, anti-diabetic, anti-inflammatory, immunomodulating, antitumoral and expectorant properties of glycyrrhetinic acid were studied as well.

Panthenol (provitamin B5) is an alcoholic analog of pantothenic acid (vitamin B5).

It is used in the pharmaceutical industry, for the preparation of cosmetics and personal care products as hydrating and wetting product, in ointments, lotions, shampoos, nasal sprays, eye drops and contact lenses cleaners.

It is used in ointments at a concentration of 2-5 % to help treating sunburns, mild burns and minor skin wounds; enhances hydration, reduces itching and skin swelling, improves skin elasticity and accelerates wound healing in the epidermis.

1. Materials and Methods

Reagents

- Panthenol CRS (SIGMA) – as internal standard.
- Glycyrrhetinic acid CRS (SIGMA) - as internal standard.
- All the other reagents in use were HPLC-grade, provided by Merck (Germany).
- Water for solutions was obtained in-house with Milli-Q system (Millipore, USA).
- All solutions were prepared on daily basis.

HPLC method

It was performed on LC Surveyor (Thermo Electron Corporation, USA) provided with quaternary pump, auto sampler, 25 µl loop and UV-VIS – diode array detector (Thermo Electron Corporation, USA).

Chromatographic peak integration was done with ChromQuest software (Thermo Electron).

The chromatographic conditions are shown in Table 1.

Table 1.
HPLC parameters for assay of panthenol and glycyrrethinic acid

Panthenol	Glycyrrhetinic acid
Stationary phase KROMASIL 60D HILIC C18 250x4.6 mm, 5µ	Stationary phase HYPERSIL BDS C18 250x4.6 mm, 5µ
Mobile phase Methanol – K_2HPO_4 20 mM pH 6 – 10/90 volumetric ratio	Mobile phase Acetonitril –phosphoric acid, 0.1% aqueous solution - 75/25 volumetric ratio
UV VIS 210 nm	UV VIS 250 nm
Temperature 30°C	Temperature 30°C
Flow 1 ml/min	Flow 1 ml/min

Preparation of standard reference solutions

The *standard working solution of glycyrrhetinic acid* had a:

- final concentration of 0.02 mg/ ml, prepared in water starting from 1 mg/ ml *stock solution of glycyrrhetinic acid* in water.

The *standard working solution of panthenol* had a:

- final concentration of 0.1 mg/ml prepared in alcohol, starting from 1 mg/ ml *stock solution of panthenol* in alcohol.

Preparation of sample solutions

To determine glycyrrhetinic acid:

- inject 5 µl whole product in the chromatographic system.

To determine panthenol in the finished product,

- dilute it 250 times in water and inject 5 µl.

2. Results and Discussions

A reversed phase HPLC method was proposed for the quantitative determination of panthenol and glycyrrhetinic acid in the finished product, selecting the best chromatographic separation conditions.

The chromatographic parameters selected as determinative in this study were: retention time, chromatogram area, resolution (selectivity of method), number of theoretical plates (efficacy of separation).

The chromatogram assay of standard reference and sample solutions revealed that there are no differences between the tested target compounds and the rest of finished product matrix (figures 1 and 2).

The HPLC assay of both active compounds in the anti-herpetic herbal supplement also involved a similar product on the market; the results were similar, confirming the reproducibility of method.

A chromatographic assay was also performed on various batches of finished product, obtaining comparable results (figure 3).

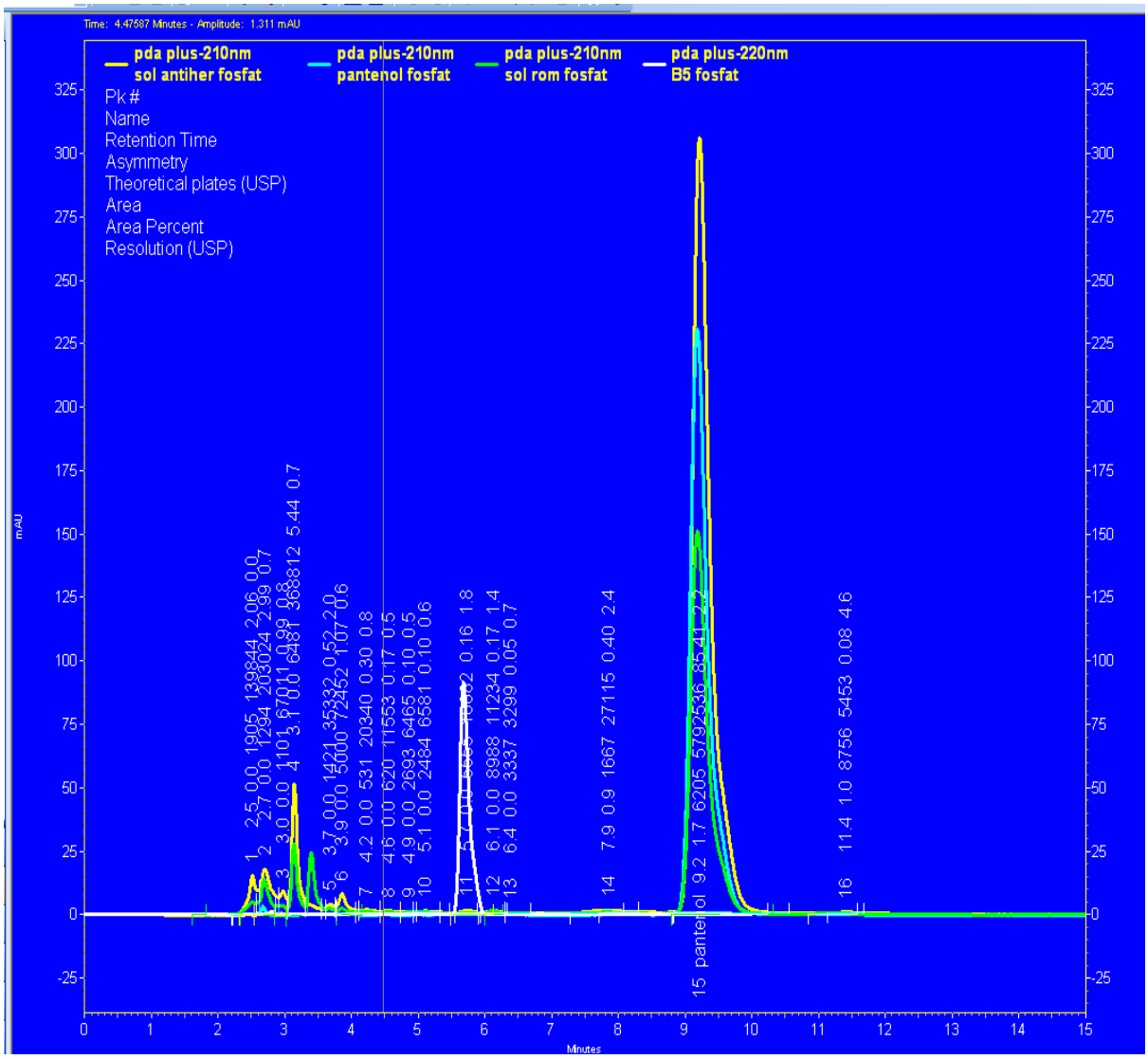

Figure 1. A comparison between ant herpetic solution and reference substance D Panthenol

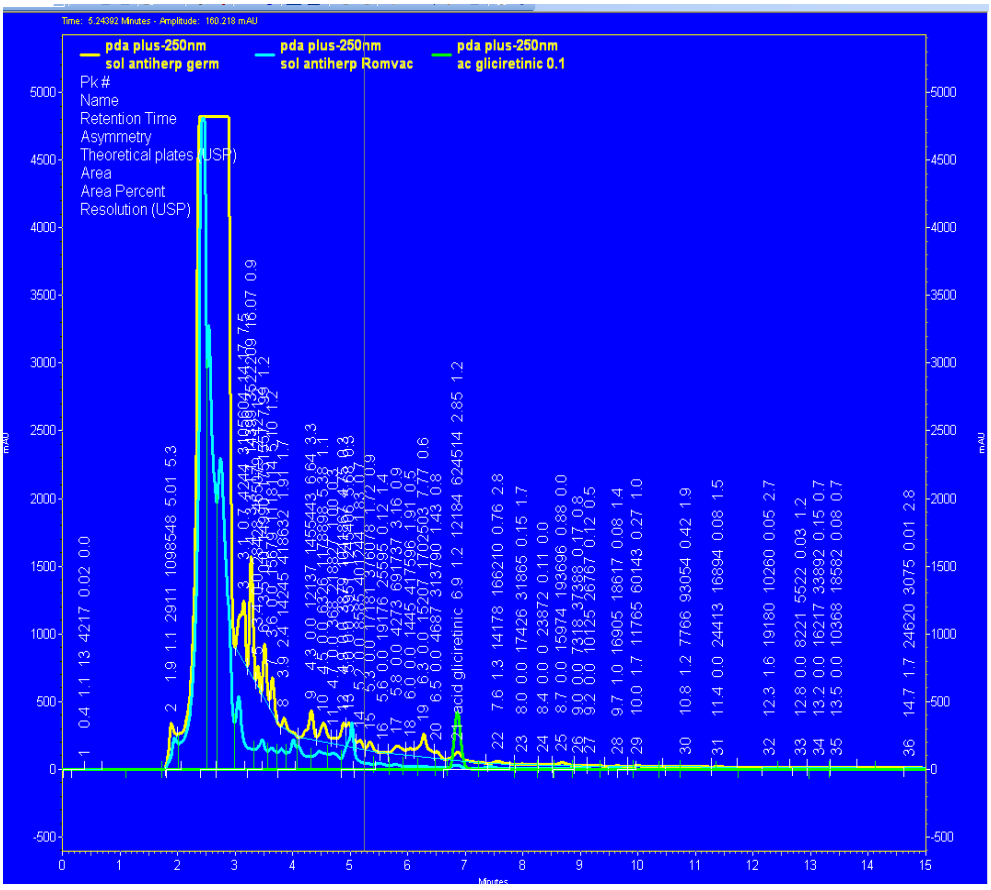

Figure 2. A comparison between ant herpetic solution and reference substance glycyrrethinic acid

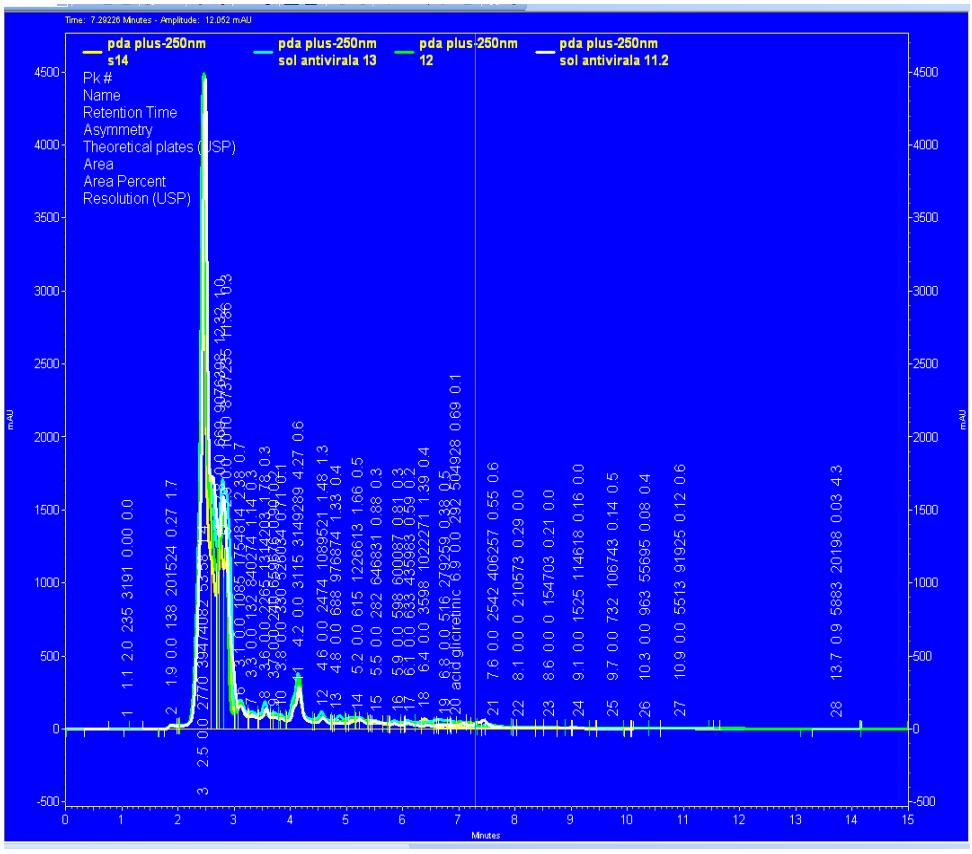

Figure 3. Reproducibility of final product batch

3. Conclusions

An HPLC method was developed for the determination of panthenol and glycyrrhetinic acid in a natural anti-herpetic product.

The chromatogram of reference substances and finished product substances confirmed the specificity of the proposed method of analysis.

The HPLC assay of successive finished product batches was a safe assay tool for the reproducibility of the manufacturing process.

The HPLC assay of panthenol and glycyrrhetinic acid was successfully performed on a similar product on the market.

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