Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF BECLOMETHASONE DIPROPIONATE AND SALBUTAMOL SULPHATE

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ABSTRACT

Objective: To develop a validated stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of Beclomethasone dipropionate (BEC) and Salbutamol sulphate (SAL) in bulk and combined dosage form.

Methods: An isocratic, RP-HPLC method was developed using Hi Q Sil C_{18} (250 x 4.6 mm, 5 µm) column and 1 mM ammonium acetate buffer and methanol (15:85 v/v) as mobile phase at flow rate of 1 ml/min and detection wavelength of 230 nm.

Results: The chromatographic conditions yield good separation between drugs with retention time (RT) of 7.75±0.03 min and 2.36±0.09 min, for BEC and SAL, respectively. The method was validated with respect to linearity, precision, accuracy and robustness. The data of linear regression analysis indicated a good linear relationship over the range of 5–30 µg/ml concentrations with a correlation coefficient (r^2) of 0.997 for both the drugs.

Conclusion: The developed method was found to be simple, sensitive, selective, accurate, and repeatable for simultaneous analysis of BEC and SAL and can be adopted for routine analysis of these drugs in bulk and pharmaceutical dosage form.

Keywords: High performance liquid chromatography (HPLC), Beclomethasone dipropionate, Salbutamol sulphate, Stability indicating, Validation.

INTRODUCTION

Beclomethasone dipropionate (BEC) chemicaly is 9 α-chloro-11β-hydroxy-16 β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropionate (fig. 1-A) and Salbutamol sulphate (SAL) chemically is (RS)-1-(4-hydroxy-3-hydroxy-methylphenyl)-2-(tert-butylamino) ethanol sulphate (fig. 1-B). Beclomethasone dipropionate is a steroidal drug used in asthma; while Salbutamol Sulphate is a β2 agonist. There may be many drugs in individual form, and in combination forms available in the market, but Salbutamol Sulphate in combination with Beclomethasone Dipropionate is a good combination in market used for the management of bronchial asthma [1].

Literature survey reveals several methods reported viz UV spectrophotometric [2, 3], simple HPLC [4-7] and Stability indicating HPLC method [8] for estimation of beclomethasone dipropionate alone and in combinations with other drugs; as well as simple UV spectrophotometric methods [9, 10], RP-HPLC method [11-13], stability indicating RP-HPLC [14, 15]and HPTLC [16] methods for estimation of Salbutamol sulphate alone and in combinations with other drugs are reported. Simple RP-HPLC method and Ultra Pressure liquid chromatography method for estimation of Beclomethasone dipropionate and Salbutamol sulphate combination [17, 18]are also reported.

To the best of our knowledge no stability indicating RP-HPLC method has been reported for simultaneous estimation of Beclomethasone dipropionate and Salbutamol sulphate. The present work describes a simple stability indicating HPLC method for the simultaneous determination of Beclomethasone dipropionate and Salbutamol sulphate in bulk and pharmaceutical dosage form (Aerocort–Rota caps), according to the International conference on harmonization (ICH) guidelines[19-21].
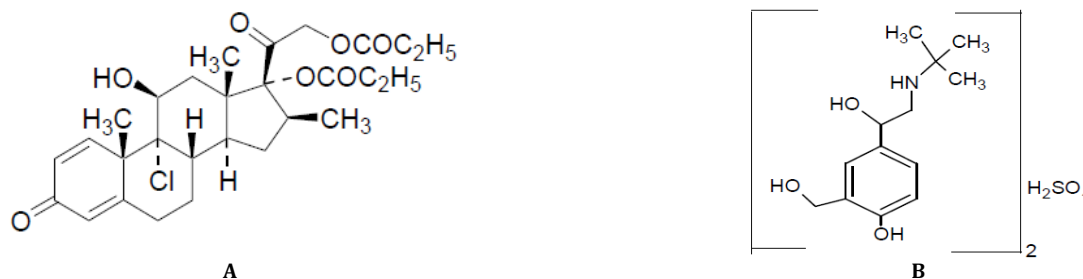

Fig. 1: Structure of A) Beclomethasone dipropionate and B) Salbutamol sulphate

MATERIALS AND METHODS

Reagents and chemicals

Authentic sample of Beclomethasone dipropionate and Salbutamol sulphate were obtained from Cipla Pharmaceuticals Ltd, Mumbai, respectively. The formulation Aerocort–Rota caps (Manufactured by-Cipla Pharmaceuticals Ltd) labeled to contain Beclomethasone dipropionate (IP) 100 µg and Levosalbutamol sulphate (IP) equivalent to salbutamol 100 µg was procured form local market.

Methanol (HPLC grade) was obtained from S. D. Fine Chem. Limited (Mumbai, India), HPLC grade water is collected at college using ELGA water purification system, ammonium acetate, glacial acetic

acid, hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) (all are AR grade) were purchased from S. D. Fine Chem. Limited (Mumbai, India).

Chromatographic condition

HPLC system used was JASCO system equipped with Model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), MD 2010 PDA detector and Borwin-PDA software (version 1.5). A chromatographic column Hi Q Sil C_{18} (250 x 4.6 mm, 5 µm) was used. Separation was carried out at a flow rate of 1 ml/min using 1 mM ammonium acetate buffer: methanol (15:85 v/v) as mobile phase and detection at 230 nm.

Preparation of 1 mM ammonium acetate buffer and mobile phase

1 mM ammonium acetate buffer was prepared by dissolving 7.71 mg of ammonium acetate in 80 ml of HPLC grade water, add 5.7 µl glacial acetic acid and make the volume up to 100 ml with HPLC grade water [22]. Mobile phase was prepared by mixing 1 mM Ammonium acetate buffer and methanol in the ratio of 15: 85 v/v. It was then filtered through 0.45 µm membrane filter paper using filtration assembly and then sonicated on ultrasonic water bath for 15 min.

Preparation of standard stock solution

Standard stock solution of each drug was prepared separately by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000 µg/ml (A). From the respective standard stock solution, working standard solution was prepared containing 100 µg/ml of Beclomethasone dipropionate and Salbutamol sulphate in mobile phase separately (B). From this further dilution was made in the mobile phase to get a final concentration of 10 µg/ml for Beclomethasone dipropionate and Salbutamol sulphate, separately.

Selection of detection wavelength

From the standard stock solution (1000 µg/ml) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that both the drug showed considerable absorbance at 230 nm. (fig. 2)
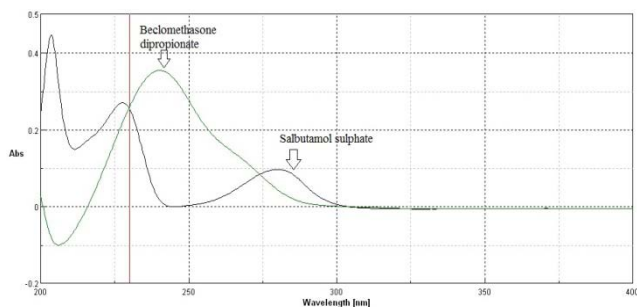

Fig. 2: Overlay UV-VIS Spectra of BEC (10 µg/ml) and SAL (10 µg/ml)

Preparation of sample solution (Rota caps formulation analysis)

Twenty capsules each containing 100 µg of Beclomethasone dipropionate and Levosalbutamol sulphate (equivalent to salbutamol 100 µg) were emptied and weighed. Powder equivalent to 10 mg of Beclomethasone dipropionate and 12.049 mg of salbutamol sulphate (equivalent to 10 mg of Salbutamol) was transferred to 10 ml volumetric flask and was diluted with methanol, sonicated for 10 min and volume made to 10 ml with methanol. Solution was filtered and further dilutions were made with mobile phase to get the final concentration of 10 µg/ml of Beclomethasone dipropionate and 12.049 µg/ml of salbutamol sulphate.

Stress degradation studies of bulk drug

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like hydrolysis, oxidation, temperature, etc. and to establish specific storage conditions, shelf-life and retest period. Dry heat and photolytic degradation were carried out in the solid state.

Alkaline hydrolysis

1 ml working standard solution of BEC (100 µg/ml) was mixed with 1 ml of 0.01 N NaOH (Methanolic) and kept aside for 6 hours at room temperature, after exposure the volume was made up to 10 ml with the mobile phase and injected; SAL was treated in a similar manner to BEC.

Acid hydrolysis

1 ml working standard solution of BEC (100 µg/ml) was mixed with 1 ml of 0.1 N HCl (Methanolic) and kept aside for 24 hours at room temperature, after exposure the volume was made up to 10 ml with the mobile phase and injected; SAL was treated in a similar manner to BEC.

Neutral hydrolysis

1 ml working standard solution of BEC (100 µg/ml) was mixed with 1 ml water, and was kept aside for 24 hours at room temperature, after exposure the volume was made up to 10 ml with the mobile phase and injected; SAL is treated in a similar manner to BEC.

Degradation under oxidative condition

1 ml working standard solution of BEC (100 µg/ml) was mixed with 1 ml of 30 % solution of H_2O_2 and was kept aside for 2 hours at room temperature, after exposure the volume was made up to 10 ml with the mobile phase and injected; SAL was treated in a similar manner to BEC.

Degradation under dry heat

Dry heat studies were performed by keeping drug sample separately in oven (60 °C) for a period of 24 hours. A sample were withdrawn after 24 hours, dissolved in methanol to get solution of 1000 µg/ml and further diluted with mobile phase to get 10 µg/ml as final concentration and was injected.

Photo-degradation studies

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux Hrs. The sample was weighed, dissolved and diluted to get 10 µg/ml and injected.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability indicating HPLC method is to achieve the resolution between BEC, SAL and its degradation products.
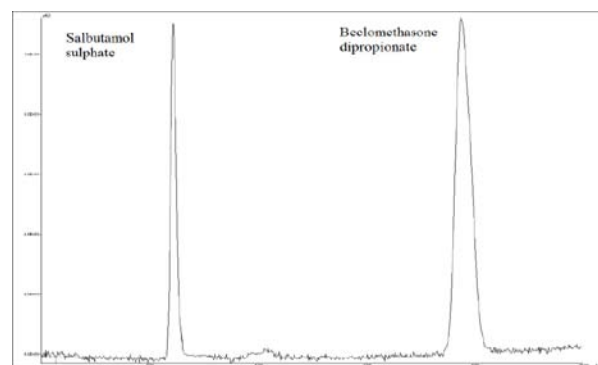

Fig. 3: Chromatogram of mixture containing BEC (10 µg/ml) and SAL (10 µg/ml).

To achieve the separation, we used a stationary phase C18 column and mobile phase 1 mM ammonium acetate buffer and methanol in the ratio 15:85, v/v. The tailing factor obtained was less than two and retention time was 7.75±0.03 min and 2.36±0.05 min for BEC and SAL, respectively (Fig.3). Forced degradation study showed the method is highly specific and no degradation products were eluted at retention time of drugs. (fig. 4 and fig. 5). Summary of stress degradation studies is summarized in table no.1
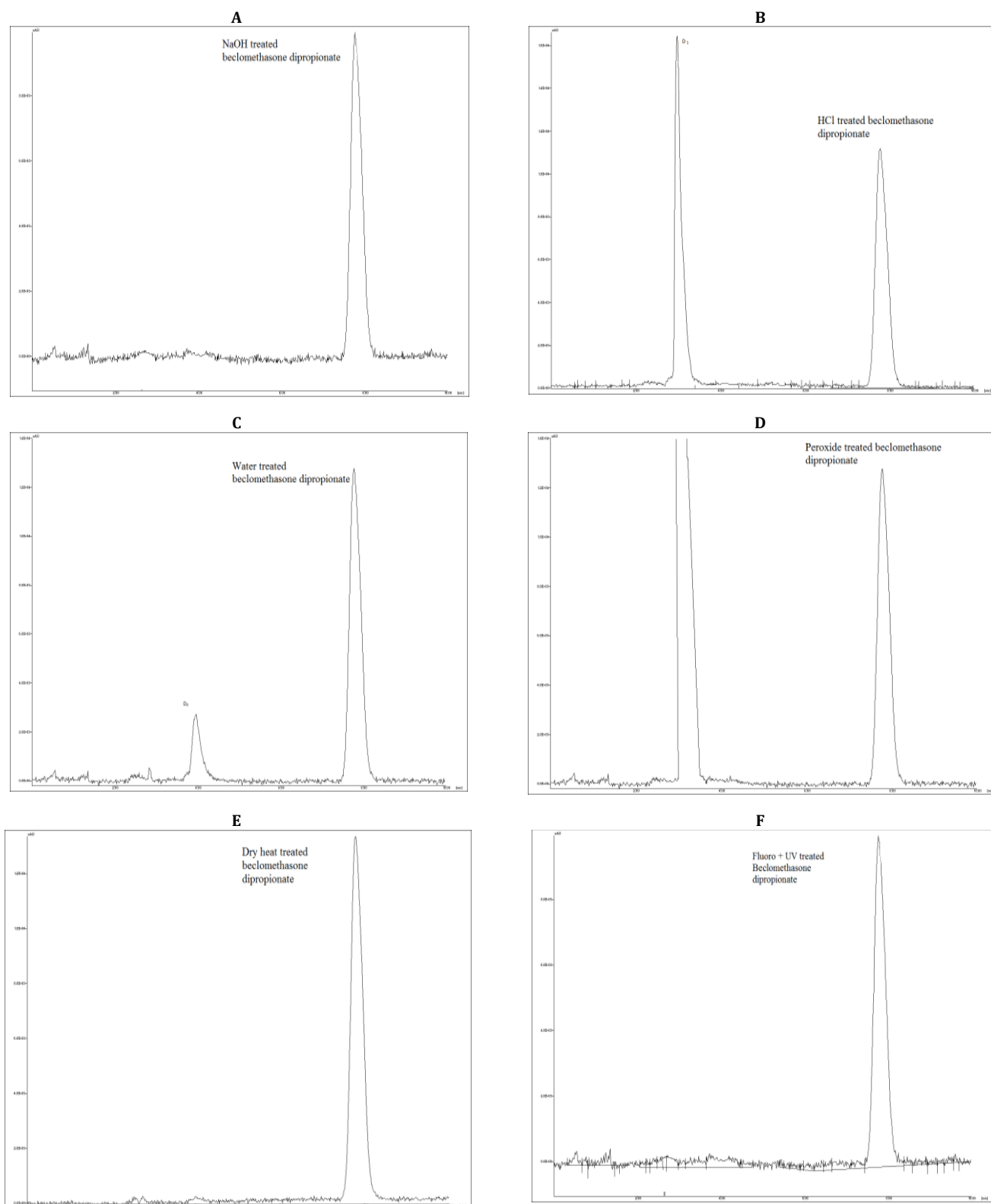
Fig. 4: Representative chromatogram of Alkali (A), Acid (B), Neutral (C), Oxidative (D), Dry Heat (E), Photolytic (F) degradation of Beclomethasone dipropionate
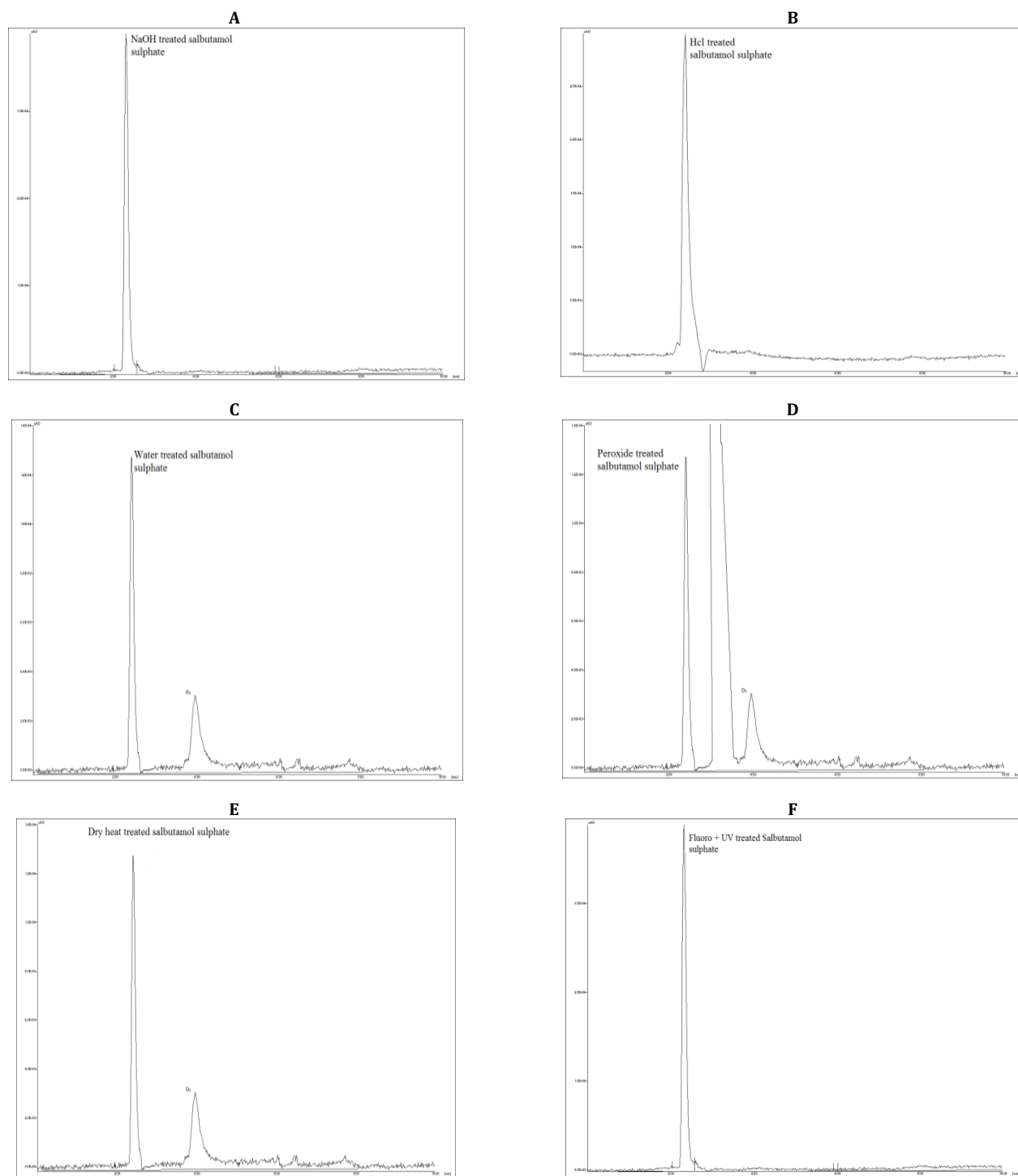
Fig. 5: Representative chromatogram of Alkali (A), Acid (B), Neutral (C), Oxidative (D), Dry Heat (E) Photolytic (F) degradation of Salbutamol sulphate

Table 1: Summary of stress degradation study of BEC and SAL (n=3)

S. No.	Stress degradation condition	% Recovery (BEC)	% Recovery (SAL)
1	Base (0.01 N NaOH Methanolic), Kept for 6 hours.	76.45	89.57
2	Acid (0.1 N HCl Methanolic), Kept for 24 hours.	65.81	80.42
3	Neutral (kept for 24 hours.)	73.37	71.31
4	H_2O_2, 30% (kept for 2 hours.)	60.99	94.68
5	Dry heat (60 °C for 24 hours.)	75.83	82.10
6	Photo stability (UV, 200 watt, hrs/square meter and Florescence, 1.2 million Lux. Hrs)	98.5	98.38

Validation of analytical method

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 991, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity

The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/ml for BEC and SAL, five replicates per concentrations were analyzed, the equation of calibration curve was found to be y = 20407 x+58659 for BEC with r^2 = 0.997 and y = 22675.4 x+8355 for SAL with r^2 = 0.997.

Precision

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies, 6 replicates of BEC (10 µg/ml) and 6 replicates of SAL (10µg/ml) were analyzed in a day and percentage relative standard deviation %RSD was calculated.

For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated.

The results obtained for Intraday and Inter day variations were found to be within limits (less than 2% RSD).

Limit of detection (LOD) and limit of quantitation (LOQ)

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S Where, σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. LOD of BEC and SAL was found to be 0.637 µg/ml and 0.221 µg/ml, respectively. LOQ of BEC and SAL was found to be 1.932 µg/ml and 0.67 µg/ml, respectively**.**

Assay

Rota caps formulation analysis was carried out as mentioned under section preparation of sample solution; Procedure was repeated for six times. Sample solution was injected and area was recorded for each drug. Concentration and % purity was determined from linear equation. (table no: 2)

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the rotacaps sample solution, at three different levels around 50, 100 and 150 %. Basic concentration of sample solution chosen was 10 µg/ml of BEC and 12.049 µg/ml of SAL. % recovery was determined from linearity equation. The results obtained are shown in (table no: 3).

Table 2: Assay of marketed formulation-rotacaps

S. No.	Beclomethasone dipropionate			Salbutamol sulphate		
	Peak area	Amount Recovered (µg/ml)	% Recovery	Peak area	Amount Recovered (µg/ml)	% Recovery
1	265159.7	10.119	101.191	281938.1	12.06	100.13
2	261497.9	9.939	99.396	279786.04	11.96	99.34
3	270145.6	10.363	103.63	277145.9	11.85	98.37
4	259927.9	9.862	98.627	280200.07	11.98	99.49
5	262412.5	9.984	99.84	279989.13	11.97	99.41
6	267852.7	10.251	104.277	281567.86	12.048	99.99
Mean	264499.4	10.087	100.87	280104.4	11.984	99.46
SD	3945.2	0.193	1.933	1695.83	0.074	0.62
%RSD	1.492	1.917	1.917	0.605	0.624	0.624

Table 3: Recovery studies of BEC and SAL

Level (%)	Sample		Standard.		% Recovery±RSD	
	BEC (µg/ml)	SAL (µg/ml)	BEC (µg/ml)	SAL (µg/ml)	BEC	SAL
50	10	12.04	5	5	100.69*±1.9	99.68*±1.56
100	10	12.04	10	10	101.18*±1.5	99.0 *±1.24
150	10	12.04	15	15	101.08*±1.7	99.61*±1.05

*Mean of three determinations

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, detection wavelength, flow rate were altered and the effect on the peak area was noted. The method was found to be robust as % RSD value found were less than 2%.

CONCLUSION

The developed method was found to be simple, sensitive, selective, accurate, and repeatable for simultaneous analysis of Beclomethasone dipropionate and Salbutamol sulphate in bulk and pharmaceutical dosage form without any interference from the excipients,

The results indicated the suitability of the method to study stability of Beclomethasone dipropionate and Salbutamol sulphate under various forced degradation conditions like hydrolysis, oxidation, dry heat and photolytic degradation.

CONFLICT OF INTERESTS

Declared None

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