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Original Article

Identification of *Centella Asiatica* Extract in a Cosmetic Cream

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Abstract

Regulatory requirements call for the identification of any claimed active ingredient in a cosmetic formulation while quantification of its amount in the product is not mandated. Quality control of products requires certain analysis. They need to assure dependable results for better identification of ingredients in cosmetic products as well as raw materials. Active ingredients for Centella cosmetic cream include extracts of *Centella asiatica*. This ingredient is a plant extract that is profiled by chromatographic analysis. The analysis is also a suitable detection method to determine presence of this active ingredient in cosmetic products. Thin layer chromatographic (TLC) analysis, a traditional method of profiling the extract of *Centella asiatica,* was employed along with the new approach of using High Performance Liquid Chromatography (HPLC). The TLC and HPLC methods used for the Centella cream were developed based on the European Pharmacopoeia monograph for *Centella asiatica* plant material. A normal phase TLC analysis was performed using Silicagel as a stationary phase and a mobile phase containing water. An RP18 column and a linear gradient were used in the HPLC method. This study reports presence of asiaticoside in samples of Centella cream both by TLC and HPLC. The presence of madecassoside was also determined by TLC. This paper reports use of these chromatographic methods for the determination of *Centella asiatica* extract in a cosmetic cream and finds it applicable for quality control purposes of other cosmetic creams containing *Centella asiatica*.

Keywords: *Centella asiatica* extract, cosmetic cream, quality control, chromatographic methods.

Introduction

Centella cosmetic creams or emulsions contain—among other actives—extracts of *Centella asiatica*. These extracts contain triterpene saponins, mainly asiaticoside and madecassoside and sapogenins (asiatic and madecassic acids) in smaller quantities. Among these compounds, asiaticoside has been reported to alter gene expression and induce the synthesis of type I collagen in human fibroblasts.[1,2]

Madecassoside has also been shown to have wound-healing properties.[3] The increase in collagen synthesis by asiaticoside in *C. asiatica* has also been found to be useful in functional cosmetics.[4]

The Argentinian (ANMAT) and Brazilian (ANVISA) health authority requires the performance of an assay that allows the identification of claimed active ingredients in cosmetic formulations, without quantifying components.

When the active ingredient is a plant extract, this identification is performed by chromatographic analysis to define a profile. This profile should be detected in the cosmetic product.

In this work, the chromatographic profile of *Centella asiatica* extract and the presence of asiaticoside and madecassoside were determined in the sample solution by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC), for comparison with a reference extract of Centella and a standard solution of asiaticoside. A simple method based on the extraction of saponins in methanol was used.

Materials and Methods

Preparation of Samples

The analysis required that sample solutions be prepared fresh and just before each analysis. The solvent, time and temperature of extraction were optimized during the development of analytical methods for this study. The following solutions were prepared in triplicate:

Sample solution: A solution of cosmetic cream in MeOH of 100 mg/ml was prepared employing an ultrasonic bath (45 °C, 10 minutes). This solution was cooled at room temperature and filtered.

Solution of Centella asiatica extract: A solution of *Centella asiatica* dry extract raw material in MeOH of 1 mg/ml was prepared using an ultrasonic bath (45 °C, 10 minutes).

Asiaticoside reference solution: A solution of 1 mg/ml in methanol of asiaticoside was prepared.

Excipients blank solution: It was prepared in the same manner as the sample solution.

The TLC and HPLC methods used for the Centella cream were developed based on the European Pharmacopoeia monograph for *Centella asiatica* plant material.

Both methods were validated using the parameters of the Argentinian Pharmacopoeia (7th Edition)[5].

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All fingerprints must be identical. The variability of the Rf values of the asiaticoside marker should not exceed 0,02 and Rt times 0,5, respectively, for repeatability.

The percent recovery of asiaticoside from the cream was 97.3% (R.S.D. 0.5%). The limit of detection (LOD) was calculated by analyzing standard solutions of decreasing concentrations, in order to establish the lowest concentration that could be detected with a signal-to-noise (S/N) ratio of 3 an LOD of 2.03 µg/ml was established.

TLC Identification

TLC analysis was performed using as a stationary phase silica gel Merk HF_{254} with 0.2 mm thickness, the mobile phase was ethyl acetate, formic acid, acetic acid, water (100:11:11:27). Anisaldehyde sulfuric reagent (heated at 100 ºC for 5–10 min) was employed as spot detection.[6]

The sample solution, the *C. asiatica* extract solution, the asiaticoside solution and the excipient blank solution were applied as a 10 mm band on TLC plate using a capillary tube. The development distance was 9.0 cm.

HPLC Identification

The HPLC method was developed and performed with a Varian® 9000 instrument using a diode array detector to verify the chromatographic peaks purity. An RP18 column (Gemini® 5µm, 150mm×4.6mm) was used. The gradient was linear and the mobile phase was constituted by solvent A) 3 ml of orthophosphoric acid in 1000 ml of water and solvent B) acetonitrile. Gradient: 22% B to 42 % B in 40 min and 42 % B to 60 % B in 5 min. The flow rate was 1 ml/min. Rheodyne injector fitted with a 20µl loop was used. Detection: 205 nm.

Results and Discussion

Plant extracts by their very nature comprise a complex mixture of compounds. Sample cleanup is important in order to eliminate interfering compounds or allowing concentration of the actives when they occur in trace amounts. It has the disadvantage of sample loss with the consequent decrease in accuracy.

Related to this, the advantage of chromatographic systems developed by TLC and HPLC is that they present an adequate resolution. Also they allow the application of the sample on the plate and the analysis of the sample solutions without the use of cleanup methods.

After revealed with anisaldehyde sulfuric reagent, similar spots at Rf and colour with the profile of *C. asiatica* extract solution were observed in the chromatographic profile of the cosmetic cream. In the lower third of the chromatogram, a dark green spot (Rf: 0.21) corresponding to asiaticoside (Figure 1) was observed. Below this band, in the chromatographic profile a purple band (Rf 0.15) corresponding to madecassoside is observed.[7]

The extract of *C. asiatica* raw material was analyzed by TLC and HPLC using asiaticoside as a marker because of its greater commercial availability and lower cost. In that way, asiaticoside, the major active principle in the raw material extract employed, was identified. This active substance is used by the pharmacopoeias for the detection and quantification of *C. asiatica.* Furthermore made-

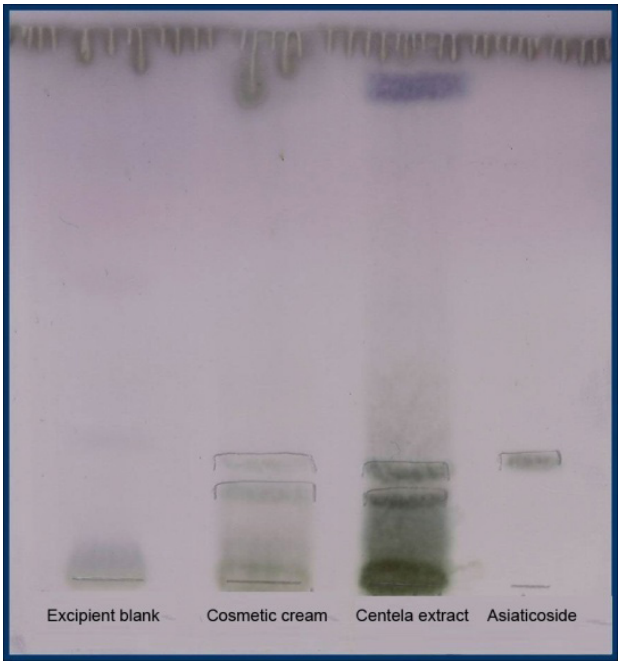

Figure 1. TLC analysis. Excipients blank; cosmetic cream; Centella extract; Asiaticoside.

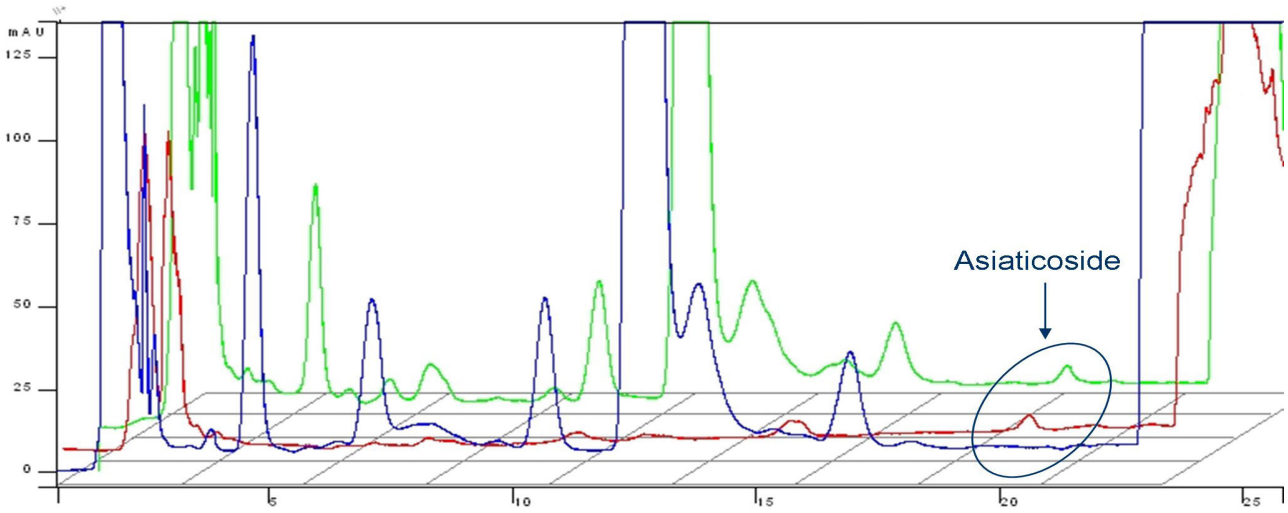

Figure 2. HPLC analysis. References: In blue, excipients blank; in red, Centella extract and in green, cosmetic cream.

cassoside was identified by TLC, by comparison with its Rf and colour in the profile described by the European Pharmacopoeia. The HPLC analysis of the cosmetic cream showed the presence of *C. asiatica* extract by the identification of asiaticoside (tr: 20 min approximately and UV spectra) and the presence of the chromatographic profile of the *C. asiatica* extract raw material. It was made by comparison with the retention times and UV spectra (Figures 2, 3) by discounting the peaks corresponding to the blank of excipients.

Conclusions

Chromatographic methods used proved to be suitable for the determination of *Centella asiatica* extract in a cosmetic cream. Therefore this work is a contribution to the quality control of cosmetic creams containing *Centella asiatica*.

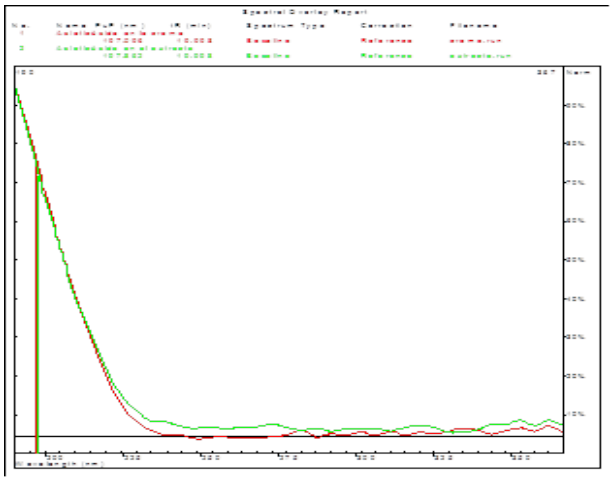

Figure 3. HPLC/UV analysis. References: In green UV spectra asiaticoside in Centella extract. In red UV spectra asiaticoside in cosmetic cream.

Acknowledgments

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