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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SALBUTAMOL AND BECLOMETHASONE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT: A new, simple, sensitive, precise, accurate and stability indicating RP-HPLC method has been developed for the simultaneous estimation of salbutamol and beclomethasone in combined tablet dosage form. The analysis was carried out at 230 nm and this chromatographic separation was achieved with Kromasil 250 C₁₈ [250 x 4.6 mm, 5 μ] column under 25 °C temperature and using mobile phase water and acetonitrile in a ratio of 55:45 v/v adjusted pH 2 with ortho phosphoric acid. The retention times of salbutamol and beclomethasone were found to be 2.030 \pm 0.01 min, 3.271 \pm 0.1min respectively. The proposed method was validated according to ICH guidelines. The linearity study of salbutamol and beclomethasone was found in concentration range of 25 - 150 μ g/ml and 50 - 300 μ g/ml and correlation coefficient (r^2) was found to be 0.999 and 0.999. The percentage recovery was obtained as 98.61 % and 100.75 % w/v for beclomethasone and salbutamol. The LOD, LOQ values were obtained from regression equations of beclomethasone and salbutamol were 1.13 μ g/ml, 2.06 μ g/ml and 3.42 μ g/ml, 6.23 μ g/ml respectively. The studies were carried out by conducting deliberate degradation of the sample with exposure to stress conditions like acidic (1M HCl), alkaline (1M NaOH), thermal, oxidizing agents (H₂O₂) and water. This method was validated and met the regulatory requirements for specificity, Linearity, LOD, LOQ, Precision, accuracy and stability for the determination of salbutamol and beclomethasone in bulk and tablet dosage form by RP-HPLC.

INTRODUCTION: Salbutamol is chemically 4-[2-(tert-butylamino)-1-hydroxyethyl] - 2- (hydroxyl methyl) phenol. It is a short-acting, selective beta-2-adrenergic receptor agonist used in the treatment of asthma and COPD. Salbutamol is generally used for acute episodes of bronchospasm caused by bronchial asthma, chronic bronchitis and other chronic broncho pulmonary disorders such as chronic obstructive pulmonary disorder (COPD).

It is also used prophylactically for exercise induced asthma. Beclomethasone is chemically 2-[(1R, 2S,10S,11S,13S,14R,15S,17S)-1-chloro-17 - hydroxy-2,13,15-trimethyl-5-oxo-14-(propanoyloxy) tetra cyclo heptadeca-3,6-dien-14-yl]-2-oxoethyl propanoate. Beclomethasone dipropionate is a prodrug of the free form, it is anti-inflammatory, synthetic corticosteroid and it is used topically as an anti-inflammatory agent and in aerosol form for the treatment of asthma and allergic rhinitis. These researches focus the simultaneous estimation of salbutamol and beclomethasone in API or combined tablet form by RP - HPLC.

The extensive literature survey revealed that there were RP-HPLC¹⁻³ and UV spectrophotometric⁴, stability indicating RP - HPLC⁵ methods were

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available for the determination of salbutamol and beclomethasone individually or in combination with other drugs⁶⁻¹⁵. But no method was reported for simultaneous estimation of salbutamol and beclomethasone in combined dosage form using RP-HPLC method. The study was thus performed with an aim to develop a simple, economic, sensitive, rapid, accurate and precise method for the determination of salbutamol and beclomethasone in combined tablet dosage form.

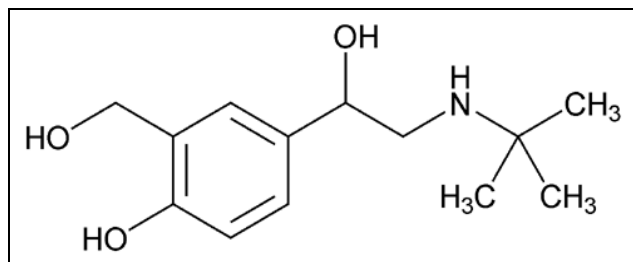


FIG. 1: STRUCTURE OF SALBUTAMOL

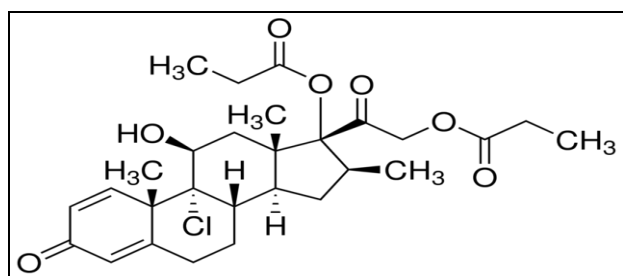


FIG. 2: STRUCTURE OF BECLOMETHASONE

MATERIALS AND METHODS:

Chemicals and Reagents: Salbutamol and Beclomethasone were purchased from Spectrum Labs, Hyderabad. Orthophosphoric acid (OPA) purchased from SD Fine Chemicals (Hyderabad, India), methanol, acetonitrile and HPLC grade water were obtained from Rankem. Salbutamol and beclomethasone containing aerotide tablets manufactured by Sun Pharmaceutical Ltd, Hyderabad, India.

Instruments: Waters (2695) HPLC using the software Empower 2. All the glass wares used were 'A' grade.

Standard stock solution: 10 mg of salbutamol and 20 mg of beclomethasone standards were accurately weighed and transferred into a 10 ml clean, dry volumetric flask, 5 ml of diluent was added, sonicated for 5 minutes and made up to the final volume with diluent. 1 ml from the above stock solution was taken into a 10 ml volumetric flask and made up to 10 ml with diluent.

Preparation of Sample Solution: 20 tablets were weighed and crushed. A powder equivalent to 10 mg of salbutamol and 20 mg of beclomethasone was taken and then transferred into a 10 ml clean, dry volumetric flask, 5 ml of diluents was added, sonicated for 5 minutes and made up to the final volume with diluent. 1 ml of the above stock solution was taken into a 25 ml volumetric flask and made up to 25 ml with diluent.

Chromatographic Conditions: Kromasil 250C₁₈ [250 x 4.6 mm, 5 μ] column was used for the chromatographic separation at a detection wave length of 230 nm, under 25 °C temperature. Mobile phase of buffer and acetonitrile pH 2 (adjusted with OPA) in a ratio of 55:45 v/v which was degassed under ultra-sonication was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was optimized to 0.9 ml/min and the injection volume 10 μ l was fixed upon the satisfactory results of various system suitability parameters such as retention time, column efficiency, tailing factor, asymmetry of the peaks. The results were shown in Fig. 3.

Method Validation: The RP-HPLC method was validated according to ICH guidelines for validation of analytical procedures for different validation parameters. The method was validated for its specificity, linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ.

System Suitability: System suitability test was carried out to verify that the analytical system was working properly and can give accurate and precise results. The overall system suitability was evaluated for the system suitability of the proposed method. Data from six injections (10 μ g/mL) were utilized for calculating parameters like theoretical plates, resolution, tailing factor and %RSD. The results were shown in Table 2.

Specificity: The specificity studies were carried out by varying specific conditions, *i.e.*, placebo study. A study conducted to demonstrate that diluent and Placebo were not interfering with the analyte peak in the proposed method. Solutions of sample, placebo and blank were prepared individually and chromatograms were obtained. The results were shown in Fig. 3, 4 and 5.

Accuracy: Accuracy was carried out by % recovery studies of salbutamol and beclomethasone at three different concentration levels (50 %, 100 %, and 150 %). Percentage recovery was calculated from the amount added and the amount recovered. The percentage recovery was within the acceptance criteria, this indicates the accuracy of the method. (Acceptance criteria: % recovery between 98 - 102%). The results were shown in **Table 3**.

Precision: Precision of an analytical procedure as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The results were shown in **Table 4**.

Linearity: Linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the samples. The linearity of the method was determined by preparing serial dilutions of minimum 5 concentrations of working stock solutions in the range of 50 - 300 µg/ml for beclomethasone and 25 - 150 µg/ml for salbutamol. The area of each injection was obtained and the peak area was plotted against actual concentration. The regression coefficient r^2 , y-intercept and slope of the regression were calculated.

Limit of Detection and Limit of Quantitation: The LOD is defined as the lowest concentration of an analyte in a sample that can be detected but not quantified. The LOQ is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy. The LOD and LOQ can be calculated based on the standard deviation of the response and the slope of calibration curve. $LOD=3.3\sigma/S$ $LOQ=10\sigma/S$ where ' σ ' is the standard deviation of the intercept of the regression lines and ' s ' is the slope of the calibration curve.

Robustness: The robustness of an analytical variation in method parameters such as flow rate, column temperature were varied within a realistic range and the quantitative influence of the variables were determined. The results were shown in **Table 5** and **Table 6**.

Stability Studies: Stability testing was established for estimating the allowed time span between

sample collection and sample analysis. It is also important to evaluate an analytical method's ability to measure drug products in the presence of its degradation products. Forced degradation studies typically involve the exposure of samples of the drugs to the relevant stress conditions of acid, base, thermal and UV. The results were shown in **Table 7**.

Acid Degradation: To 1 ml stock solution of salbutamol and beclomethasone, 1ml of 2N hydrochloric acid was added and refluxed for 30 mins at 60 °C. The resultant solution was diluted to obtain 100 µg/ml and 200 µg/ml solutions and 10µl solutions were injected into the system and the chromatogram were recorded to assess the stability of drug substances. The results were shown in **Fig. 9**.

Oxidation Degradation: To 1 ml of stock solution of salbutamol and beclomethasone, 1 ml of 20 % hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60 °C. For HPLC study, the resultant solution was diluted to obtain 100µg/ml and 200 µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of drug substances. The results were shown in **Fig. 10**.

Alkali Degradation: To 1 ml of stock solution of salbutamol and beclomethasone, 1 ml of 2N sodium hydroxide was added separately. The solutions were kept for 30 min 60 °C. For HPLC study, the resultant solution was diluted to obtain 100µg/ml and 200µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of drug substances. The results were shown in **Fig.11**.

Thermal Degradation: The standard drug solution was placed in oven at 105 °C for 6hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 100µg/ml & 200µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of drug substances. The results were shown in **Fig. 12**.

Photo Degradation: The photochemical stability of the drug was also studied by exposing the 1000 µg/ml and 2000 µg/ml solution to UV light by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/m² in photo stability chamber. For HPLC study, the resultant solution was diluted to

obtain 100 μ g/ml and 200 μ g/ml solutions and 10 μ l were injected into the system and the chromatograms were recorded to assess the stability of drug substances. The results were shown in **Fig. 13**.

RESULTS AND DISCUSSION: Optimization of the method was carried out by performing various trials by change in mobile phase composition, column and flow rate *etc.* The chromatographic conditions were achieved by water and acetonitrile in a ratio of 55:45 v/v adjusted pH 2 with ortho phosphoric acid was selected as mobile phase and Kromasil 250C₁₈ [250 x 4.6 mm, 5 μ] column as stationary phase because of better resolution, number of Theoretical plates and symmetric peaks. Salbutamol and beclomethasone were found to show appreciable absorbance at 230 nm determined spectro-photometrically and hence it was selected as the detection wavelength. **Fig. 3** represents chromatogram of mixture of standard solutions.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC PARAMETERS

Optimized chromatographic conditions	
Mode of separation	Isocratic
Mobile phase	Water : acetonitrile (55:45)
Column	Kromasil 250C18 (250 x 4.6 mm, 5 μ)
Column temperature	25 °C
Detection wavelength	230 nm
Run time	6 min
Injection volume	10 μ l
Flow rate	0.9 ml/min

Method Validation:

System Suitability: An RP -HPLC method was developed by monitoring the system suitability

parameters, *i.e.* Tailing factor (T), the number of theoretical plates (N), the runtime and the cost effectiveness. System suitability method acceptance criteria set in each validation run were: tailing factor ≤ 2.0 and theoretical plates > 2000 . In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was $< 2.0\%$. A chromatogram obtained from reference substance solution was presented. System suitability parameters were shown in **Table 2**. All the system suitability parameters are found to be satisfactory. The peak is reasonably symmetrical. High numbers of theoretical plates indicate the efficient performance of the column with reasonable retention times.

Specificity: The blank chromatogram showed no interference peaks at the retention time of beclomethasone and salbutamol. This indicates that diluent solution used in sample preparation do not interfere in the estimation of beclomethasone and salbutamol. Similarly the placebo sample chromatogram showed no interference peaks at the retention time of beclomethasone and salbutamol respectively, which indicates the specificity of the proposed method. The results were given in **Fig. 4, 5 and 6**.

TABLE 2: RESULT OF SYSTEM SUITABILITY PARAMETERS

Parameters	Beclomethasone	Salbutamol
Linearity range (μ g/ml)	50-300 μ g/ml	25 - 150 μ g/ml
Retention time (RT)	3.267min	2.032 min
Number of theoretical plates (n)	10251	7288
Correlation coefficient (r^2)	0.999	0.999
Tailing factor (T)	1.2	1.2
Limit of detection (μ g/ml)	1.13	2.06
Limit of Quantitation(μ g/ml)	3.42	6.23

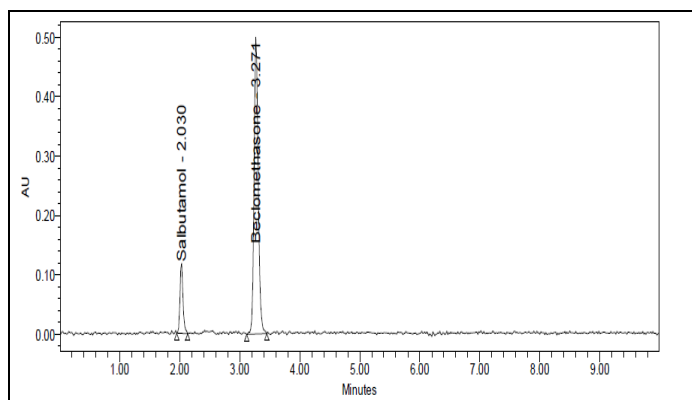


FIG. 3: OPTIMIZED CHROMATOGRAM OF SALBUTAMOL AND BECLOMETHASONE

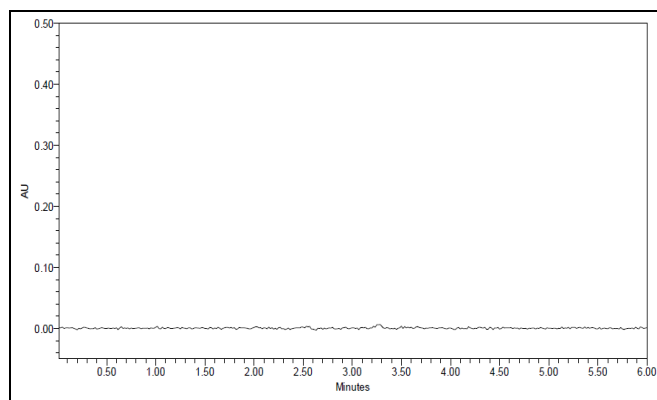


FIG. 4: CHROMATOGRAM OF BLANK

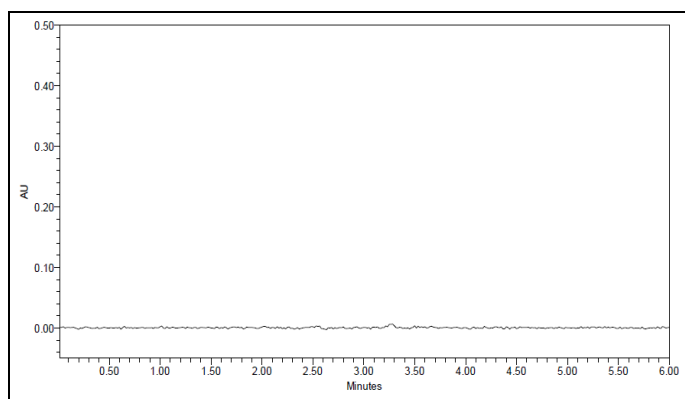


FIG. 5: CHROMATOGRAM OF PLACEBO

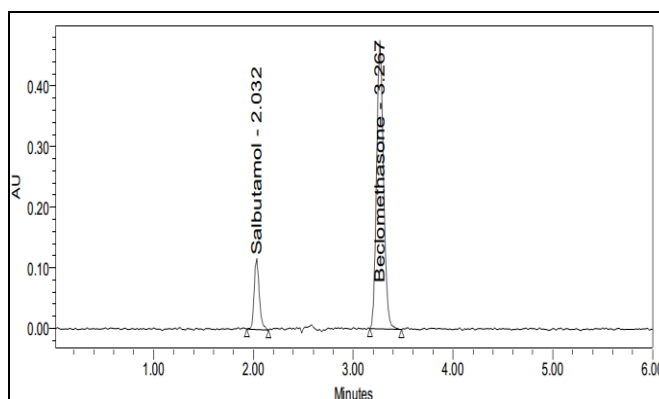


FIG. 6: CHROMATOGRAM OF STANDARD

The blank and placebo peaks are not interfering with the analyte so the method was specific.

Linearity: The concentration range of 50-300µg/ml for beclomethasone and 25-150 µg/ml of Salbutamol were found to be linear with correlation coefficients 0.999 and 0.999 for beclomethasone and Salbutamol respectively. The results were given in Fig.7 and 8.

Accuracy: The accuracy of an analytical method is the closeness of that results obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known added amount of analyte. % Recovery

should be in the range of 98.0% to 102 %. The observed data was within the required range, which indicates good recovery values and hence the method was accurate. The results were shown in Table 3.

Precision: The percentage relative standard deviation was calculated for the peak areas of the drug and it was found to be 0.3 % and 0.7 % for salbutamol and beclomethasone respectively. The % RSD for the area of six standard injections were should not be more than 2 % and the method was found to be precised. The results were shown in Table 4.

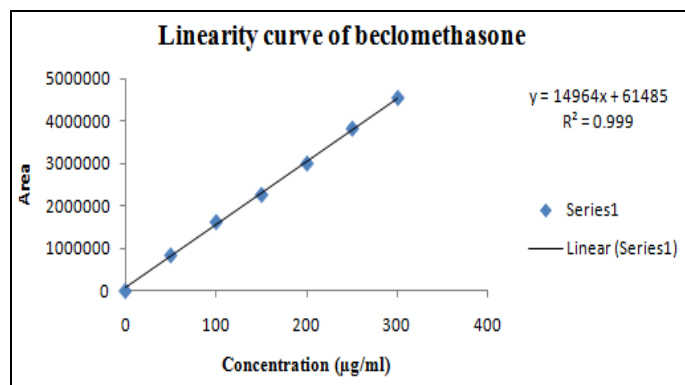


FIG. 7: CALIBRATION PLOT OF BECLOMETHASONE

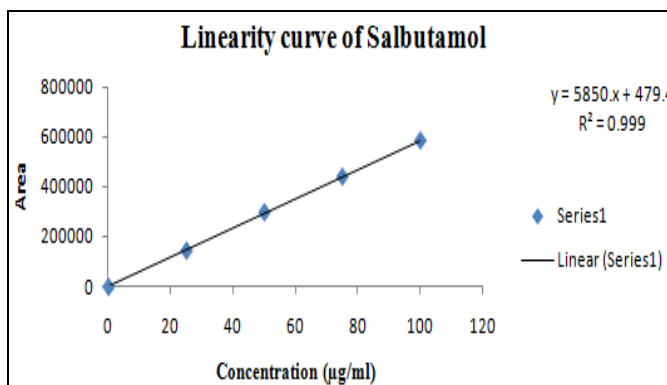


FIG. 8: CALIBRATION PLOT OF SALBUTAMOL

TABLE 3: DATA FOR ACCURACY

Sample	The amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	% RSD
Beclomethasone	100	99.6µg/ml	99.61	0.94
	200	198.9µg/ml	99.48	0.69
	300	295.1µg/ml	98.39	0.25
Salbutamol	50	49.1µg/ml	98.38	0.06
	100	98.7µg/ml	98.77	0.49
	150	100.8µg/ml	100.54	0.63

TABLE 4: PRECISION RESULTS

Analytes	Mean*	% RSD
Salbutamol	0.6	0.3
Beclomethasone	0.7	0.7

*six determinations

Linearity: Linearity of detector response shows the linear relationship between the concentration and the detector response. The coefficient of salbutamol and beclomethasone correlation of was found to be 0.999. The linearity was found in the concentration range of 50 - 300 µg/ml of beclomethasone, 25 - 150 µg/ml of salbutamol.

Regression equation of the beclomethasone and salbutamol are found to be, $y = 14964x + 61485$, and $y = 5778.7x + 3827.3$ and the regression coefficient was 0.999 respectively. The correlation coefficient value was < 1 shows that the method was linear.

Limit of Detection: Limit of detection of target assay concentration of salbutamol and beclomethasone by using the formula method was found to be 2.06 µg/ml and 1.13 µg/ml respectively.

Limit of Quantification: Limit of quantification of the target assay concentration of salbutamol and beclomethasone by using the formula method was found to be 6.23 µg/ml and 3.42 µg/ml respectively.

Robustness: Robustness of the method tested by keeping the ratio of mobile phase as constant and the chromatograms of drug solution were recorded with different flow rates such as 1.0 ml/min, 0.8 ml/min and 0.9 ml/min and different column temperatures such as 20 °C, 25 °C, 30 °C. The peak

was observed as sharp with good resolution and passes all system suitability parameters indicating the method was robust. The results were reported in **Table 5** and **6**.

TABLE 5: RESULT OF ROBUSTNESS STUDIES (FOR VARIANT FLOW RATE)

Analytes	Flow rate(ml)	Plate count	Tailing
Salbutamol	1.0	5840	1.26
	0.9	10251	1.19
	0.8	6535	1.21
Beclomethasone	1.0	8180	1.08
	0.9	7197	1.10
	0.8	7615	1.12

TABLE 6: RESULT OF ROBUSTNESS STUDIES (FOR VARIANT COLUMN TEMPERATURE)

Analytes	Column temperature (°C)	Plate count	Tailing
Salbutamol	20	6695	1.22
	25	5834	1.24
	30	5098	1.14
Beclomethasone	20	7438	1.09
	25	7519	1.07
	30	7197	1.10

Degradation Studies: The degradation was determined by analyzing both drug solutions in presence of acid, base, hydrogen peroxide, thermal and UV. The results of peak area of salbutamol and beclomethasone was changed hence the drug was degraded but the % degradation was less than 10% w/v. The results were within the limit as per ICH guidelines shown in **Table 7**.

TABLE 7: FORCED DEGRADATION STUDY RESULTS FOR SALBUTAMOL AND BECLOMETHASONE

Type of degradation	Beclomethasone			Salbutamol		
	Area	Recovery in %	% Degraded	Area	Recovery in %	Degraded Drug %
Acid	2186597	95.59	4.41	380928	95.29	4.71
Base	2224596	97.3	2.75	388735	97.24	2.76
Peroxide	2249771	98.4	1.64	393297	98.38	1.62
Thermal	2267595	99.1	0.87	397025	99.32	0.68
UV	2273344	99.4	0.61	396403	99.16	0.84
Water	2270637	99.3	0.73	396885	99.28	0.72

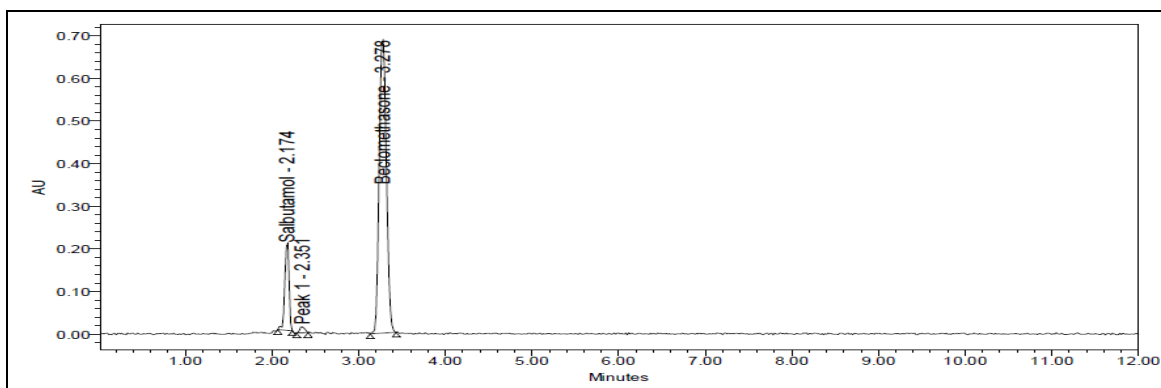


FIG. 9: TYPICAL CHROMATOGRAM OF ACID DEGRADATION OF SALBUTAMOL AND BECLOMETHASONE

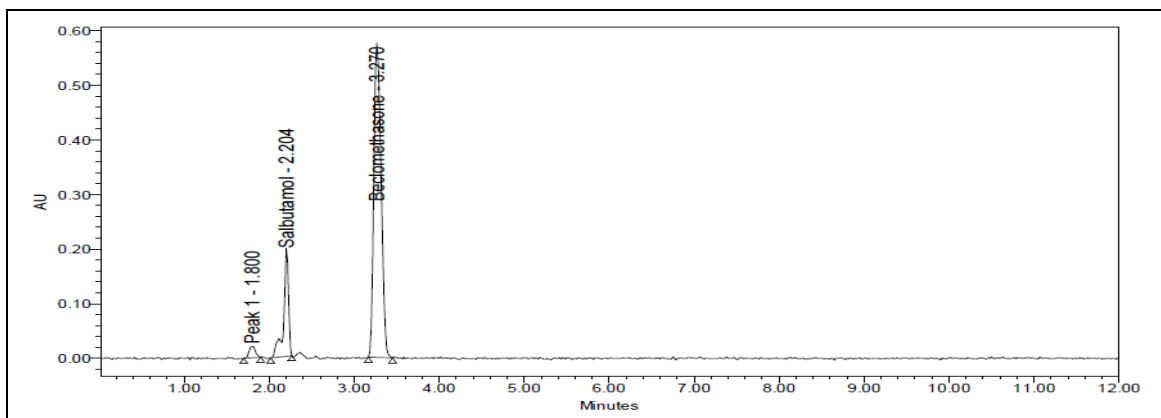


FIG. 10: TYPICAL CHROMATOGRAM OF BASE DEGRADATION OF SALBUTAMOL AND BECLOMETHASONE

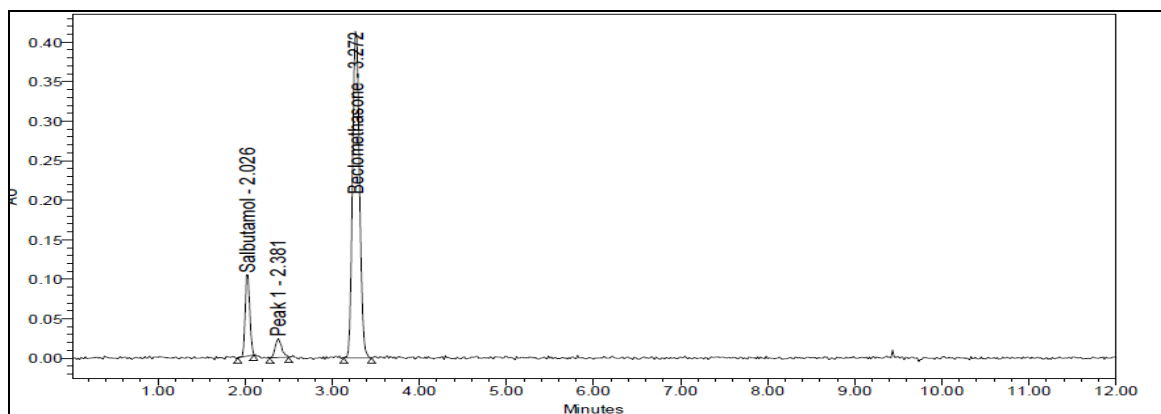


FIG. 11: TYPICAL CHROMATOGRAM OF PEROXIDE DEGRADATION OF SALBUTAMOL AND BECLOMETHASONE

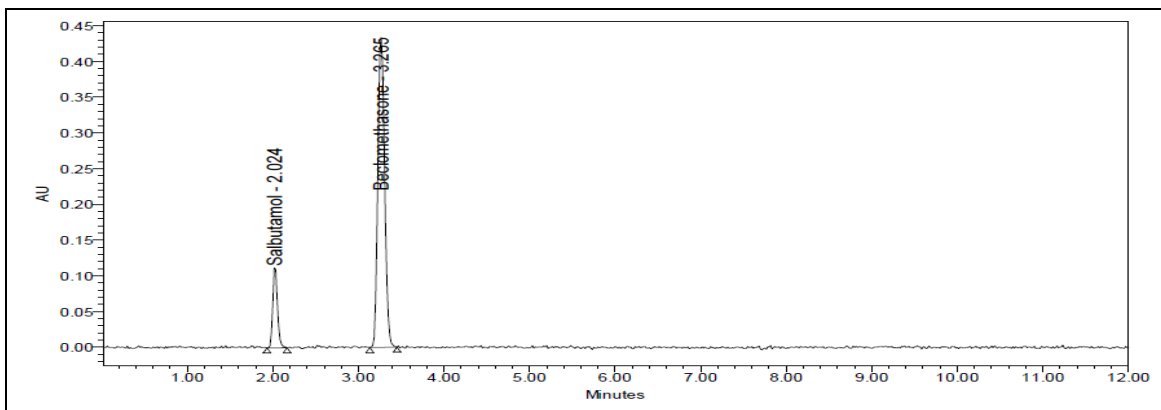


FIG. 12: TYPICAL CHROMATOGRAM OF THERMAL DEGRADATION OF SALBUTAMOL AND BECLOMETHASONE

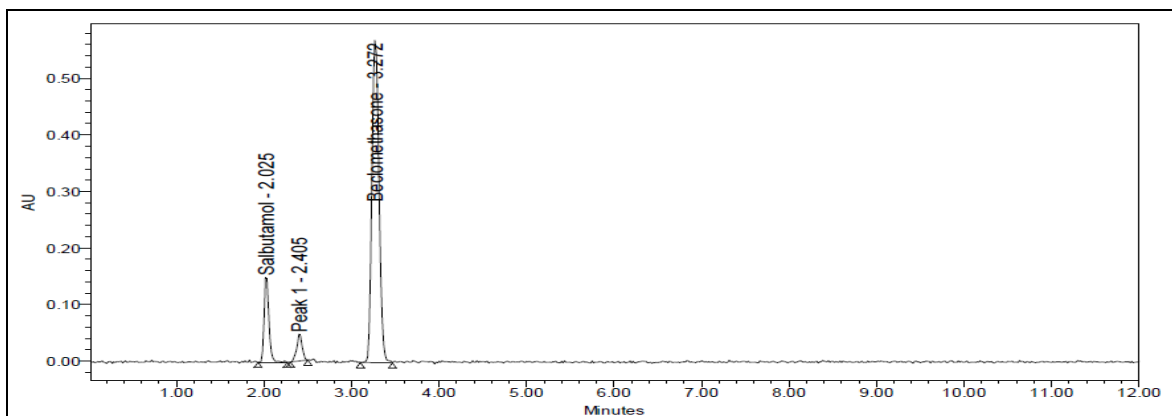


FIG. 13: TYPICAL CHROMATOGRAM OF UV DEGRADATION OF SALBUTAMOL AND BECLOMETHASONE

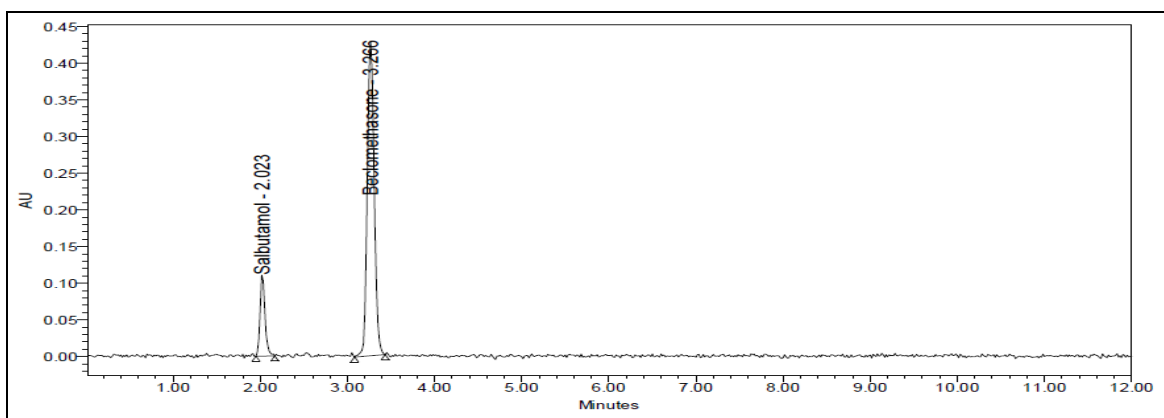


FIG. 14: TYPICAL CHROMATOGRAM OF SALBUTAMOL AND BECLOMETHASONE IN WATER

CONCLUSION: The study was undertaken in order to develop and validate the stability indicating RP-HPLC method for estimation of salbutamol and beclomethasone in pharmaceutical formulations. The method was developed and validated by means of accuracy, precision, linearity, LOD and LOQ and robustness as per ICH guidelines. The results of the study indicate that the proposed RP-HPLC method can be used in quality control departments with respect to routine analysis for the assay of the tablets containing salbutamol and beclomethasone.

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CONFLICTS OF INTEREST: Nil

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