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Stability indicating RP-HPLC method for simultaneous determination of Beclomethasone Dipropionate and Clotrimazole from dosage form

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Abstract : A simple and economical isocratic reverse phase liquid chromatographic (RP-HPLC) method for fast, effective, accurate and simultaneous determination of Beclomethasone Dipropionate and Clotrimazole has been reported. Separation was achieved using methanol and 20 mM potassium dihydrogen orthophosphate at pH 6.8 maintained with dilute orthophosphoric acid solution on Inertsil - ODS 3V column having 250 x 40 mm i.d and particle size 3 µm with isocratic programme as eluent at a constant rate of 1.0 ml per minute. The peaks were analyzed at 254 nm. Retention time of Beclomethasone dipropionate and Clotrimazole was 3.6 and 4.5 minutes. The proposed method was validated and successfully used for estimation of Beclomethasone dipropionate and Clotrimazole.
Key words : RP-HPLC, Beclomethasone Dipropionate, Clotrimazole and Validation.

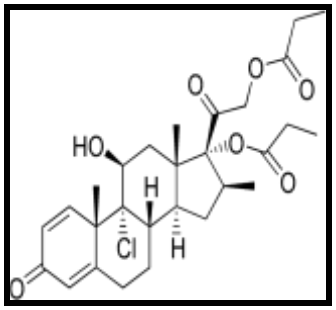
Introduction

Beclomethasone Dipropionate is chemically 9-chloro-11β, 17, 21- trihydroxy-16β - methyl pregna-1, 4-diene -3, 20 - dione 17, 21-dipropionate and is a corticosteroid with glucocorticoid activity. It is anti-inflammatory (topical) and hence antiallergic and antiasthmatic. It is a white crystalline powder and is slightly soluble in water. **Clotrimazole** is chemically 1– (α- chloro,-α, α-diphenylbenzyl)imidazole. It is colourless, crystalline, weakly alkaline and practically insoluble in water but soluble in acetone, chloroform and ethanol. It forms stable salts with both organic and inorganic acids and is used topically in superficial fungal infections[1] like pityriasis versicolor and dermatophytosis[2]. Both molecules have been officially reported in IP[5], EP[6] and USP[4]. Formulations containing both components are available in market in the form of cream.

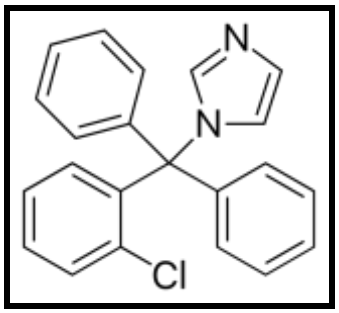
Several methods[11] have been reported for the effective separation of Beclomethasone Dipropionate (I) in pharmaceutical formulations. In the present work, a new, simple, fast and accurate stability indicating HPLC method for simultaneous determination of Clotrimazole in presence of Beclomethasone Dipropionate has been discussed and validated[4-5]. Better separation efficiency and lower consumption of organic solvent can be achieved by using new chromatographic columns with unique stationary phases such as hybrid materials chosen due to their wide pH range and specialized separation.

Few Chromatographic methods such as HPLC[3, 8,9,10] **and** HPTLC[11] have been reported for the simultaneous determination of various pharmaceutical combinations but literature survey has revealed very few chromatographic methods for Beclomethasone dipropionate and Clotrimazole combination. The main objective of study is to provide a simple, rapid, efficient, reliable and economic method for the simultaneous determination of Beclomethasone dipropionate and Clotrimazole. The proposed developed method has been subsequently validated as per ICH guidelines [3-4].

Structure:



Beclomethasone dipropionate



Clotrimazole

Materials and Methods (Experimental)

Chemicals and Reagents

Standard Beclomethasone Dipropionate and Clotrimazole were obtained from local pharmaceutical company with claimed purity 99.7%. All the solutions were prepared in double distilled water. Methanol used for mobile preparation was from E.Merck Science (Germany) and ortho-phosphoric acid (AR grade) was supplied by Zen Chemicals, Thane. Mobile phase was filtered using 0.45µm syringe filter made by Millipore and Whattman filter paper No.41 (purchased from local market) was used in the preparation of sample solution

Apparatus and Chromatographic Conditions

The LC system (Thermo Separation Products) consists of P-2000 Binary Pump solvent delivery system, a Rheodyne injector (7725i) fitted with a 20 µl loop, column oven and a photodiode array detector. The output signal was monitored and processed using a Chromquest version 3.0 software. Shimadzu UV Visible double beam spectrophotometer was used to scan the two drugs in order to select the wavelength for analysis.

Chromatographic Mode	Isocratic
Column	Inertsil-ODS 3 V, 25cm length x 3.5 mm i.d 3µm particle size
Wavelength	254 nm
Column oven temperature	40°C
Injection Volume	20.0 µl
Flow rate	1.0 ml/min
Mobile Phase A	Buffer solution, Filter and Degas
Mobile Phase B	Methanol
Buffer Solution	20 mM potassium dihydrogen orthophosphate in distilled water. Adjust pH of above solution to 6.8 with orthophosphoric acid. Filter and degas.
Diluent	Methanol (HPLC grade)

Praparation of Standard Solution

Weigh accurately 100 mg of Beclomethasone Dipropionate WS and 100 mg Clotrimazole WS, transfer it into a 100 cm^3 standard flask, add 30 ml of diluent and sonicate to dissolve. Allow it to cool to room temperature, mix well and make up to the volume with diluent.

Analytical Method Validation [3-4]

System Suitability

System suitability tests are used to ensure reproducibility of the equipment. System suitability has been checked by recording Theoretical plates and Tailing factor for both Beclomethasone Dipropionate and Clotrimazole which is given in **Table.1**

Specificity

The specificity of method was confirmed by observing the chromatograms of both the combined standard solution and sample solution. The chromatograms obtained from the sample solution were found to be identical to those obtained for standard solution.

The addition of the standard solution to the drug sample solution did not change the characteristics of chromatograms. This gives the validity of method for the determination of both the drugs from combined pharmaceutical formulation

Linearity and Range

The linearity for Beclomethasone Dipropionate and Clotrimazole was observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges of 100 µg/mL to 300 µg/mL for both. The calibration curves were constructed with concentration (C) against peak area. The slope, intercept, regression equation and correlation coefficient for both was obtained as given in **Table-1.**

Table. 1: Method validation parameters for the determination of Beclomethasone Dipropionate and Clotrimazole.

Parameters	Values	
	Beclomethasone Dipropionate	**Clotrimazole**
System suitability **Theoretical Plates-** **Tailing Factor-**	 More than 5500 1.1	 More than 8900 1.30
Linearity range (µg/mL)	100 to 300 µg/mL	100 to 300 µg/mL
Slope (m) [a)]	3619.05	7110.442
Intercept(c) [a)]	-180952	-355590
Correlation coefficient (R^2)	1.000	1.000
LOD (µg/mL)	10 µg/mL	10 µg/mL
LOQ (µg/mL)	30 µg/mL	30 µg/mL
Intraday precision (n=5)	0.59%	0.65%
Interday precision (n=5)	0.79%	0.71%
Assay	98% to 102%	98% to 102%
Recovery	98% to 102%	98% to 102%

LOD and LOQ

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ respectively. The LOD and LOQ for both the drugs were 10 µg/mL and 30 µg/mL as given in **Table-1.**

Intra-Day and Inter-Day Precision

The intra-day and inter-day precision was used to study the variability of the method. It was checked by recording the chromatograms of sample solutions of Beclomethasone Dipropionate and Clotrimazole at three different levels i.e. 75% ,100% and 125% both at intra-day (five times within 24 hour) and inter-day (two times

each, during 3 day intervals) to check the precision. The mean % RSD for intra-day and inter-day precision was found to be less than 1% for both. Results of intra- and inter- day precision studies have been given in **Table-1.**

Robustness

Robustness of the method was examined by the consistency of peak height and peak shape. Since it is a measure of its capacity to retain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage, robustness of the method was performed by intentionally modifying the chromatographic conditions such as composition of mobile phase, change in flow rate and change in oven temperature.

Chromatographic parameters of each analyte such as retention time, tailing factor, resolution and theoretical plates were measured at each changed conditions.

Accuracy (Recovery)

Recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of standard addition. A fixed volume of standard solution was mixed with different concentrations of preanalyzed sample solutions and mixtures were analyzed by proposed method. Percentage recovery was determined at different levels i.e. from 10 % to 30 % level. The results of recovery analysis for Beclomethasone Dipropionate and Clotrimazole have been shown in **Table-2.**

Table 2. Results of Recovery studies of Beclomethasone Dipropionate and Clotrimazole

Standard	Level	Amount of Std added	Amount of Std Found	RSD (%) (n = 5)	Recovery (%)
Beclomethasone Dipropionate	10%	20.0	20.20	0.49	101.0 %
	20%	40.0	40.18	0.40	100.45%
	30 %	60.0	59.85	0.55	99.75 %
				Mean	**100.40%**
Clotrimazole	10%	30.0	19.80	0.61	99.00%
	20%	40.0	40.35	0.42	100.87%
	30 %	60.0	60.80	0.53	101.33 %
				Mean	**100.40%**

Result and Discussion

In the present work, conditions were optimized for development and validation of a simple and accurate RP-HPLC method for simultaneous quantification of Beclomethasone Dipropionate and Clotrimazole in pharmaceutical drug formulation. Method development was right from optimization of the condition and parameters i.e. selection of system, column, mobile phase. Different compositions of mobile phases have been tried and finally Buffer **:** Methanol in the ratio 600 **:** 400 was found to be the most appropriate composition because both the components were eluted with good resolution and good peak shape. Under the described experimental conditions, sharp peaks belonging to Beclomethasone Dipropionate and Clotrimazole were obtained at retention time of 3.6 min and 4.5 min respectively. The regression analysis was found 1.0 for Beclomethasone Dipropionate and Clotrimazole which shows the response is linear from 100 ppm to 300 ppm. Selectivity showed no interference or overlapping of peaks either due to excipients or degradation products.

The developed chromatographic method was validated using ICH guidelines. A new chromatographic method has been developed and subsequently validated for the simultaneous quantification of both the analytes from a combined drug formulation. The advantages of this method for analytical purposes lie in the rapid determination, its cost effectiveness, easy preparation of the sample, good reproducibility. In addition to this, proposed method is found to be more simple, economic, accurate and practical. Thus the present method can be

recommended for routine stability analysis in quality control of Beclomethasone Dipropionate and Clotrimazole along with its degradation products in cream.

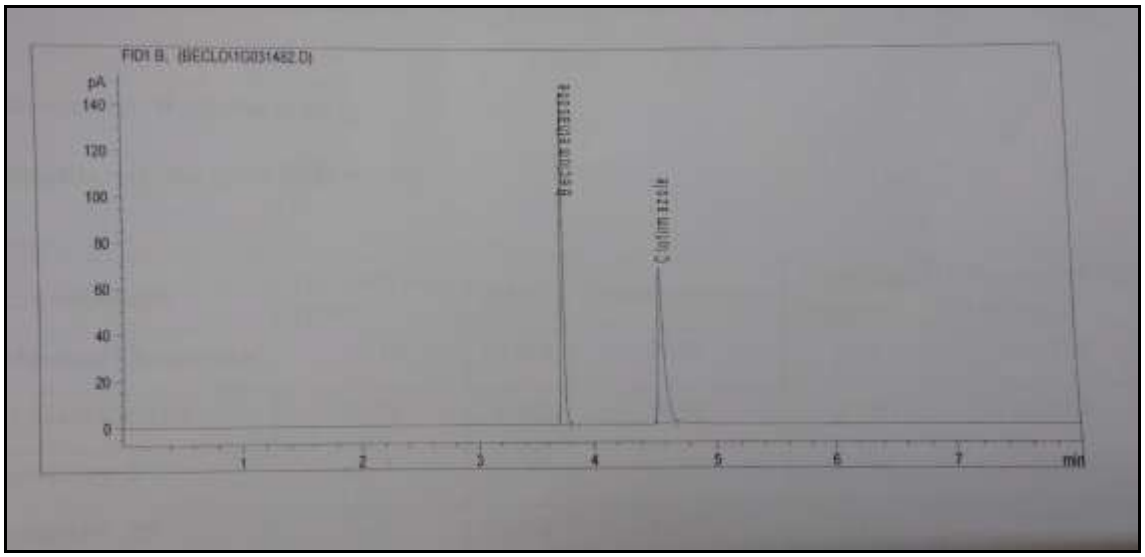


Figure-1. Chromatogram for Beclomethasone Dipropionate and Clotrimazole standard

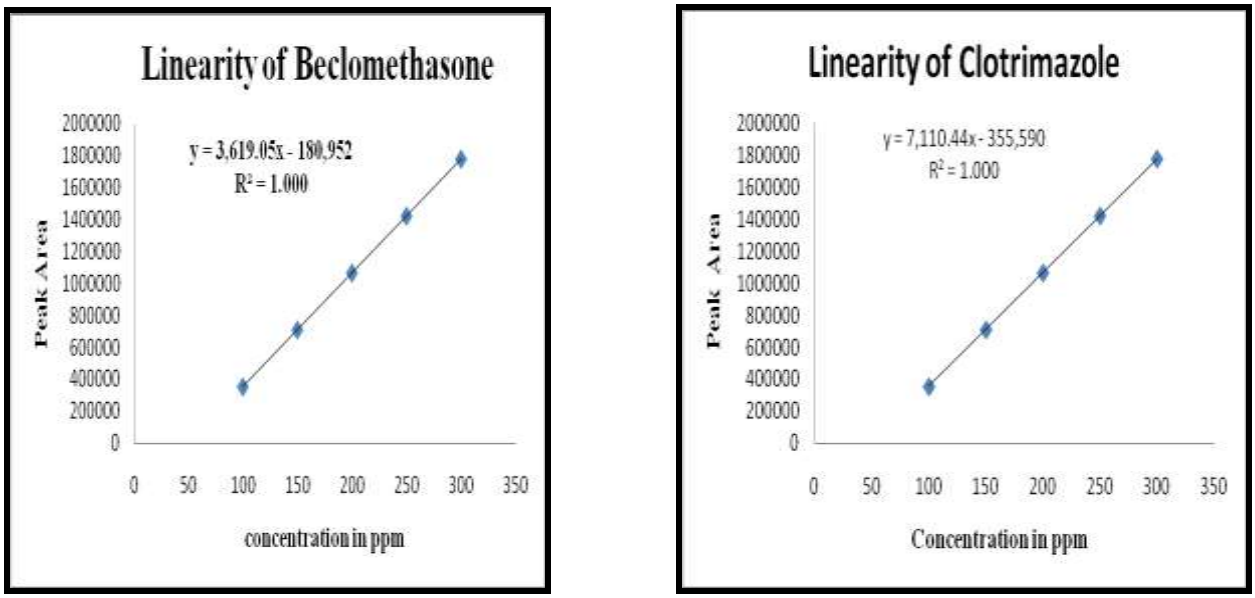


Figure-2. Linearity graph for Beclomethasone Dipropionate and Clotrimazole standard

Acknowledgement

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