DOI: 10.55522/jmpas.V14I4.6914 ISSN NO. 2320 – 7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

UPLC method development and validation for the simultaneous determination of Beclometasone and Formoterol in pharmaceutical dosage form

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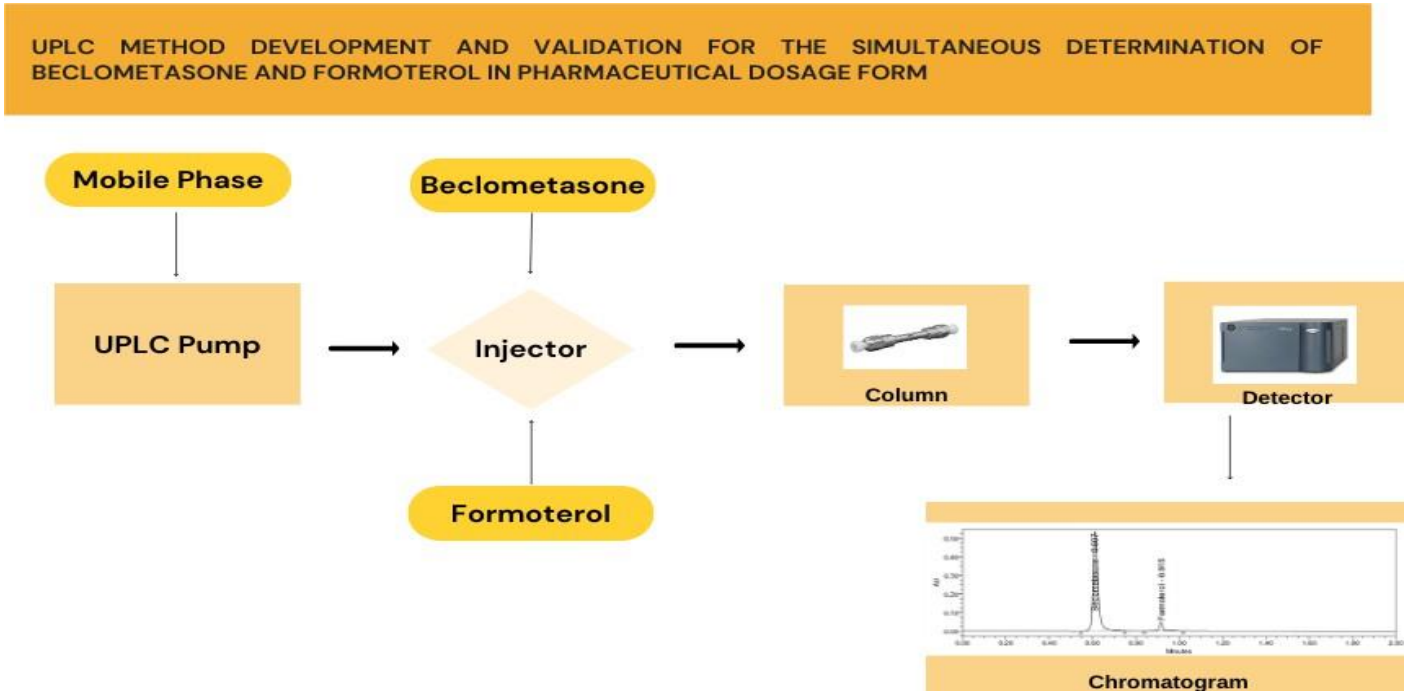
Received - 21-05-2025**, Revised** - 08-06-2025, **Accepted** - 29-08-2025 **(DD-MM-YYYY)**

Refer This Article

T Mallika, P Aparna, K Bhavyasri , 2025 UPLC method development and validation for the simultaneous determination of Beclometasone and Formoterol in pharmaceutical dosage form. Journal of medical pharmaceutical and allied sciences, V 14 - I 4, Pages - 01 – 07. Doi: https://doi.org/10.55522/jmpas.V14I4.6914.

ABSTRACT

A new, simple, Accurate, precise UPLC method was developed for the simultaneous estimation of the Beclometasone and Formoterol in pharmaceutical dosage form. The chromatographic separation was carried out using an HSS C18 column (2.1 × 100 mm, 1.8 µm particle size), maintained at a temperature of 30°C. The mobile phase consisted of orthophosphoric acid and acetonitrile in a 57:43 volume ratio, delivered at a constant flow rate of 0.3 mL/min. Detection of both analytes was performed at 220 nm using a UV detector. Retention time of Beclometasone and Formoterol were found to be 0.607 min and 0.915 min. The developed method was validated as per ICH guidelines. All parameters met the acceptance criteria and this method can be applied for use in routine quality control, formulation analysis, and stability monitoring in pharmaceutical environments.



Keywords: Beclometasone, Formoterol, UPLC method, Asthma, COPD, Quality Control, ICH guidelines.

INTRODUCTION

Beclometasone is a synthetic glucocorticoid commonly used as an anti-inflammatory agent in the treatment of respiratory diseases such as asthma and allergic rhinitis [1]. It functions by binding to glucocorticoid receptors, which results in the modulation of gene expression to suppress the release of pro-inflammatory mediators and reduce airway inflammation [2-3]. Administered primarily via inhalation, beclometasone helps control chronic symptoms and prevents exacerbations by decreasing airway hyper-responsiveness and mucosal edema. Its localized delivery through inhalers minimizes systemic side effects compared to oral corticosteroids. Over several decades, beclometasone has been established as a mainstay in asthma management guidelines, providing effective long-term control when used regularly [4-5].

Formoterol is a long-acting beta2-adrenergic receptor agonist (LABA) widely used in the management of respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD) [6-7]. It works by selectively stimulating beta2 receptors in the airway smooth muscle, leading to relaxation and bronchodilation, which helps relieve airflow obstruction. Unlike short-acting beta2 agonists, Formoterol has a prolonged duration of action, typically lasting about 12 hours, making it suitable for maintenance therapy [8] . It is often administered via inhalation, either alone or in combination with inhaled corticosteroids, to improve lung function, reduce symptoms, and prevent exacerbations. Its rapid onset of action, comparable to that of short-acting agents, distinguishes it from other LABAs, providing both quick relief and sustained control. Safety profiles and efficacy of Formoterol have been well-documented, making it a cornerstone in modern asthma and COPD treatment strategies [9-10].

Figure 1: Chemical structure of Beclometasone

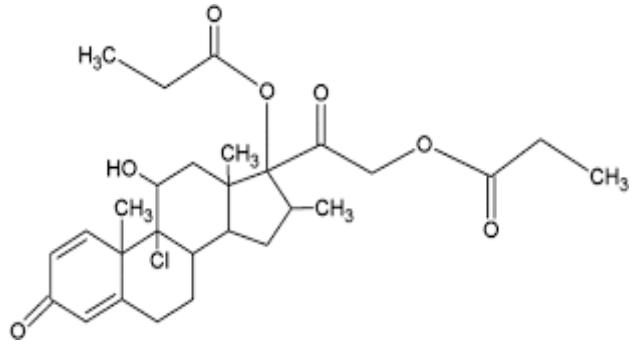
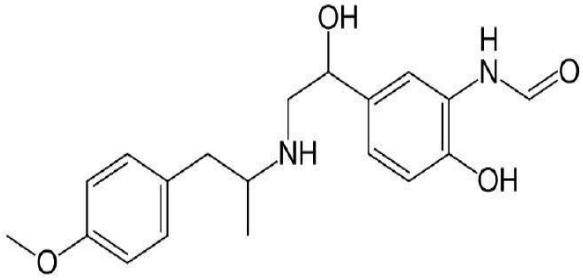


Figure 2. Chemical structure of Formoterol



The combination of Beclometasone and Formoterol is widely used in asthma and COPD treatment due to their complementary anti-inflammatory and broncho dilating effects [11-13]. Accurate estimation of both drugs in a single method is essential for quality assurance but is often complicated by their differing chemical characteristics and low dosage levels [14-16]. Existing methods like UV, HPLC, LCMS methods are time-consuming or lack sensitivity and there is no reported UPLC method [17-18]. UPLC technology offers faster analysis, better resolution, and higher sensitivity compared to traditional HPLC. Developing an efficient UPLC method will support reliable quality control, regulatory compliance, and product development for combination therapies [19-20].

This study aims to develop and validate a fast, precise, and sensitive UPLC method for the simultaneous analysis of Beclometasone dipropionate and Formoterol fumarate in combined pharmaceutical formulations [21]. Objectives include optimizing key chromatographic parameters such as mobile phase, flow rate, and detection settings to ensure effective separation within a short run time. The method will be validated following ICH guidelines, evaluating linearity, precision, accuracy, specificity, detection limits, and quantification limits. Ultimately, the goal is to establish a dependable method for routine quality control and stability analysis [22-23].

MATERIALS AND METHODS

Pure reference standards of Beclometasone dipropionate and Formoterol fumarate were obtained from Spectrum pharma research solutions, Hyderabad and used as received. Marketed formulation - Bekform 400mcg/6mcg Rotacap, Cipla ltd was obtained from local pharmacy store. Analytical-grade / HPLC grade solvents and reagents including HPLC grade water, acetonitrile, methanol, phosphate buffer, potassium dihydrogen orthophosphate, and orthophosphoric acid were procured from Rankem (India) and used without further purification. All chemicals met the criteria for chromatographic analysis.

The chromatographic analysis was performed using a Waters ACQUITY UPLC system equipped with a quaternary solvent delivery pump, a UV (TUV) detector, and an auto sampler, all controlled by Empower 2 software for data acquisition and processing. A Sartorius electronic analytical balance was used for precise weighing of materials. pH adjustments were made using a Labman digital pH meter (India), and sample degassing was carried out using a Labman ultrasonic bath. Additionally, a UV-Visible spectrophotometer with 2 mm and 10 mm matched quartz cells, integrated with UV Win 6 software, was utilized to measure the absorbance of Beclometasone and Formoterol solutions during preliminary method development.

Buffer preparation (0.01 N KH_2PO_4)

An accurately weighed quantity of 1.36 g of potassium dihydrogen orthophosphate was transferred into a 1000 mL volumetric flask. Approximately 900 mL of Milli-Q water was added, and the solution was degassed by sonication. The volume was then made up to the mark with Milli-Q water, and the pH was adjusted to 3.0 using dilute orthophosphoric acid.

Preparation of beclometasone standard stock solution

A quantity of 10 mg of Beclometasone was precisely weighed and transferred into a 25 mL volumetric flask. About three-fourths of the volume was filled with the prepared diluent (a mixture of buffer and acetonitrile in a 50:50 v/v ratio), and the solution was sonicated for 10 minutes to ensure complete dissolution. The final volume was adjusted with the same diluent to obtain a standard stock concentration of 400 µg/mL

Preparation of formoterol standard stock solution

Accurately weighed 0.6 mg of Formoterol was placed into a 100 mL volumetric flask, followed by the addition of approximately 75% of the diluent. After sonication for 10 minutes, the solution was brought to volume with diluent, yielding a standard stock concentration of 6 µg/Ml.

Preparation of standard working solution (100% level)

From the respective stock solutions, 1 mL of Beclometasone (400 µg/mL) and 1 mL of Formoterol (6 µg/mL) were pipetted into a 10 mL volumetric flask. The volume was made up with diluent to obtain a final working solution containing 40 µg/mL of Beclometasone and 0.6 µg/mL of Formoterol.

Preparation of sample stock solutions

Contents of 10 rotacaps (each equivalent to Beclometasone (400mcg)) and Formoterol (6mcg)) were transferred into a 10 ml volumetric flask, 2.5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by 0.45µm filters.

Preparation of sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (40µg/ml of Beclometasone and 0.6µg/ml of Formoterol).

Method validation

The developed UPLC method for the simultaneous quantification of Beclometasone and Formoterol was validated as per ICH Q2(R1) guidelines. The following parameters were assessed:

System suitability

System performance was evaluated by injecting six replicates of a mixed standard solution containing Beclometasone and Formoterol. Key metrics such as retention time, theoretical plate count, resolution, and tailing factor were monitored to ensure consistent chromatographic performance.

Linearity

Calibration curves for both Beclometasone and Formoterol were established with concentrations of Beclometasone (10-60µg/ml) and Formoterol (0.15-0.9µg/ml), suitable for their dosage in combined formulations. A plot of peak area versus concentration was generated, and the correlation coefficients (r^2) were calculated to confirm linearity, with an acceptable limit of $r^2 \geq 0.999$ for both drugs.

Accuracy

Recovery studies were performed by spiking known quantities of Beclometasone and Formoterol into pre-analyzed matrix samples at three concentration levels (50%, 100%, and 150%). The percentage recovery for each analyte was calculated to determine method accuracy.

Precision

Repeatability (intra-day precision) was assessed by analyzing six replicates of a fixed concentration of the combined standard solution containing both Beclometasone and Formoterol under the same conditions on a single day.

Intermediate precision (inter-day) was evaluated by repeating the experiment on different days using different analysts. The %RSD was calculated for each set of data.

Specificity

Specificity was verified by analyzing the chromatograms of blank, placebo, and standard solutions and peaks were assessed for purity and resolution.

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

The LOD and LOQ for Beclometasone and Formoterol were calculated using the standard deviation of the response and the slope of the calibration curve. The formulae used were:

$$LOD = 3.3 \times (\sigma/S)$$
$$LOQ = 10 \times (\sigma/S)$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve for each analyte.

Robustness

Robustness of the method was evaluated by introducing deliberate variations in critical parameters, including flow rate (±0.1 mL/min), mobile phase composition (±5% organic content), and column temperature (±5°C). Standard solutions were injected and effect on retention time, peak area, and resolution of Beclometasone and Formoterol was evaluated to ensure the reliability of the method under slight variations.

RESULTS AND DISCUSSION

Method development

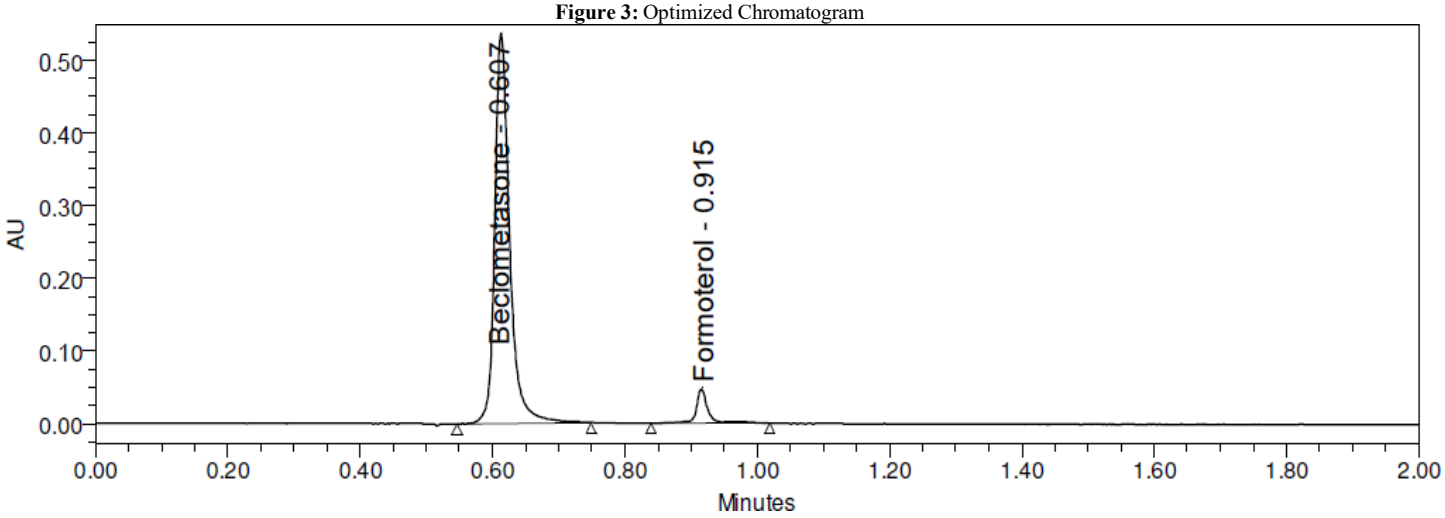
Initial method development focused on identifying suitable chromatographic conditions for the simultaneous analysis of Beclometasone and Formoterol. Various stationary phases and mobile phase combinations were systematically evaluated to achieve efficient separation with sharp, symmetrical peaks and minimal interference. Trials using different proportions of acetonitrile, methanol, and

phosphate buffer were conducted under both isocratic and gradient modes.

Finally, a precise and efficient UPLC method was developed for the simultaneous analysis of Beclometasone and Formoterol. The chromatographic separation was carried out using an HSS C18 column (2.1 × 100 mm, 1.8 µm particle size), maintained at a temperature of 30°C. The mobile phase consisted of orthophosphoric10.

acid and acetonitrile in a 57:43 volume ratio, delivered at a constant flow rate of 0.3 mL/min. Detection of both analytes was performed at 220 nm using a UV detector. A sample injection volume of 2.00 µL was utilized, with the total run time set to 2 minutes for effective separation. The diluent used for preparing the samples was a 1:1 mixture of buffer and acetonitrile, providing adequate solubility and compatibility with the chromatographic system and the chromatogram is given in the Figure 3.

Figure 3: Optimized Chromatogram



System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines and the data is given in the Table 1.

Table 1: System suitability parameters for Beclometasone and Formoterol

Property	Beclometasone	Formoterol
Retention time	0.607 min	0.915 min
Theoretical plates (N)	3578	4822
Tailing factor (T)	1.27	1.18

Specificity

Specificity was verified by analyzing the chromatograms of blank, placebo, and standard solutions and confirmed that there was no interference at the retention times of Beclometasone and Formoterol.

Linearity

Six linear concentrations of Beclometasone (10-60µg/ml) and Formoterol (0.15-0.9µg/ml) were injected in a triplicate manner. Average areas were mentioned and linearity equations obtained for Beclometasone was y = 42226x + 43717 and of Formoterol was y = 107763x + 405. Correlation coefficient obtained was 0.999 for the two drugs and the data is given in the Figure 4 and Figure 5.

Precision

Repeatability

Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.4% and 0.4% respectively for Beclometasone and Formoterol. As the limit of Precision was less than "2" the system precision was passed in this method and the data is given in the Table 2.

Figure 4: Calibration curve of Beclometasone

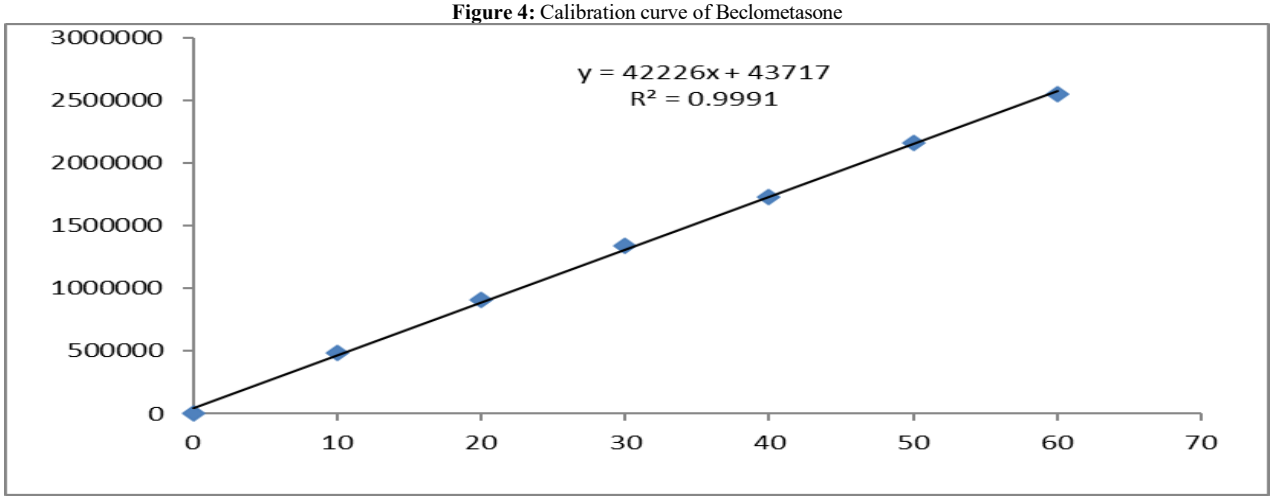


Figure 5: Calibration curve of Formoterol

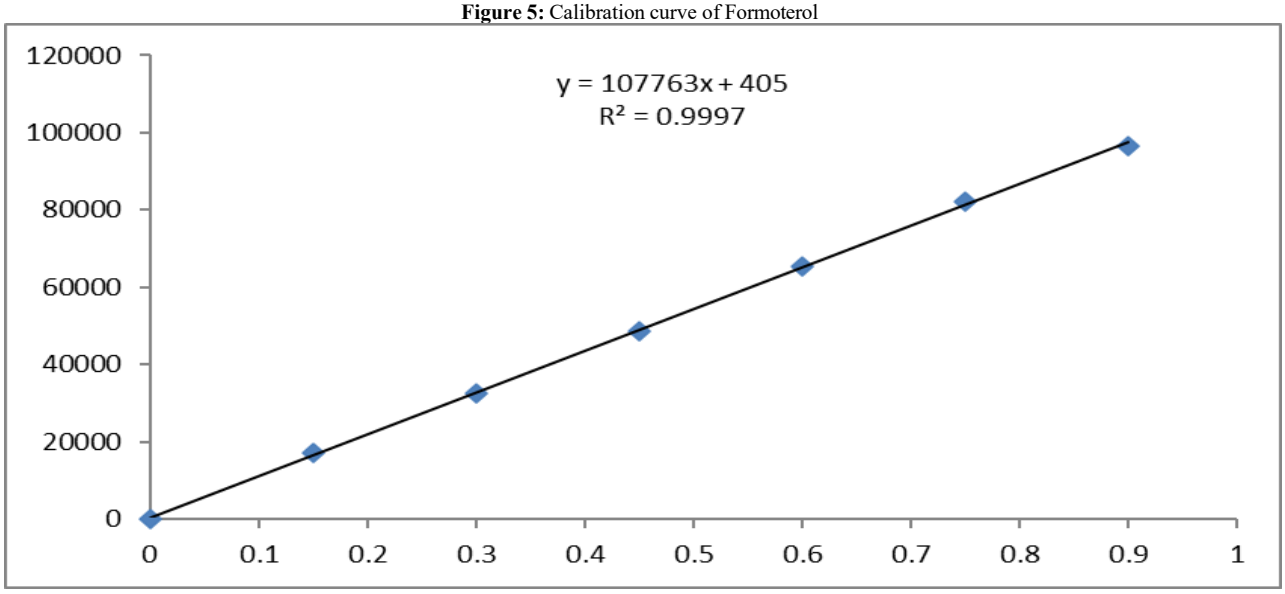


Table 2: Repeatability table of Beclometasone and Formoterol

	Area of Beclometasone	Area of Formoterol
	1782793	65869
	1796185	65950
	1798419	66259
	1787684	65417
	1794884	65807
	1782846	65818
Mean	1790469	65853
S.D	6930.4	271.3
%RSD	0.4	0.4

Intermediate precision

Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.9% and 0.5% respectively for Beclometasone and Formoterol. As the limit of Precision was less than "2" the system precision was passed in this method and the data is given in the Table 3.

Table 3: Intermediate precision table of Beclometasone and Formoterol

	Area of Beclometasone	Area of Formoterol
	1741288	65869
	1749951	65150
	1763449	65259
	1720323	65417
	1755599	65007
	1732220	65018
Mean	1743805	65287
S.D	15841.2	324.3
%RSD	0.9	0.5

Accuracy

The mean %Recovery was obtained as 99.64% and 100.13 % for Beclometasone and Formoterol, respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The low detection and quantification thresholds achieved for both analytes highlight the method's robustness and suitability for routine quality control, where precise quantification at minimal concentrations is often required and the data is given in the Table 4.

Table 4: Limit of Detection (LOD) and Limit of Quantitation (LOQ) values of Beclometasone and Formoterol

Molecule	LOD	LOQ
Beclometasone	0.22	0.66
Formoterol	0.003	0.011

Robustness

Robustness of the method was evaluated by introducing deliberate variations in critical parameters, including flow rate (±0.1 mL/min), mobile phase composition (±5% organic content), and column temperature (±5°C). Samples were injected in triplicate under each condition. The system suitability parameters remained consistent, and all results complied with acceptance criteria. The %RSD values for both analytes were within the prescribed limits, confirming the method's reliability under minor operational changes and the data is given in the Table 5.

Table 5: Robustness data for Beclometasone and Formoterol.

Condition	%RSD Beclometasone	%RSD Formoterol
Flow rate Minus	0.9	0.8
Flow rate Plus	0.9	0.9
Mobile phase Minus	0.8	0.7
Mobile phase Plus	0.7	0.8
Temperature Minus	0.3	0.4
Temperature Plus	1.0	0.9

Assay

The assay results demonstrated that both active pharmaceutical ingredients (APIs) were present within the acceptable limits of their labeled claim, as per pharmacopeial standards. Beclometasone dipropionate was found to be 100.11% of the labeled amount, and Formoterol fumarate was 99.68% indicating consistent drug content and confirming the formulation's uniformity. These values reflect the method's ability to accurately quantify both APIs despite their presence at significantly different concentrations in the dosage form and the data is given in the Table 6.

Table 6: Assay Data of Beclometasone and Formoterol

	% Assay of Beclometasone	% Assay Formoterol
	99.68	99.70
	100.43	99.82
	100.56	100.29
	99.96	99.01
	100.36	99.60
	99.69	99.62
Avg	100.11	99.68
Stdev	0.39	0.41
%RSD	0.39	0.41

Degradation studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

CONCLUSION

A simple, Accurate, precise UPLC method was developed for the simultaneous estimation of the Beclometasone and Formoterol in pharmaceutical dosage form. The chromatographic separation was carried out using an HSS C18 column (2.1 × 100 mm, 1.8 µm particle size), maintained at a temperature of 30°C. The mobile phase consisted of orthophosphoric acid and acetonitrile in a 57:43 volume ratio, delivered at a constant flow rate of 0.3 mL/min. Detection of both analytes was performed at 220 nm using a UV detector. Retention time of Beclometasone and Formoterol were found to be 0.607 min and 0.915 min. The developed method was validated as per ICH guidelines. All parameters met the acceptance criteria and this method can be applied for use in routine quality control, formulation analysis, and stability monitoring in pharmaceutical environments.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my research guide for their invaluable guidance, support, and encouragement throughout this work. I also extend my heartfelt thanks to Jawaharlal Nehru Technological University (JNTU) for providing the necessary facilities and support to carry out this research.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this study.

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