Extraction and Analysis of Cosmetic Active Ingredients from an Anti-Cellulitis Transdermal Delivery System by High-Performance Liquid Chromatography

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Abstract

A new transdermal delivery system that controls cellulitis is evaluated using reversed-phase high-performance liquid chromatography coupled with photodiode array detection. An extraction procedure and the validation of the analytical method to assay the active excipients from the *Centella asiatica* plant (asiaticoside, madacessic acid, and asiatic acid) are described. Excellent results are obtained in terms of linearity, accuracy, and specificity of the analytical method.

Introduction

The treatment of cellulitis has already been widely reported upon, and numerous ways to treat it (oral products, creams, or injections), even though they are widely spread throughout the market, are more or less efficient. Oral administration, injections, or cream applications present a particular inconvenience for the patient, because they need to be repeated in single doses due to the variation with time (as a Gaussian curve) of the drug concentration (1,2).

Presently, the transdermal therapeutic system (TTS) that remedies this major drawback of dependence is more and more often used. This medical product, consisting of a combination of an adhesive component and a drug, is applied to the skin. Then, the drug migrates from the system through the skin and into the body, resulting in a constant drug concentration in the body for a prolonged time. The obvious benefit of TTS is that it maintains a constant, prolonged, and therapeutically effective drug level in the body.

Actually, this product is already well-known in the therapy of hypertension, motion sickness, and smoking cessation, corresponding to TTSs of clonidine, scopolamine, and nicotine, respectively, which are commercially available (3).

In this paper, a new transdermal delivery cosmetic system (TDCS) that controls cellulitis is evaluated. The triterpene saponins and aglycone extracted from the *Centella asiatica* L. plant, whose eutrophic action in the treatment of cellulitis is widely reported, are used as the active principles of the TDCS (4,5). Important aspects, such as the extraction of the drug from the patch and the assessment of the efficiency of the procedure, are discussed. Chemical analysis monitoring at 200 nm is performed using an efficient reversed-phase high-performance liquid chromatography (HPLC) procedure and a diode array detector (6). Obviously, detection at such a low wavelength exhibits a few difficulties due to the absorbance of any transparent solvent at this wavelength. The goal was to demonstrate, using analytical tools, that this TDCS aimed at treating cellulitis is efficient and reliable.

Experimental

Materials

Ortho-phosphoric acid (99% crystal, Merck, Darmstadt, Germany) and bidistilled water (Carlo Erba, Milan, Italy) were used to prepare the phosphoric acid solution at 0.3%; this solution and acetonitrile (gradient-grade for chromatography, Merck) were both freshly filtered on 0.2-µm membrane filters (Merck) and used as the HPLC eluent. The eluent was degassed with helium throughout the chromatographic run.

Standards

Reference samples of asiatic acid, madecassic acid, and asiaticoside (Indena, Milan, Italy) were used to set up the analytical method.

Instrumentation

An HPLC system (Star 9012 SDS, Varian, Palo Alto, CA) possessing an integrated proportioning solvent delivery system was used. The system was equipped with a photodiode array detector (9065 polychrom, Varian). The entire analytical

apparatus was controlled by an LC Star Workstation (Varian) that also processed data. A Varian MICROPAK 5-µm C_{18} column (40 mm × 12.5 cm) was used.

Chromatographic conditions

Chromatographic runs were carried out with an acetonitrile–water gradient elution system. The solvents were phosphoric acid at 0.3% (solvent A) and acetonitrile (solvent B). From the beginning up to 4 min, the mobile phase was constant (H_3PO_4–CH_3CN, 70:30). From then to 20 min, the gradient was linear from 30 to 50% acetonitrile. The flow rate from the beginning to 20 min went from 1 to 1.2 mL/min. A re-equilibration time of 5 min was necessary before starting a new run. The separation was performed at the room temperature (6–8).

Cosmetic patch

The cosmetic anticellulitis patch was prepared using 5 mg of an extract of *Centella asiatica* in which asiatic acid, madecassic acid, and asiaticoside were the active components. This new TDCS prepared for the study can be defined as a matrix system, which means that the active ingredient is contained in a reservoir as a semi-solid solution, and the skin predominantly controls the rate of release of active ingredient from the system. In the course of its production, the next layers were loaded into the system: an impermeable backing membrane to contain the system, an adhesive layer where the active principle is dispersed in polyacrylate, and a protective liner that enables the storage of the system in a package before its use (3,9).

Preparation of the adhesive–active principle combination

The most important stage of the production of the TDCS is the preparation of the adhesive and active principle. Indeed, it must be taken into account that the adhesive used has to be compatible with the active principle to enable the system integrity during coating and drying.

A stability study, such as a hydrolysis or a photolysis of the labile functional groups of the active ingredients present in the patch, would be required in order to evaluate the degradation of excipients used. In this report, such a study will not be taken into account.

In this case, a special acrylic polymer suitable for medical use in TTS was employed (2,9). *Centella asiatica* L. extract (1 g) was dissolved in the minimum amount of CH_3OH, the solution was concentrated, and 34 g of adhesive was added to the solution. The combination was turbid but perfectly homogeneous.

For this experimental study, the different layers of the patch were first spread manually onto a silicone sheet, and after a 24-h period for drying, they were directly transferred onto the PVC impermeable backing membrane. In the future, obviously, the patch production will be performed by an automatic process in order to have the best reproducibility.

Results and Discussion

Specificity of the analytical method

The determination of the specificity of this analytical method ensures that the signal measured in the analytical conditions only comes from the substance to be analyzed, which means that no interference comes from the excipients, degraded products, or impurities. Specificity was studied using a standard of the three active principles and a patch

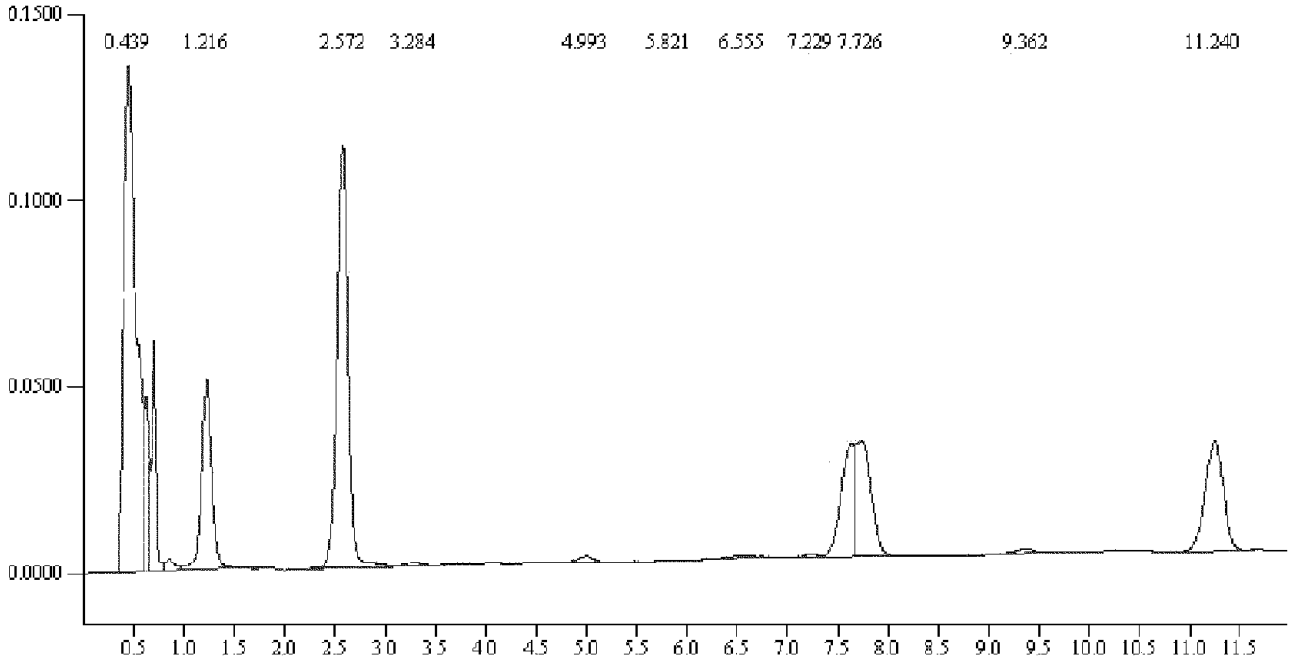

Figure 1. Chromatogram of *Centella asiatica* L. extract.

containing the active cosmetic ingredients.

The chromatograms corresponding to the samples are shown in Figures 1 and 2. Each of them illustrates a separation of the three active principles (asiaticoside, madecassic acid, and asiatic acid) contained in *Centella asiatica* L.

It should be observed that the madecassic peak is doubled. Indeed, in this case, it is possible that two chemically stable isomeric forms are present either in the *Centella asiatica* L. extract or in the pure standard. In the course of this study, the improvement of the low resolution of these peaks will not be attempted, because the most important factor is to assess the total quantity of active principles.

Linearity

In order to prove the capacity of the method to give results directly proportional to the concentration of the substances analyzed in the sample in the considered range, a linearity study was performed by injecting (in triplicate) 5 solutions whose active ingredient concentration varies from 80 to 120% of the product theoretical concentration. Whereas for asiaticoside, the study range is 1–2 mg/patch, for madecassic acid and asiatic acid, the concentration range varies from 0.75 to 1.5 mg/patch, because the respective proportions are 40, 30, and 30% of the *Centella asiatica* extract powder (10).

A mother solution of 0.6 g/L in active principle was prepared. Pure standards of asiaticoside, madecassic acid, and asiatic acid were weighed in such quantity to have a final proportion of 40, 30, and 30%, respectively, of the total mass of the mother solution. Then, 4 dilutions from 1:2 to 1:5 were created in order to have 5 solutions of each compound to analyze.

The linear regression curves corresponding to the 3 compounds are shown in Figure 3. In the case of the madecassic acid curves, the peak height was considered instead of the peak area because of the poor resolution of the peaks corresponding to the two stable isomeric forms of this compound, as it was stated previously. Furthermore, curves have been drawn for the total peak height obtained when adding the peak height of two isomers.

Optimization of the extraction procedure

Triterpenes extraction

Five mg of the *Centella asiatica* extract were introduced on each patch. Asiaticoside, asiatic acid, and madecassic acid were present at 40, 30, and 30%, respectively, in the active mixture and were extracted from new and used (meaning after its application to skin) patches after 1, 2, or 3 days. After comparison with pure standards, the quantity of each active compound in the *Centella asiatica* extract was determined, and the extraction recovery was calculated.

Seven extractions were performed, leaving the patches under agitation in 10 mL of 3 different solvents: CH_3OH, dimethylformamide (DMF), and a mixture of toluene and pentane-dione (90:10). The results obtained with this third mixture showed very poor recovery, perhaps because of a weak solubility of the active ingredients in this solvent. On the other hand, chromatograms obtained when analyzing the samples coming from the DMF extraction showed numerous peaks all along the chromatographic separation. Indeed, DMF is a very strong solvent, being able to extract most of the components present in the patch. The best recovery was obtained with CH_3OH, which acts as a specific solvent for the present active principle. It is important to notice that all experiments were performed with patches containing 4 mg of *Centella asiatica*.

Figure 4 and Table I show the extraction recovery as a func-

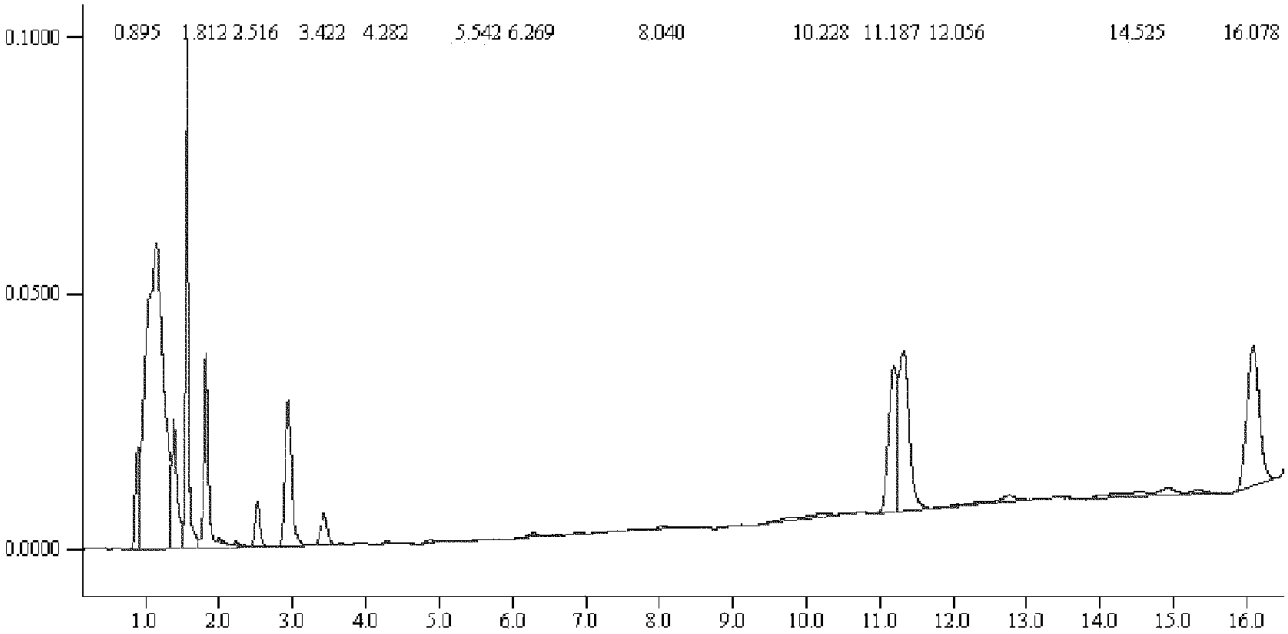

Figure 2. Chromatogram of a new patch containing the active ingredients.

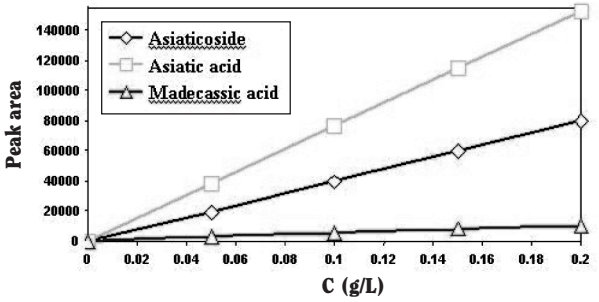

Figure 3. Linear range of the study for the triterpenes of *Centella asiatica*.

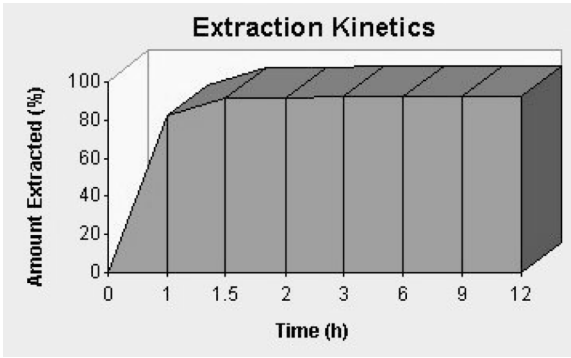

Figure 4. Optimization of triterpenes extraction time on new patches.

Table I. Optimization of the Extraction Time

	Extraction time		
	3 h	6 h	24 h
Asiaticoside (mg)	1.38	1.40	1.39
Madecassic acid (mg)	0.97	0.95	0.97
Asiatic acid (mg)	1.23	1.21	1.31
Total mass extracted (mg)	3.58	3.60	3.67
Extraction recovery (%)	89.5	90	92

Table II. Extraction Procedure Validation of Used Patches

	Recovery (%)		
	1.0-mg patches	2.5-mg patches	5.0-mg patches
Extract 1	86	92	89
Extract 2	99	93	88
Extract 3	99	83	94
Extract 4	97	87	90
Extract 5	97	83	89
Extract 6	99	90	88
Average	96.167	88.000	89.667
Standard deviation	5.076	4.382	2.251
Coefficient of variation (%)	5.278	4.979	2.510
Average recovery for the three concentrations		91.440	
Adjustment factor		1.0936	

tion of the extraction duration. It can be noticed that after only 3 h, 89.5% of the total active ingredient mass was already reached. Furthemore, increasing the extraction time of 3 h results in only a small gain in terms of extraction recovery. Therefore, it was useless to continue this study with an extraction time longer than 24 h. For these experiments, an extraction duration of 3 h was maintained.

Extraction procedure validation

In order to have a method suitable to check the active principle content in the patch, it was important to validate the extraction procedure and optimize its recovery. A method was needed to evaluate the active principle content in a used patch. Therefore, the main objective was to evaluate the recovery of the extraction procedure. On the other hand, it was necessary to validate the procedure over a wide concentration range (10). For this purpose, patches having an active ingredient content of less than 5 mg were produced (2.5 and 1 mg) in order to simulate used patches with a different active ingredient absorption degree (50 and 80%, respectively).

The results in terms of recovery are shown in Table II. It can be concluded that the extraction is perfectly reliable on either new or used patches.

Accuracy

The accuracy parameter is required to evaluate the homogeneity of the content of produced patches in order to control the production step. This study was used to verify that the values found experimentally are consistent with the theoretical values.

According to the *United States Pharmacopoeia*, the determination of accuracy must be performed on 10 samples, regardless of the size of the lot considered. In the case of the

Table III. Accuracy of the Analytical Method

	Mass (mg)			
	Asiaticoside	Asiatic acid	Madecassic acid	Total mass
Extract 1	1.83	1.29	1.34	4.46
Extract 2	1.81	1.33	1.33	4.47
Extract 3	1.73	1.49	1.50	4.72
Extract 4	1.78	1.43	1.40	4.61
Extract 5	1.78	1.34	1.37	4.49
Extract 6	1.78	1.33	1.32	4.43
Extract 7	1.80	1.40	1.35	4.55
Extract 8	1.79	1.45	1.40	4.64
Extract 9	1.83	1.30	1.44	4.57
Extract 10	1.81	1.32	1.30	4.43
Average	1.794	1.368	1.375	4.537
Standard deviation	0.0295	0.0692	0.0611	0.0978
Coefficient of variation (%)	0.0164	0.0506	0.0444	0.0215
Accuracy (%)	10.3	8.8	8.3	9.2

transdermal system, the verification of the uniformity of dosage units can be carried out on 10 samples if the three following conditions are met: the active ingredient must lie within the range of 85.0–115.0% of the label claim in no less than 9 of the 10 dosage units (patches); no unit should be outside the range 75.0–125.0 % of the label claim; and the relative standard deviation of the 10 dosage units must be less than or equal to 6.0% (10).

The results are summarized in Table III. It is important to note that the results obtained here are very satisfying when speaking in terms of a manual production.

Conclusion

The assessment method that has been developed and validated is reliable for the assay of the active ingredients of *Centella asiatica* L. extract. Unfortunately, the absorption into the skin was very poor; however, this was only a preliminary study. Considering the excellent results gained in terms of accuracy and precision of the of the analytical method, this study on TDCS will be further developed to increase the active ingredient released by the addition of new excipients capable of improving the penetration through the skin (11). In that case, a stability study will be certainly necessary because of a more important number of ingredients being together.

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