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High resolved stability indicting liquid chromatography method for the simultaneous quantification of Beclomethasone Dipropionate and Formoterol Fumarate dihydrate in pharmaceutical formulations

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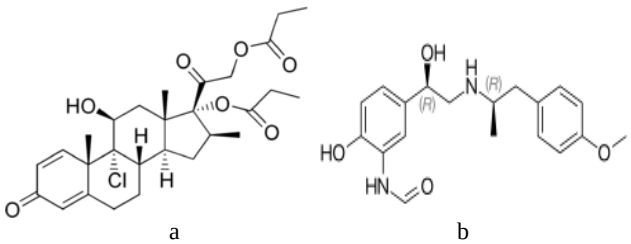
Abstract
A simple, precise isocratic stability indicating RP- HPLC method was developed for the determination of Beclomethasone and Formoterol in pure and its pharmaceutical formulations. In the developed method, Methanol and Phosphate buffer in the ratio of 85:15 (v/v) as mobile phase and Waters C-18 (250mm x 4.6mm, 5µm) column as stationary phase were used. The flow rate and detection wavelength were 1.0 mL/min and 215 nm respectively. The method was validated as per ICH guidelines for specificity, linearity and range, precision, accuracy, robustness, solution stability, limit of quantification and limit of detection. The stability-indicating capability was established by forced degradation experiments. The results of all the validation parameters were well within their acceptance limits and also the degradation products formed during the different stress conditions in stability studies were separated from both drugs and also from individual degradation products. This validated method was applied for the simultaneous estimation Beclomethasone and Formoterol in commercially available formulation sample.

Keywords: Beclomethasone, Formoterol, RP HPLC, method development and validation

1. Introduction
Beclomethasone is a corticosteroids class steroid medication prescribed to prevent and control symptoms caused by asthma. It works by reducing the swelling of the airways in the lungs to make breathing easier. The inhaled form is used in the long-term management of asthma [1]. The cream may be used for dermatitis and psoriasis [2]. The pills have been used to treat ulcerative colitis. The nasal spray is used to treat allergic rhinitis and nasal polyps [3].
Dry/irritated throat, hoarseness, or coughing may occur during the usage of Beclomethasone along with bad taste in the mouth or voice changes [4].
Formoterol is a long-acting bronchodilator used as a long-term treatment to prevent or decrease wheezing and trouble breathing caused by asthma or ongoing lung disease (chronic obstructive pulmonary disease-COPD, which includes chronic bronchitis and emphysema). It should only be used long-term if asthma symptoms are not controlled by other asthma medications. Formoterol works like other β2 agonists, causing bronchodilation by relaxing the smooth muscle in the airway so as to treat the exacerbation of asthma [5].



a b

Fig 1: Chemical structure of Beclomethasone (a) and Formoterol (b)

Literature review reveals that there are only one HPLC [6], one UV spectrophotometer [7] and one HPTLC method [8] reported for simultaneous determination of Beclomethasone and Formoterol in pharmaceutical dosage forms. The other methods available were found to be estimation of Beclomethasone in single or in other combined dosage forms or Formoterol in single or in other combined dosage dorms using different analytical techniques [9-25]. Hence the present work aimed to develop a simple, stable and robust HPLC method for the separation and simultaneous quantification of Beclomethasone and Formoterol in pharmaceutical dosage forms.

2. Materials and Methods
2.1 Chemicals and Materials
Methanol and water: HPLC Grade and purchased from Thermo Fisher Scientific India private limited, Mumbai. Acetonitrile: HPLC Grade and purchased from Merck chemicals private limited, Mumbai. The membrane filters 0.22 µm and syringe filters 0.45µm for the analysis were supplied by Millpores (Millipores Ltd. Banglore). Denver electronic analytical balance (SI-234) was used to weigh the standard and sample drugs. Analytically pure Beclomethasone and Formoterol were obtained as gift sample from reputed pharmaceutical companies. Formulations of FULLFORM© rota cap having 400mcg containing a combination of Beclomethasone and Formoterol were purchased from local market.

2.2 Equipment
Agilent 1100 series HPLC with Quaternary G1311 A pump, COLCOM G1316A thermostat column temperature control, Thermostatic auto sampler G 1329A with sample volume of

0. 1 – 1500 µL and variable programmable UV detector G 1314 A. The instrument was operated and integrated with Agilent chem. station LC software. The LC was coupled with Water mass detector model LAA 1369.

2.3 Preparation of mobile phase
The mobile phase was prepared by mixing Methanol and Phosphate buffer in the ratio of 85:15 (v/v) and sonicated for 15min. Mobile phase was filtered through 0.22µm membrane filter. The pH of the mobile phase was found to be 5.2.

2.4 Preparation of standard solutions
10mg of drug was into a 10ml volumetric flask. Methanol was used to dissolve the drug and it is dissolved completely then final volume was made up to 10ml with same diluents. 1000µg/ml stock solution was obtained. 1ml of the above stock solution was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent to get 100 µg/ml solutions.
Same procedure was fallowed for the preparation of Beclomethasone and Formoterol standard solutions separately. 1ml for the each of the selected concentration was mixed separately to get working standards for method development and validation studies.

2.5 Preparation of sample solution
FULLFORM rota cap©having400mcg of Beclomethasone and 6mcg of Formoterol was used for formulation analysis. Weighed accurately and was dissolved in 10mLmobile phase. Then it was filtered and made up to 10mL with same diluents to make 1000µg/mL stock solution. From this by proper dilution, appropriate concentrations of the two drugs in the dosage form were prepared.

2.6 Method development
Various chromatographic conditions like mobile phase ratio, mobile phase solvents, column, pH of the mobile phase, wavelength of the detector etc. have been optimized in order to achieve separation and identification of Beclomethasone and Formoterol. Chromatographic conditions with best system suitability conditions were selected. The developed method was validated in terms of system suitability, specificity, linearity and range, precision, accuracy, limit of detection, limit of quantification, solution stability and robustness as per USP and ICH guidelines.

2.7 Forced degradation studies
To perform the forced degradation study 50 mg each drugs were subjected to acidic, alkaline, oxidizing, thermal and photolytic conditions. For acidic degradation the drug was heated under reflux with 0.1 M HCl at 80^0C for 2 h and the mixture was neutralized. For alkaline degradation the drug was treated with 0.1 M NaOH at 80^0C for 2 h and the mixture was neutralized. For degradation under oxidizing conditions the drug was heated under reflux with (30%, v/v) H_2O_2 at 80ºC for 2 h. For thermal degradation the powdered drug was heated to 70ºCfor 48 h. For photolytic degradation the powdered drug was exposed to sunlight for 48 h. The placebo was also subjected to the same stress conditions to determine whether any peaks arose from the declared excipients. After completion of the treatments the solutions were left to return to room temperature and diluted with solvent mixture to furnish standard concentration solutions. The purity of the drug peak obtained from the stressed sample was measured using UV detector and compared with the chromatogram of untreated drugs in tablet solution.

3. Results and Discussion
3.1 Method development
The RP-HPLC chromatographic conditions were optimized to get best resolution and peak shape. The selection of mobile phase was based on peak parameters like symmetry and theoretical plates. Symmetrical peaks with good separation (retention time for Beclomethasone is 7.96 and Formoterol is 5.16min) were obtained with reverse phase Waters C-18 (250mm x 4.6mm, 5µm) column. The mobile phase containing Methanol and Phosphate buffer in the ratio of 85:15 (v/v) was used at a flow rate of 1.0mL/min. The optimum wavelength for detection and quantification was at 215nm, at which good detector response was obtained for both the drugs the results are given in Table 1 and the standard chromatogram was given in Figure 2.

Table 1: Optimized chromatographic conditions for the analysis of Beclomethasone and Formoterol

S. No.	Parameter	Results
1	Mobile phase	Methanol and Phosphate buffer in the ratio of 85:15 (v/v)
2	Wavelength	215nm
3	Stationary phase	Waters C-18 (250mm x 4.6mm, 5µm) column
4	pH of mobile phase	5.2
5	Flow rate	1.0mL/min
6	Pump mode	Isocratic
7	Pump pressure	10.7±5MPa

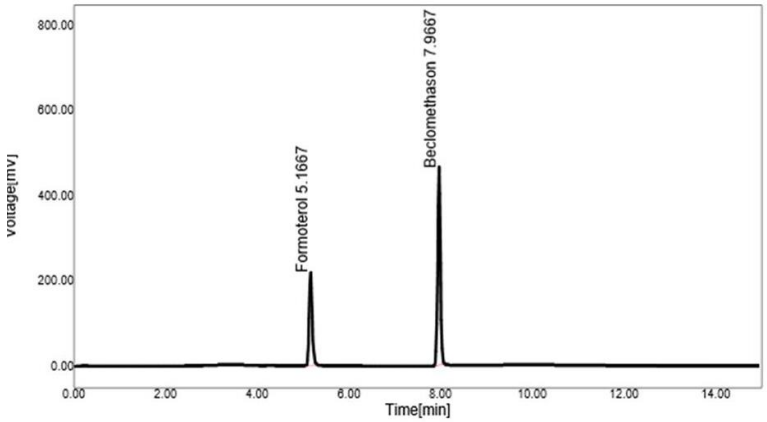


Fig 2: Standard Chromatogram

3.2 Method Validation

System suitability parameters like number of theoretical plates (N), peak asymmetry factor (As), resolution etc., were studied in the developed method. The results are given in Table 2.

Table 2: System Suitability Results

S. No	Parameter	Results
1	Active pharmaingradient concentration	Beclomethasone – 400µg/mL Formoterol - 6µg/mL
2	Retention time	Beclomethasone – 7.96min Formoterol - 5.16min
3	Resolution	Beclomethasone – ……. Formoterol – 12.68
4	Area	Beclomethasone – 682540.9 Formoterol - 247469.3
5	Theoretical plates	Beclomethasone – 7534 Formoterol - 5430
6	Tailing factor	Beclomethasone – 0.68 Formoterol - 1.26

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 100-600µg/mL(1424x + 10444) for Beclomethasone and 1.5-9 µg/mL (35924x + 30091) for Formoterol. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. Correlation coefficients were found to be 0.999 and 0.999 for Formoterol (Fig 3) and Beclomethasone (Fig 4) respectively. The results are given in Table 3

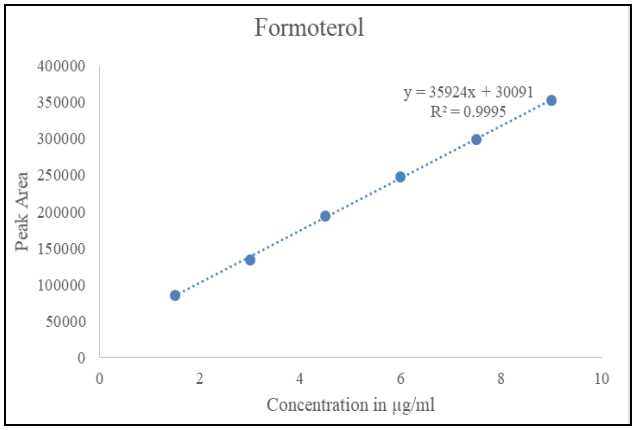


Fig 3: Formoterol calibration curve

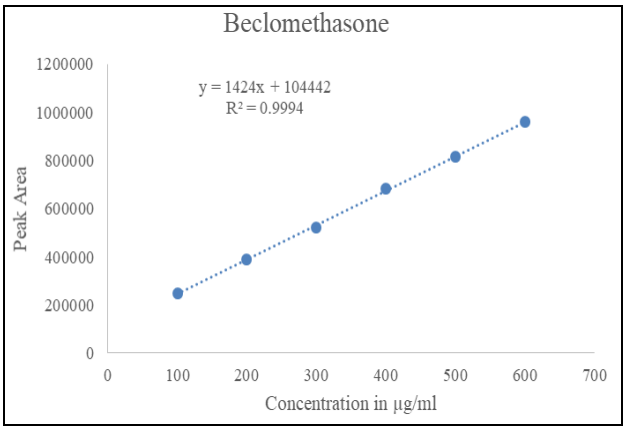


Fig 4: Beclomethasone calibration curve

The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done at two levels (intraday and inter day). Intraday precision was done by analyzing the intermediate concentration of each drug for six times. Interday precision was measured over three consecutive days for the same drug concentrations for six times. The %RSD values were calculated for each of them. The intraday precision study for six sample preparations in marketed samples showed a RSD of 0.31 and 0.90 for Beclomethasone and Formoterol respectively. For the interday precision, a study carried out by the same analyst working on different days. The inter-day RSD values (For Standard) were found to be to 0.90 and 0.65 by Beclomethasone and Formoterol respectively. The same study was carried out for different analysts (n=6 number of samples per analyst) obtaining a RSD of 0.34 and 0.93 (ruggedness) respectively for Beclomethasone and Formoterol. These results show that the proposed analytical technique has a good intermediate precision. Results are summarized in Table 3. Robustness of the method was determined by small deliberate changes in detector wavelength, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

Recovery studies were carried out by applying the method to drug sample to which known amount of standard Beclomethasone and Formoterol corresponding to 50%, 100% and 150 % of label claim had been added. At each level of three determinations were performed.LOD and LOQ decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated from standard calibration curves. The proposed procedures were successfully applied for the simultaneous estimation of Beclomethasone and Formoterol in the formulation and the drug contents in each sample were calculated by comparison with the appropriate standard solution of the drug. The results obtained were in agreement with label claim. The summaries of results of analysis are given in Table 3. The chromatogram for formulation was shown in Fig.5.

Table 3: Summary results

Parameter	Results	
	Beclomethasone	**Formoterol**
Linearity range(µg/ml)	100-600	1.5-9
Correlation coefficient	0.999	0.999
Slope	1424	35924
Intercept	10444	30091

LOD(µg/ml)	1.50	0.025
LOQ(µg/ml)	5.0	0.09
Recovery (%)		
50	98.68	98.23
100	99.28	98.84
150	99.31	98.99
Precision (RSD %)		
Intraday(n=6)	0.31	0.90
Interday(n=6)	0.34	0.93
Ruggedness(n=6)	0.51	1.04
Robustness (% Change)		
Mobile Phase 1[Methanol and Phosphate buffer in the ratio of 90:10 (v/v)]	0.45	0.66
MP 2[Methanol and Phosphate buffer in the ratio of 80:20 (v/v)]	0.11	0.26
pH 1 (5.1)	0.01	0.12
pH 2 (5.3)	1.41	0.68
Wave Length 1 (210nm)	0.84	0.56
Wave Length 2 (220nm)	0.95	0.45
Formulation assay	99.32	98.43

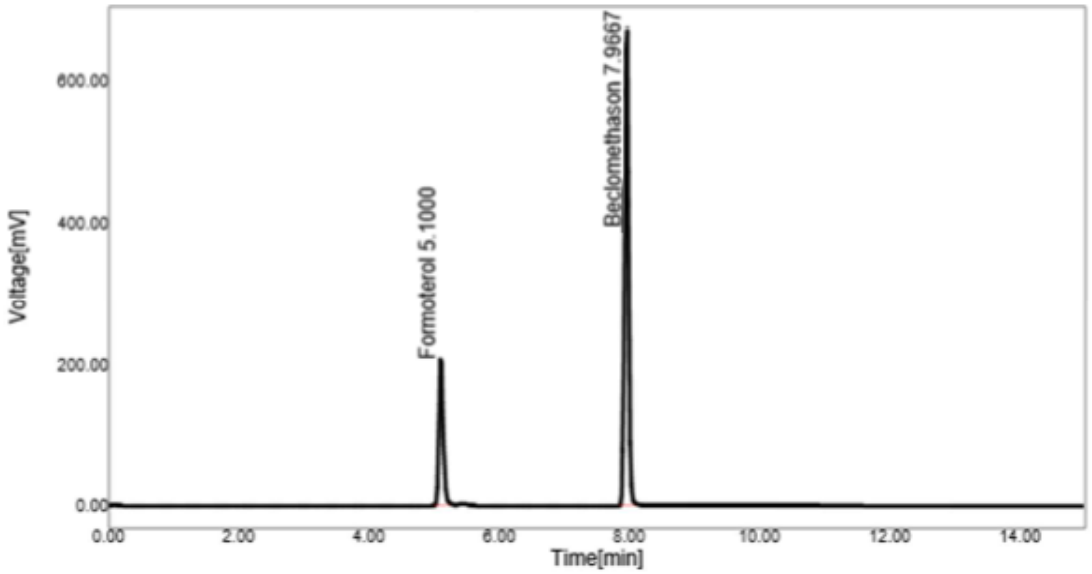


Fig 5: Formulation Chromatogram

3.3 Forced degradation studies

Stability of Beclomethasone and Formoterol was carried out by forced degradation study. The chromatograms of samples degraded with acid, base, hydrogen peroxide and light showed well separated spots of pure Beclomethasone and Formoterol as well as some additional peaks at different R_t values. The chromatogram of acid degradation study showed additional peak at R_t value 2.81, 7.50 and 10.13 min (Fig.6) and 1.26, 2.18, 9.50min in base degraded samples. The sample degraded with hydrogen peroxide (Fig. 7) showed additional peak at R_t value of 0.61, 3.18 and 11.33min. The sample degraded in dry heat showed additional peak at Rt 1.16, 1.33, 1.91 and 11.33 min and in UV light at Rt 1.91, and 9.38 min (Fig. 10). The percentage recovery in the degradation studies was also calculated for Beclomethasone and Formoterol in the optimized method. More than 90% recovery was obtained for both the drugs in all the degradation conditions except Beclomethasone in base degradation (88.97%).Hence the method was stable in all the stress degradation conditions studied. The results were given in Table 4.

Table 4: Forced degradation studies results

Condition	No of additional peaks observed	Formoterol			Beclomethasone		
		Area Obtained	% Recovered	% Degradation	Area Obtained	% Recovered	% Degradation
Acid	3	219857	88.82	11.18	640958	93.91	6.90
Base	3	228930	92.51	7.49	651763	95.49	4.51
Peroxide	3	230118	92.99	7.01	648024	94.94	5.06
Thermal	4	235819	95.29	4.71	604829	88.62	11.38
UV	2	221039	89.32	10.68	639987	93.77	6.23

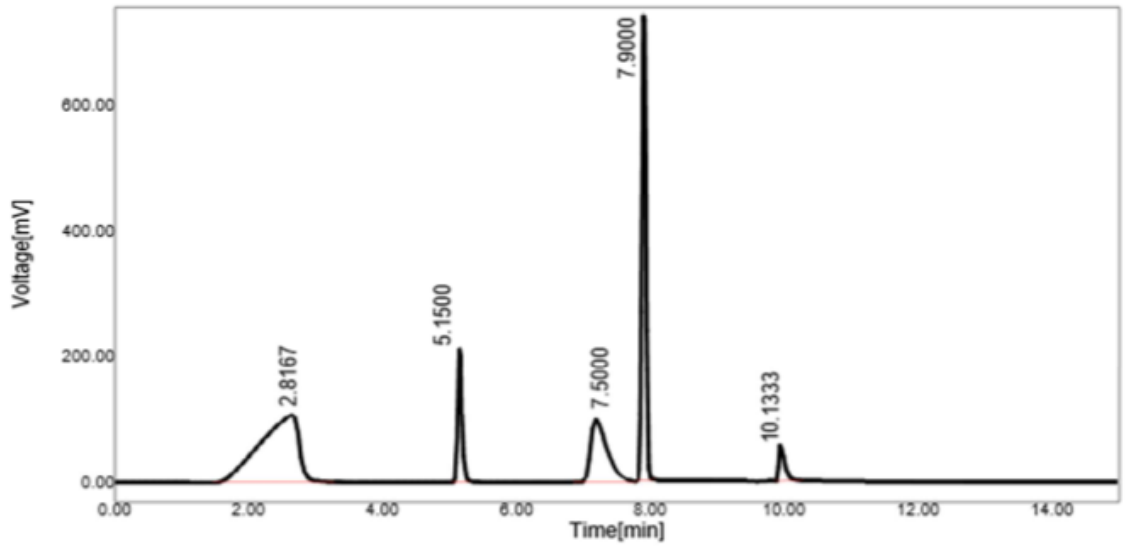


Fig 6: Acid degradation chromatogram

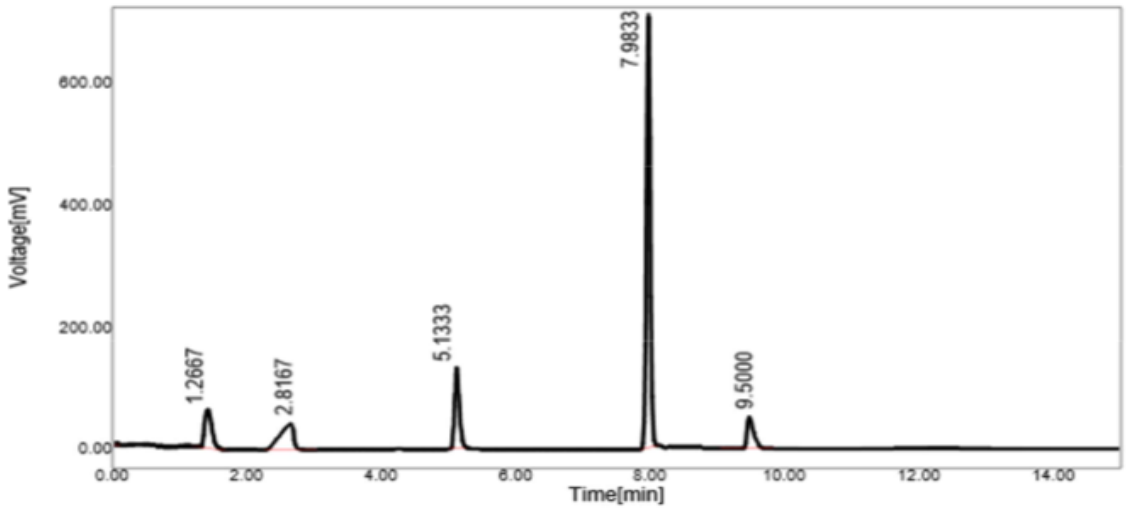


Fig 7: Base degradation chromatogram

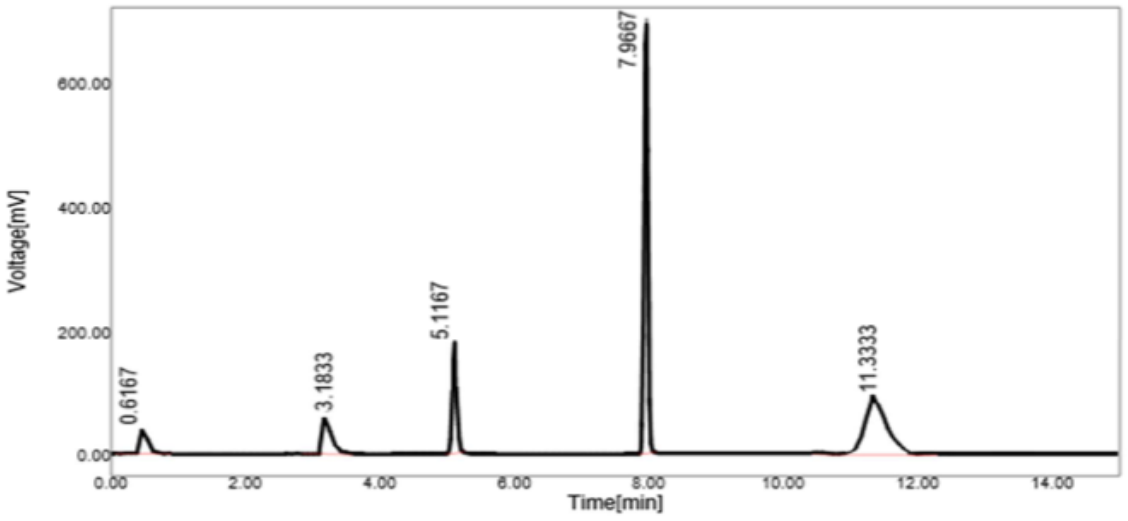


Fig 8: Peroxide degradation chromatogram

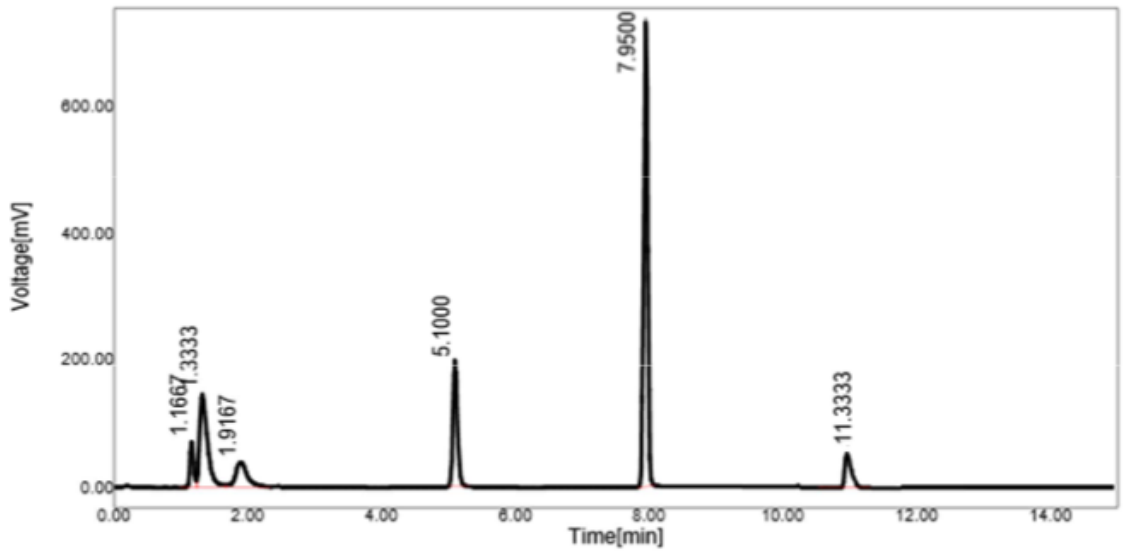


Fig 9: Thermal degradation chromatogram

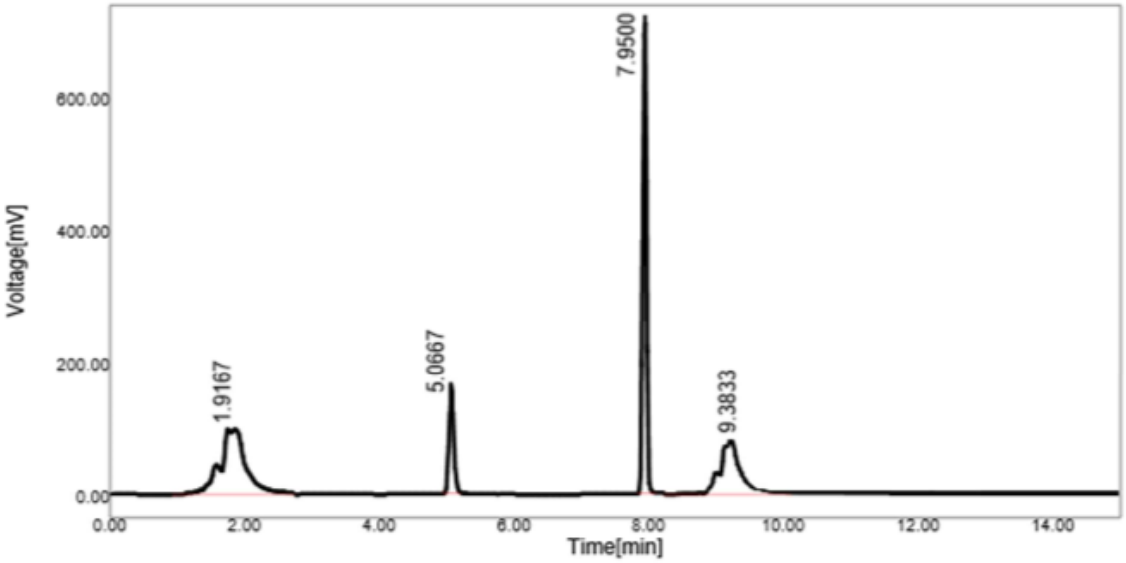


Fig 10: UV light degradation chromatogram

4. Conclusion

An isocratic stability-indicating HPLC-UV method has been developed for the estimation of Formoterol and Beclomethasone in bulk and pharmaceutical formulations. Separation was achieved on Waters C-18 (250mm x 4.6mm, 5µm) column using mobile phase of Methanol and Phosphate buffer in the ratio of 85:15 (v/v) at a flow rate of 1.0 mL/min. UV detection was carried at a wavelength of 215nm.The method is successively applied to pharmaceutical formulation. No chromatographic interferences from the tablet excipients were found. The suitability of this HPLC method for quantitative determination of the compounds is proved by validation in accordance with the requirements of ICH guidelines. Statistical data showed that RP-HPLC methods are robust, rugged, sensitive and accurate as compared to existing analytical methods.

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