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Development of Analytical Procedures for Quantification of Psorantron C®

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ABSTRACT

Background: Psorantron C® is an ointment containing dithranol (1,8-dihydroxy-9-anthrone) and salicylic acid as active ingredients, and it stops the uncontrolled division of cells in tissues affected by psoriasis. It is an original medication developed at the Tbilisi State Medical University (TSMU) I. Kutateladze Institute of Pharmacochemistry. The primary active ingredient in the ointment is dithranol. The salicylic acid determines keratolytic properties, which improves the therapeutic effect of the drug. Dithranol and salicylic acid were selected as chemical and biological markers for the development of a method for quantification of the ointment.

Objectives: This study aimed to develop a new method for quantifying the ointment Psorantron C®, developed at the Tbilisi State Medical University (TSMU) Iovel Kutateladze Institute of Pharmacochemistry.

Methods: High-performance liquid chromatography (HPLC) separation was performed on an Eclipse plus C-18 column (250mm x 4.6mm, 5µm) with a solvent system of acetonitrile-water (60:40) under isocratic conditions, and UV detection was performed at 254 nm and 354 nm.

Results: The dependence of the concentration of solutions on the peak area is linear R^2=0.999. Method Standard Deviation (SD)≤1% and Relative Standard Deviation (RSD) ≤1.5%.

Conclusions: The method provided is accurate, sensitive, and reproducible, which can be successfully used for the quantification of Psorantrone C® ointment.

Keywords: Dithranol; high-performance liquid chromatography (HPLC); Psorantron C®; salicylic acid.

BACKGROUND

Psoriasis is a chronic inflammatory multi-factorial skin disease. The causes of the disease are not yet known, but scientists believe that a hereditary factor may contribute to its development. Various theories exist, with the most well-founded being those related to the nervous, infectious, and immune systems. The disease can appear at any age, but it is more common during puberty. Not infrequently, psoriasis has a family character. Men get sick more often than women. There are different forms of psoriasis: diffuse, droplet, and erythrodermic.[1-3]

Psoriasis does not affect the internal organs, except for the arthropathic form, which causes damage to the joints. Psoriasis treatment includes both internal and external therapy. According to statistical data, patients with psoriasis account for 3-10% of all skin patients.

Dithranol (1,8-dihydroxy-9-anthrone) has been actively used in the topical treatment of psoriasis for over a century and can provide prolonged remission.[4] Psorantron C®, a dithranol-based drug for the treatment of psoriasis, was developed at the TSMU I. Kutateladze Institute of Pharmacochemistry, for which a Patent was issued. The active substances are dithranol and salicylic acid. The former prevents the uncontrolled division of cells in psoriatic tissue, and the latter confers keratolytic properties to the medication, thereby enhancing the overall therapeutic effect of the drug. Ointments with 0.5% and 1.5% dithranol and 3% salicylic acid are recommended in medical practice.[5-7]

METHODS

Study materials

Dithranol - chemically pure (Sigma-Aldrich, Co). Salicylic acid - chemically pure (Sigma-Aldrich, Co). HPLC-grade acetonitrile was purchased from VWR. Purified water for high-performance liquid chromatography (HPLC) analysis was obtained from a Millipore Classic system.

Study equipment

Agilent Technologies Model 1260 liquid chromatograph, equipped with vacuum degasser, binary pump, auto-sampler, and photodiode detector (DAD). The system was piloted with the Chemstation computer program. Chromatographic separation was achieved using an Eclipse plus C-18 column (6.0x250 mm; 10 mm). Mobile phase: water and acetonitrile (40:60, v/v). Mobile phase flow rate - 1 ml/min. The injection volume was 10 µl. All analyses were performed at room temperature. UV spectra were recorded in the range of 200-400 nm. Quantification was performed at the following wavelengths: 254 nm and 354 nm.

Preparation of standard solutions

A standard solution of Psorantron C® was prepared in methanol at 1.0 mg/ml. A series of dithranol and salicylic acid

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working solutions (n=5) with different concentrations (0.0019-1.0 mg/ml) were prepared. All prepared standard solutions were filtered through a 0.45µm membrane filter (Millipore, ref HVPL04700).

Preparation of the test solution

20.0 mg of Psorantron C® is added to a flask with a volume of 100 ml in methanol; after opening the test object, the volume is filled with methanol to the brim. 2 mL of the preparation was filtered through a membrane filter (0.45 mm Millipore) into an HPLC vial.

The content of the active components was calculated based on the following formula:

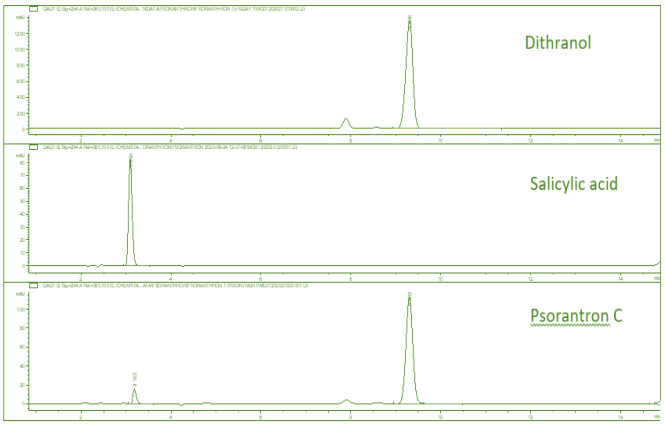
$$C = \frac{S1 \text{ x } m2}{S2 \text{ x } m2} \text{ x } 100$$

where S1: sample peak area; S2: standard peak area; m1: sample mass; m2: standard mass.

RESULTS

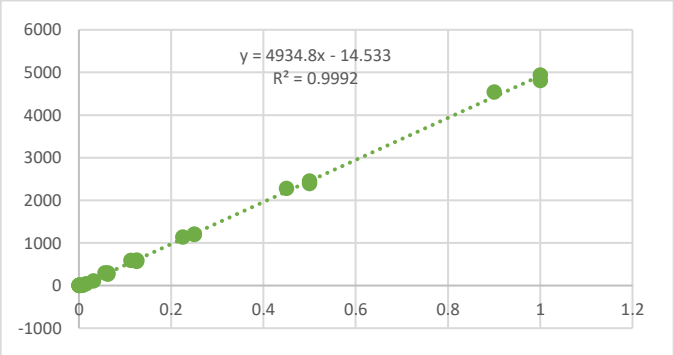
HPLC separation conditions were optimized to achieve optimal peak resolution. Optimal separation of Psorantron C® was achieved on a reversed-phase Eclipse plus C-18 (250mm x 4.6mm, 5µm). To ensure optimal separation, the effect of the mobile phase - acetonitrile-water 60/40, v/v ratio - was studied. A solution of dithranol and salicylic acid was prepared in methanol. The retention times of dithranol and salicylic acid were found to be 9.3- and 3.1-min. Measurements at 354 and 254 nm showed sufficient sensitivity and a satisfactory chromatographic baseline. As a result, under the optimized conditions, base separation was achieved at 9.3 and 3.1 min with symmetrical, sharp, and well-defined peaks of dithranol and salicylic acid. A chromatogram showing the complete separation of dithranol and salicylic acid in Psorantron C® is presented in Figure 1.

FIGURE 1. High-performance liquid chromatography (HPLC) profile of Dithranol and salicylic acid and Psorantrone C® ointment



The HPLC analysis method was developed for the quantitative determination of dithranol and salicylic acid ointment Psorantron C®. The calibration curves were linear over the different concentration ranges. Correlation coefficients were greater than 0.999 for dithranol and salicylic acid (Fig.2).

FIGURE 2. Calibration curve and linearity of Dithranol



The inter-day % RSD was less than 2.5%, and the intra-day % RSD was less than 3.36%, showing good precision of the method. The recoveries determined for each component ranged from 99.68 to 100.29% for Psorantron C®, and the RSDs were less than 1.4%, indicating good precision. The detection limit was 0.78 µg/ml. The limit of quantification was 3.8 µg/mL for Dithranol and Salicylic acid.

DISCUSSION

As a drug of choice for treating psoriasis, dithranol has been used in various formulations for many decades. The development of convenient and straightforward assay methods for each formulation is essential for drafting normative documentation and, thus, the subsequent quality control of a given drug. Psorantron C®, a dithranol and salicylic acid-containing ointment for the treatment of psoriasis, developed at the TSMU I. Kutateladze Institute of Pharmacochemistry, required a simple and convenient quantitative analysis method.[8]

Compared to other methods, HPLC assays are rapid, convenient, and cost-effective, allowing for the simultaneous assessment of multiple components. Therefore, they are perfect for quantitative analyses of multicomponent medications. Several HPLC methods have been developed to determine dithranol and its main degradation products in various formulations.[9-12]

The described HPLC method for quantifying 1.5% Psorantron C® ointment, developed and validated in this study, is fast, sensitive, and simple, requiring no complicated sample

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pre-treatment and is reproducible; hence, it can be included in the normative documentation of the drug.

CONCLUSIONS

A simple, isocratic, reversed-phase HPLC method for the determination of dithranol and salicylic acid in the ointment Psorantron C® has been developed and validated. The proposed method is particular, accurate, and precise, requiring minimal sample preparation with a short run time, making it suitable for routine quantitative analyses. The validation confirms the method's reliability and reproducibility, underscoring its practical value.

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