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Simultaneous HPLC Analysis of Betamethasone and Clotrimazole in Cream Formulations

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Abstract

An HPLC method for the simultaneous quantitative determination of betamethasone and clotrimazole in cream formulation has been developed. The method utilizes a reversed-phase C18 (250 X 4.0 mm) stationary phase, with a mixture of methanol-acetate buffer-acetonitrile (33:27:40, v/v) as a mobile phase, and spectrophotometric UV detection at 254 nm. The method has been validated for cream formulations containing betamethasone and clotrimazole with linear range of 0.025 to 0.075 mg/ml for betamethasone with a correlation coefficient of 0.9996, and linear range of 0.25 to 0.75 mg/ml for clotrimazole with a correlation coefficient of 1.000. The results demonstrated that this method is accurate, precise, specific, linear, reliable, sensitive, and fast.

Keywords: HPLC; Betamethasone; Clotrimazole; Pharmaceutical preparations

Introduction

Betamethasone is a potent synthetic glucocorticoid that is widely used for the treatment of inflammation, allergies and other diseases related to glucocorticoid deficiency [1]. Clotrimazole is a chlorinated synthetic imidazole derivative having antifungal and antibacterial activities, which are used in the treatment of some infections [2]. The combination of betamethasone and clotrimazole is used for the treatment of candidiasis, vulvovaginal candidiasis and other species of Candida [3-5] and provides anti-inflammatory action.

In the scientific literature, analysis of betamethasone and clotrimazole has been reported as individual ingredients [1-2,6-14] and in combination products [15]. Betamethasone has been determined in different pharmaceutical preparations by HPLC [1,6-7]. Clotrimazole has been determined in different pharmaceutical preparations by: Titration method [2], gas liquid chromatography [8], high performance TLC (HPTLC) [9], micellar electrokinetic chromatography (MEKC) [10] and by HPLC [11-14]. Reversed-phase LC for the simultaneous determination of betamethasone and clotrimazole in cream formulations has been described in the USP [15]. However, sample preparation of the cream in this USP method is time consuming (about one hour), tedious (requires centrifuge and heating). The main objective of this study is, therefore, to develop and validate an HPLC method involving minimum sample preparation, good resolution, reasonable analysis time, good accuracy, high precision, good specificity, good linearity, and excellent reliability.

Material and Methods

Equipments and settings

The HPLC measurements were carried out using a Merck Hitachi HPLC (Hitachi, Ltd. Tokyo, Japan) equipped with a manual loop injector that was connected to a photo diode array detector, and a recorder.

An analytical column with C18 stationary phase (250 X 4.0mm i.d.) bonded onto 5µm silica gel manufactured by Merck (Darmstadt, Germany) was used for chromatographic separation. Degassing of the mobile phase was performed using Sonnicator (Fisher Scientific FS 220). Instrumental HPLC settings were as follows: flow rate 1.5 ml/min; injection volume 5µl, column temperature ambient; and wavelength 254nm.

Reagents

All the active and inactive ingredients of the cream were kindly supplied by Jerusalem Pharmaceuticals Co. Ltd., Al-Bireh, Palestine, and were of British Pharmacopoeia (BP) or Untied States Pharmacopoeia (USP) quality, and were used without further purification. Acetonitrile and methanol (HPLC grade) are from J. T Baker (NJ, USA). All other chemicals were of analytical reagent grade and they are from Merck (Darmstadt, Germany). Water used was distilled and deionised by passing through water purification system.

Acetate buffer with a pH of 6.8 was prepared by dissolving 25.0 g ammonium acetate in 1000 ml of distilled deionised water. Diluent was prepared by mixing 990 ml of methanol and 10 ml of acetic acid. The mobile phase, standard and sample solutions were filtered using 0.45µm microporous filters type polyamid.

Standards and sample preparation

A standard solution having 0.05 mg/ml and 0.5 mg/ml of betamethasone and clotrimazole, respectively was prepared as follows: 100 mg of betamethasone was dissolved in 100 ml diluent (Solution A), 50 mg of clotrimazole was dissolved in 10 ml diluent (Solution B). Then, 5 ml of Solution A and 10 ml of Solution B was diluted to 100 ml with diluent.

The sample was prepared by weighing 5.0 g of the cream which is equivalent to 5.0 mg of betamethasone and 50.0 mg of clotrimazole in a100 ml-beaker, and then an adequate volume of diluent was added with stirring until homogeneous solution was obtained. The solution was transferred to a 100 ml volumetric flask and the volume was completed to 100 ml with diluent.

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Solutions for validation study

Linearity and range: Stock standard solution having 0.5 mg/ml and 5.0 mg/ml of betamethasone and clotrimazole, respectively was prepared by dissolving 50 mg of betamethasone and 500 mg of clotrimazole in 100 ml diluent. Five different concentrations of betamethasone and clotrimazole were prepared from the sock solution as follows: 5 ml of stock solution was diluted to 100 ml with diluent (0.025 mg/ml of betamethasone and 0.25 mg/ml of clotrimazole), 15 ml of stock solution was diluted to 200 ml with diluent (0.0375 mg/ml of betamethasone and 0.375 mg/ml of clotrimazole), 10 ml of stock solution was diluted to 100 ml with diluent (0.05 mg/ml of betamethasone and 0. 5 mg/ml of clotrimazole), 25 ml of stock solution was diluted to 200 ml with diluent (0.0625 mg/ml of betamethasone and 0.625 mg/ml of clotrimazole), and 15 ml of stock solution was diluted to 100 ml with diluent (0.075 mg/ml of betamethasone and 0.75 mg/ml of clotrimazole).

Accuracy (Recovery): For recovery study, the placebo of the cream formulation was prepared according to the formulation procedure. Then, to the required quantity of the placebo, a known quantity of betamethasone and clotrimazole was added to get three concentration levels of betamethasone and clotrimazole (50%, 100%, and 150% of the working concentration of betamethasone and clotrimazole).

	% recovery	
Concentration level	Betamethasone	Clotrimazole
50	99.6	101.0
100	100.3	100.6
150	98.6	99.8
	Average: 99.5	Average: 100.5

Table 1: percentage recovery of betamethasone and clotrimazole at three concentration levels.

Day	% Betamethasone*	% Clotrimazole*
1	98.3±0.86	99.7±0.52
2	98.6±0.59	98.3±0.65
3	98.2±0.50	99.3±0.77
4	99.2±0.62	98.6±0.34
5	98.5±0.78	98.5±0.70
6	99.3±0.89	99.3±0.72

*Mean ± R.S.D. for four samples

Table 2: Intermediate-precision of the method for analyzing betamethasone and clotrimazole in cream formulations.

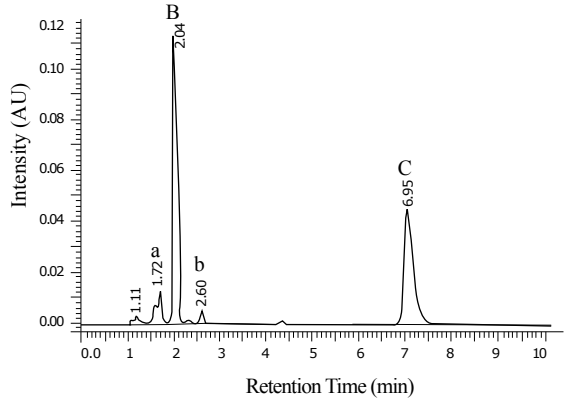

Figure 1: Typical chromatogram of pharmaceutical combination containinig 0.05 mg/ml of betamethasone, and 0.5 mg/ml of clotrimazole. Column: C18 (25cm X 4.0mm i.d). Mobile phase: methanol/buffer/acetonitrile (33:27:40, v/v). Flow rate: 1.5ml/min; λ: 254 nm. Peaks identification, B: Betamethasone, C: Clotrimazole, a, b: Excipients from the cream preparation.

Results and Discussion

Method development

Reversed-phase LC-method was employed for the chromatographic separation of betamethasone and clotrimazole. To this end, reversed-phase C8 and C18 columns using mixture of organic solvents (acetonitrile, and methanol) and aqueous buffer as a mobile phase was tested. While C8 column does not show enough resolution between these two analytes, C18 column shows adequate resolution. In order to optimize the chromatographic parameters, the effects of the buffer, methanol, and acetonitrile volume fractions on the separation of betamethasone and clotrimazole were studied. The optimum composition of the mobile phase was selected based on obtaining stable baseline, sharp peaks in reasonable time, and good separation of betamethasone and clotrimazole from each other and from the excipients present in the cream formulation. The selected composition was methanol/acetate buffer (pH = 6.8)/acetonitrile with a ratio of 33:27:40 by volume. A typical chromatogram of betamethasone and clotrimazole (prepared from the cream preparation) is shown in Figure 1.

Method validation

After method development, validation of the current method was performed in accordance with USP requirements for assay determination (Category-I: Analytical methods for quantitation of active ingredients in finished pharmaceutical products) which include accuracy, precision, selectivity, linearity and range.

Linearity and range: To evaluate linearity of the method, five calibration standards of betamethasone and clotrimazole containing 0.025 to 0.075 mg/ml of betamethasone and 0.25 to 0.75 mg/ml of clotrimazole were analyzed. A plot of peak area vs. amount injected was linear in the range of 0.025 to 0.075 mg/ml of betamethasone with a correlation coefficient of 0.9996, and in the range of 0.25 to 0.75 mg/ml of clotrimazole with a correlation coefficient of 1.000.

Accuracy (Recovery): Percentage recovery of betamethasone and clotrimazole using this method was determined by analyzing the three samples of the cream (prepared as in section 2.4.2) and the percentage of betamethasone and clotrimazole in the samples was calculated at the three concentration levels (50%, 100%, and 15%), by simple proportion from peak areas of the sample and a standard. Results have shown that the mean recovery of betamethasone and clotrimazole is within 100 ± 2.0%, see Table 1.

Precision: Instrumental precision of this method was determined by injecting the standard solution of the two analytes six times. The RSD of peak areas of betamethasone and clotrimazole for the six replicates was found to be 0.73% and 0.52% for betamethasone and clotrimazole, respectively.

Intermediate-precision of the method was also evaluated by analyzing six samples of the two analytes at six days. Results which are represented in Table 1 show good intermediate-precision of the method (average percentage is 98.7% and 99.0% for betamethasone, and clotrimazole, respectively). Furthermore, RSD of the four samples was found to be less than 1.0%, see Table 2.

Selectivity: Selectivity of the current method was demonstrated by good separation of betamethasone and clotrimazole. Furthermore, betamethasone and clotrimazole are good separated from the excipients of the cream preparation as seen in Figure 1.

Conclusion

The method represents a fast analytical procedure for simultaneous determination of betamethasone and clotrimazole in cream formulations with good accuracy, precision, reproducibility, linearity, selectivity, and reliability. The sample preparation is simple, and the elution is isocratic. The method is amenable to the analysis of large number of samples with excellent precision and accuracy.

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