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COMMUNICATION

Determination of Betamethasone Dipropionate and Salicylic Acid in Pharmaceutical Preparations by High-Performance Liquid Chromatography

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ABSTRACT

The simultaneous determination of betamethasone dipropionate (BD) and salicylic acid (SA) in both ointment and topical solution was developed using high-performance liquid chromatography (HPLC). The method was standardized using a LiChrospher® 100 RP-18 (125 × 4 mm, 5 μm) column, acetonitrile–tetrahydrofuran–acetic acid 1% (25:20:55 v/v), apparent pH 3.3, as mobile phase, and UV detection at 254 nm. The peak area response versus concentration was linear in a concentration range from 5.0 to 50.0 μg/ml of BD and from 20.0 to 200.0 μg/ml of SA. The correlation coefficients were 0.9997 for BD and 0.9987 for SA, and the relative standard errors of estimates were 1.38% for BD and 3.27% for SA. The coefficient of variation and the recovery average were, respectively, 0.41–1.15% and 100.09% for BD, and 0.57–0.95% and 99.79% for SA.

INTRODUCTION

Betamethasone dipropionate (BD) is a glucocorticoid used only in topical therapy because of its high oil–water partition coefficient (1). The association of BD in topical pharmaceutical preparations is efficient in the treatment of various dermatoses because of the anti-inflammatory property of BD and the antibacterial and antifungal actions of salicylic acid (SA) (2).

Although many chromatographic methods have been reported for the determination of SA in pharmaceutical preparations containing aspirin (3–7), just a few methods have been found in the literature for the determination of BD in topical preparations (8–11).

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A chromatographic method for the simultaneous determination of BD and SA in ointment and topical solution, which presented a very good resolution in the chromatographic separation of both drugs, was developed in this work.

EXPERIMENTAL

Apparatus

HPLC separations were made on a system comprising a CG solvent delivery pump (model 480-C) and a CG variable UV detector set at 254 nm (0.32 aufs) connected to a CG integrator (model CG-200) (Instrumentos Cientificos CG Ltda., São Paulo, Brazil). The system was equipped with a Rheodyne 7125 injection valve fitted with a 20-µl loop.

Reference Substances, Reagents, and Solutions

Betamethasone was donated by Shering-Plough S/A (Brazil) and salicylic acid was of pharmaceutical grade. These substances were used as reference substances without further purification. All reagents and solvents were of analytical grade. The solvents used in the mobile phase were of HPLC grade. Distilled water was purified using a Milli-Q system (Millipore, Milford, MA). All solutions were filtered through a hydrophilic Millipore® Durapore filtration membrane (0.22 µm pore size).

Chromatographic Conditions

The mobile phase used was acetonitrile–tetrahydrofuran–acetic acid 1% (25:20:55 v/v), apparent pH 3.3. The analytical column was a LiChrospher® 100 RP-18 (125 × 4 mm, 5 µm) column in a LiChro CART® (125-4) (Merk, Darmstadt, Germany). All analyses were done under isocratic conditions at a flow rate of 1 ml/min and at room temperature.

Samples

Sample 1 was a commercially available sample (topical solution) and sample 2 was a simulated sample (topical solution), both with the following composition: 0.64 mg of BD, 20.0 mg of SA, and excipient q.s.p. 1.0 ml. Sample 3 was a commercially available sample (ointment) and sample 4 was a simulated sample (ointment), both containing 0.64 mg of BD, 30.0 mg of SA, and excipient q.s.p. 1.0 g.

Sample Preparation

Topical Solutions

A sample amount containing 1.28 mg of BD and 40.0 mg of SA was weighed. The sample was transferred to a 50-ml volumetric flask and completed to volume with the mobile phase. A 10-ml aliquot was transferred to a 50-ml volumetric flask and completed to volume with the mobile phase. The solution contained 5.12 µg of BD/ml and 160.0 µg of SA/ml. The solution was filtered and 20 µl was injected.

Ointment

A sample amount equivalent to 1.28 mg of BD and 60.0 mg of SA was weighed. The sample was transferred to a 125-ml Erlenmeyer flask, 50 ml of mobile phase was added, and the mixture was heated at 70°C in a water bath until complete fusion of the ointment. After the mixture cooled, this procedure was repeated twice, then the mixture was cooled in an ice-bath for 10

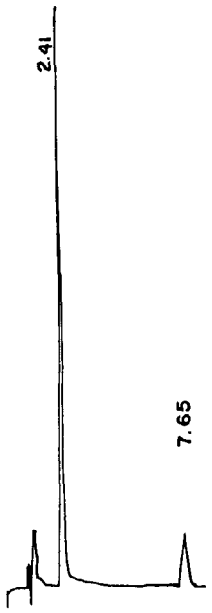

Figure 1. Chromatogram of the commercially available topical solution (sample 1): 160.00 µg of SA/ml, (retention time [RT] = 2.41 min); 5.12 µg of BD/ml, (RT = 7.65 min). Conditions: LiChrospher® 100 RP-18 (5 µm) in LiChroCART® (125-4) column; mobile phase: acetonitrile–tetrahydrofuran–acetic acid 1% (25:20:55 v/v) apparent pH 3.3, at room temperature; loop: 20 µl, detection at UV 254 nm; flow rate: 1.0 ml/min.

Table 1

Results Obtained in the Recovery Tests, Coefficient of Variation, and Confidence Limit for p = 95%, *Using the HPLC Method*

	Recovery[a] (%)		Coefficient of Variation (%)		Confidence Limit[b]	
Sample	BD	SA	BD	SA	BD	AS
1	99.98	99.69	1.15	0.95	96.38 ± 0.79	98.13 ± 0.67
2	100.06	99.90	0.56	0.79	100.39 ± 0.40	99.61 ± 0.56
3	100.30	99.61	0.41	0.72	104.38 ± 0.31	103.66 ± 0.53
4	100.02	99.96	0.66	0.57	100.58 ± 0.47	99.94 ± 0.41

[a]Average of two determinations
[b]Average of 10 determinations.

min, filtered into a 50-ml volumetric flask, and completed to volume with the mobile phase if necessary. The solution contained 25.60 μg of BD/ml. For the determination of SA, a 3.0-ml aliquot of the last dilution was transferred to a 25-ml volumetric flask and completed to volume with the mobile phase. This solution contained 144.0 μg of SA/ml. Both solutions were filtered and 20 μl of each solution was injected.

Standard solutions were prepared in the same concentrations. The final concentrations of BD and SA in the samples were calculated by comparison of sample and standard peak area obtained with the average of three injections of standard solutions.

RESULTS AND DISCUSSION

The wavelength of 254 nm was selected in order to permit the simultaneous determination of both active substances in the topical solutions (samples 1 and 2), because SA is present in a much higher concentration than BD and shows minimum absorption in this wavelength, and BD presents maximum absorption.

The mobile phase containing acetonitrile–tetrahydrofuran allowed the elution of BD with adequate retention time and the addition of acetic acid permitted the SA to remain in a non-ionized form with an increase in its retention time (Fig. 1).

The calibration curve was obtained in a concentration range from 5.0 to 50.0 μg of BD/ml and from 20.0 to 200.0 μg of SA/ml. The regression curve was calculated by the least-squares method. The correlation coefficients were 0.9997 for BD and 0.9987 for SA, and the relative standard errors of estimates were 1.38% for BD and 3.27% for SA.

The recovery tests were performed according to the Association of Official Analytical Chemists (AOAC) (12). BD and SA standard solutions were added to sample solutions and the recovery data are presented in Table 1. Recovery tests confirmed the accuracy of the proposed method and there was no excipient interference. The coefficient of variation and the confidence limits (p = 95%) of the results are also presented in Table 1.

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