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Development and Validation of a Viable and User-Friendly Spectrophotometric Analytical Method for Estimation of Assay of Formoterol and Beclomethasone in Metered Dose Inhaler Formulation

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ABSTRACT: The primary aim of this research endeavor is to innovate a viable and user-friendly UV spectroscopic approach for quantifying formoterol and beclomethasone within metered dose inhalers (MDIs). These formulations, amalgamating Beclomethasone Dipropionate (BDP) and Formoterol Fumarate (FF), are extensively employed in managing bronchial asthma and chronic obstructive pulmonary diseases. Although analytical techniques already exist for assessing formoterol and beclomethasone in combination dry powder inhalers, this study concentrates on devising and validating a UV spectrophotometric method for simultaneously quantifying formoterol fumarate and Anhydrous Beclomethasone Dipropionate in Pressurized Metered Dose Inhalers (pMDIs).

The estimation is performed at a wavelength of 240 nm for Beclomethasone Dipropionate and 216 nm for Formoterol Fumarate. Both drugs demonstrate satisfactory linearity within the concentration range of 10-30 µg/ml for BDP and 0.3 to 0.9 µg/ml for FF at wavelengths of 240 nm (r^2=0.999) and 216 nm (r^2=0.999), respectively. The Beer-Lambert law is adhered to within the concentration range of 10-30 µg/ml for BDP and 0.3-0.9 µg/ml for FF.

The proposed methods are subjected to validation following ICH guidelines to assess accuracy, method precision, specificity, among other parameters. As a result, the proposed method can be effectively employed for the simultaneous determination of Beclomethasone Dipropionate and Formoterol Fumarate in routine analytical applications, offering a rapid and reliable solution for pharmaceutical analysis in the field.

KEYWORDS: Analytical Method, Beclometasone Dipropionate, Development and Validation, Formoterol Fumarate, pMDI, Spectroscopic.

1.0 INTRODUCTION

Asthma, a chronic condition instigated by airway inflammation, manifests with symptoms such as coughing, wheezing, chest tightness, and breath shortness. This inflammation prompts heightened reactivity of smooth muscles, inducing forceful contractions (bronchospasm) in response to various triggers. Asthma typically exhibits phases of asymptomatic periods interspersed with acute exacerbations. In the context of the present study, the concurrent administration of formoterol fumarate and beclomethasone dipropionate via pressurized metered dose inhalers (pMDIs) demonstrates a notable synergistic effect in the management and regulation of asthma. Chronic Obstructive Pulmonary Disease (COPD) constitutes a complex ailment involving emphysema within the lung parenchyma, inflammation of large central airways, dysfunction of the mucociliary apparatus, bronchiolitis, and structural alterations in small airways. These changes lead to persistent respiratory symptoms and airflow limitation, attributed to either airway or alveolar abnormalities due to substantial exposure to harmful particles and gases.

A pressurized metered dose inhaler (pMDI) comprises a multi-dose system encompassing an aluminium canister housed within a plastic actuator, facilitating drug delivery through the actuator's orifice. The canister accommodates either a suspension of micronized drug crystals or a drug solution with a co-solvent, suspended in a propellant or occasionally in a blend of propellants. Suspension formulations primarily incorporate a surfactant (e.g., oleic acid or lecithin) to mitigate particle agglomeration. The components of an MDI encompass the active ingredient combined with the propellant, the surfactant/solvent termed as excipient, and the drug container. This container integrates a metering valve crimped onto it, an actuator linking the metering valve to the spray nozzle, and a mouthpiece. Moreover, a retention chamber or spacer may also be integrated into the delivery system by

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connecting it to the actuator mouthpiece. A precisely measured volume (typically ranging from 20 to 100µL) of the drug, excipient, and propellant mixture is expelled from the canister through a valve, rapidly traversing through the actuation port where atomization occurs. [1]

Beclometasone dipropionate, chemically represented as (11β,16β)-9-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy) pregna-1,4-diene-3,20-dione (see Fig. 1), is classified as a glucocorticosteroid. It is utilized to aid in reducing inflammation associated with Chronic Obstructive Pulmonary Disease (COPD. [2,3,4]

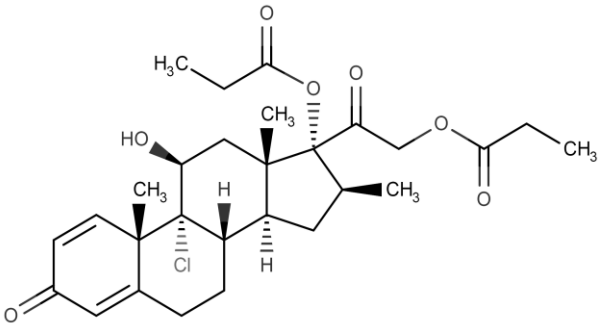

Fig. 1 Chemical Structure of Anhydrous Beclomethasone Dipropionate

Formoterol fumarate dihydrate, molecularly denoted as (2E)-but-2-enedioic acid bis(N-{2-hydroxy-5-[(1R)-1-hydroxy-2-{[(2R)-1-(4-methoxyphenyl)propan-2yl]amino} ethyl]phenyl} formamide) dihydrate (refer to Fig. 2), belongs to the class of long-acting beta 2 agonists. Formoterol fumarate dihydrate serves to dilate and relax the air passages within the lungs, aiding in respiratory function improvement. [5,6,7]

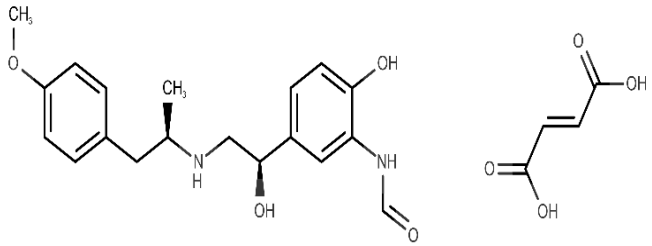

Fig. 2. Chemical Structure of Formoterol Fumarate

The synergistic action of both Beclometasone dipropionate and Formoterol fumarate dihydrate collaboratively works to maintain airway patency and enhance respiratory ease. Extensive literature surveys confirm the existence of numerous analytical techniques for the quantification of Formoterol Fumarate, either individually or in conjunction with other bronchodilator medications. Additionally, several methodologies have been documented for the determination of Beclometasone, either in isolation or in combination with other therapeutic agents, utilizing UV spectrophotometry.

Moreover, analytical methods specifically tailored for the estimation of Formoterol and Beclometasone within combination dry powder inhalers have been extensively explored and reported in scientific literature. These methods contribute to the advancement of pharmaceutical analysis, ensuring accurate and reliable quantification of these active pharmaceutical ingredients in inhalation formulation. Indeed, an endeavor was undertaken to develop and validate a UV spectrophotometric method for the concurrent estimation of Formoterol fumarate and Anhydrous Beclomethasone Dipropionate within pressurized metered dose inhalers (pMDIs). This effort represents a significant advancement in pharmaceutical analytical techniques, aiming to provide a reliable and efficient means of quantifying these active ingredients in inhalation formulations. The simultaneous estimation of Formoterol fumarate and Beclomethasone Dipropionate in pMDIs via UV spectrophotometry signifies a valuable contribution to pharmaceutical research and development. Such methodologies play a crucial role in ensuring the quality, safety, and efficacy of inhalation therapies for the management of respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD).[8-14]

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2.0 MATERIALS AND METHODS [15-18]

2.1 *Instrument Used*

The analysis was conducted utilizing a UV-visible double beam Spectrophotometer manufactured by Agilent Technologies, specifically the Cary®UV model. Quartz cells with a path length of 10 mm were employed for the analysis. The absorption spectra of both the reference and test solutions were measured using a 1 cm quartz cell, covering the wavelength range from 200 to 400 nm. This instrumentation setup ensures precise and accurate measurement of absorbance within the specified wavelength range, facilitating the reliable determination of the concentrations of Formoterol fumarate and Anhydrous Beclomethasone Dipropionate in pressurized metered dose inhalers.[15-16]

2.2 *Reagents And Chemicals*

Pure drug samples of Formoterol Fumarate (FF) and Anhydrous Beclomethasone Dipropionate (BDP) were generously provided as gift samples by Vamsi Labs Ltd. in Solapur and Avik Pharma Pvt. Ltd. in Vapi, Gujarat, respectively. Polyethylene glycol 1000 (PEG 1000) was obtained as a gift sample from Croda Chemicals in Mumbai. The HFA 134a Propellant System, metered valve, and aluminium canisters were graciously provided as gift samples by Medisol Lifescience Pvt. Ltd. in Vapi, Gujarat.

Methanol of analytical grade and Milli-Q water of HPLC grade were utilized throughout the study for various analyses and formulations. The innovative combination metered dose inhaler formulation of Formoterol and Beclomethasone was developed utilizing these high-quality materials, paving the way for enhanced therapeutic outcomes in respiratory care. [15-16]

2.3 *Preparation of Standard Stock Solution*

Precisely weighed standard quantities of Beclomethasone Dipropionate (BDP) and Formoterol Fumarate (FF), each 10 mg, were separately transferred into individual 100 mL volumetric flasks. Subsequently, 10 mL of methanol (MeOH) was added to each flask to dissolve the drugs. The flasks were then shaken manually for 5 minutes to ensure complete dissolution of the drugs. Following dissolution, the volume was adjusted by adding additional methanol to achieve the appropriate concentrations of Beclomethasone Dipropionate (BDP) and Formoterol Fumarate (FF) at 100 µg/mL each. This meticulous process ensures accurate preparation of standard solutions with the desired concentrations for subsequent analyses and formulation development.

2.4 *Determination of Absorption Maxima (λmax)*

From the standard stock solutions of Beclomethasone Dipropionate (BDP) and Formoterol Fumarate (FF) with a concentration of 100 µg/mL, 1 mL of each solution was pipetted out and transferred into two separate 10 mL volumetric flasks. The volume in each flask was then made up to the mark with the respective solvent to achieve a final concentration of 10 µg/mL for both BDP and FF. Subsequently, both solutions were subjected to UV-visible spectrophotometric analysis in spectrum mode over the wavelength range of 200-400 nm to determine their absorption maxima. This step is crucial for identifying the wavelengths at which maximum absorbance occurs for each compound, providing essential information for subsequent quantitative analysis and method development.

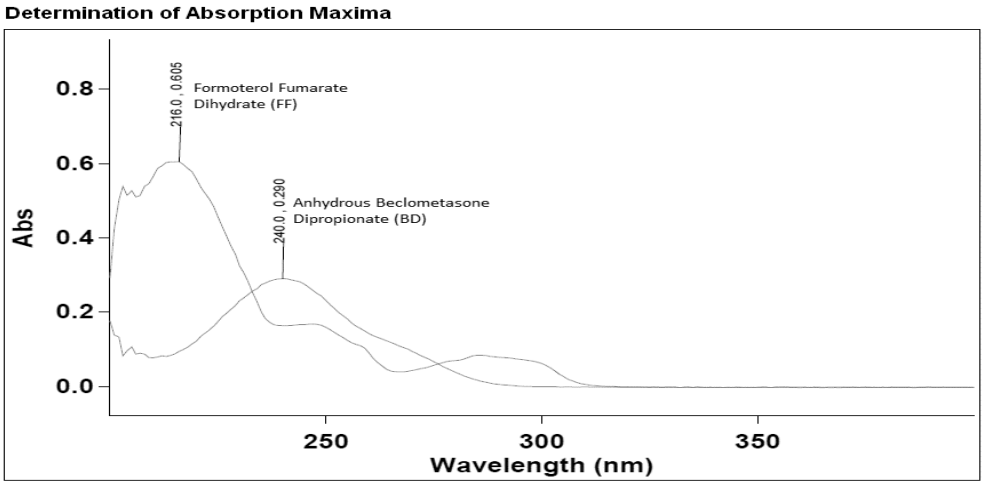

Fig. 3. Overlain Spectrum of Beclometasone and Formoterol in MeOH

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2.5 *Preparation of Sample Solution*

Place Teflon /Steel base plate/ and a central circular indentation with a hole about 1.5 mm in diameter in a small beaker (100mL) suitable for shaking and add the 35ml diluents (MeOH) in 100 mL beaker. Shake vertically the pressurized canister for about 30 seconds and place it inverted in 100 ml beaker, actuate immediate 1st spray, followed by subsequent 9 sprays below the surface of the 35ml diluent maintaining the pressurized canister in the vertical plane through the hole in the centre of the base plate. Quantitatively transfer contents to a 100 ml volumetric flask and add diluents. Rinse the beaker and canister up to ferrule with water and quantitatively transfer to a 100 ml volumetric flask and make up to the volume with diluents. Repeat process Middle 10 sprays assay and end 10 spray of canister.

2.6 *Validation Methods*

The assay method for quantifying Formoterol and Beclomethasone underwent validation according to the stringent criteria outlined in the ICH Q2 R1 Guidelines, for various parameters such as specificity and system suitability, Linearity and Range, Accuracy, and System / Method Precision. [17-20]

2.6.1 *Linearity and range*

Linearity refers to the ability of an analytical method to yield test results that are directly proportional to the concentration of the analyte within a defined range, typically spanning from 50% to 150%. This range, known as the linearity range, encompasses the upper and lower concentration limits within which the method reliably demonstrates linear behaviour. For this study, standard solutions containing Beclomethasone Dipropionate (BDP) at concentrations of 10, 16, 18, 20, 22, 24, and 30 µg/ml, and Formoterol Fumarate (FF) at concentrations of 0.30, 0.48, 0.54, 0.60, 0.66, 0.72, and 0.90 µg/ml were meticulously selected to establish the linearity range. These concentrations represent the upper and lower levels of the analytes that exhibit linearity when analysed using the described method. A calibration curve was constructed by plotting the concentration of the standard solutions against the corresponding absorbance values, with regression analysis employed to determine the regression equation. The least squares method facilitated the calculation of the slope, intercept, and correlation coefficient (which is expected to exceed 0.99), thus providing a robust assessment of the method's linearity across the specified concentration range (Table I).

Table I. Dilutions for Linearity and Range

Linearity Level	*Solution to be taken from 'Solution(C)'mL*	*Final Volume(mL)*	*Conc. of BDP(µg/mL)*	*Conc. of FF (µg/mL)*
Linearity -50%	5.0	100	10.0	0.30
Linearity -80%	8.0	100	16.0	0.48
Linearity -90%	9.0	100	18.0	0.54
Linearity -100%	10.0	100	20.0	0.60
Linearity -110%	11.0	100	22.0	0.66
Linearity -120%	12.0	100	24.0	0.72
Linearity -150%	15.0	100	30.0	0.90

2.6.2 *Detection and Quantification Limits*

The Limit of Detection (LOD) and Limit of Quantification (LOQ) are derived from the standard deviation of the response and the slope of the constructed calibration curve. The LOD is determined as 3.3 times the standard deviation of the response divided by the slope of the calibration curve. On the other hand, the LOQ is calculated as 10 times the standard deviation of the response divided by the slope of the calibration curve. These parameters provide valuable insights into the sensitivity and reliability of the analytical method, allowing for precise determination of lower concentrations of the analytes with acceptable levels of accuracy and precision. LOD = 3.3σ/S and LOQ = 10σ/S, where S=Slope and σ = Std. deviation.

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2.6.3 ***Specificity and system suitability***

The analytical capability to measure the active ingredients accurately and selectively within a metered dose inhaler (MDI), in the presence of other components like excipients, was investigated. A placebo solution was prepared using a sample containing polyethylene glycol and propellant HFA. Subsequently, standard solutions for Beclomethasone (solution-1 at 400.0 ppm) and Formoterol (solution-2 at 120.0 ppm) were prepared. A final standard stock solution was created by blending these solutions to achieve concentrations of 20.0 ppm for Beclomethasone and 0.60 ppm for Formoterol.

The blank, placebo, standard solutions, sample replicates (initial, middle, end), and bracketing standard solution were all analyzed using the established analytical method on a UV spectrophotometer. This analysis aimed to identify any interference from the placebo with the absorbance readings of Formoterol and Beclomethasone. It was ensured that both the blank and placebo solutions exhibited no interference at the wavelengths corresponding to those of Beclomethasone and Formoterol. Additionally, the % Relative Standard Deviation (%RSD) of the standard absorbance was required to be within 2.0%, ensuring consistency and precision in the measurements.

2.6.4 ***Accuracy***

To assess the accuracy of the analytical method, percentage recovery studies were conducted at three distinct levels: 50%, 100%, and 150%. Standard solutions of Beclomethasone Dipropionate (BDP) at a concentration of 1000 µg/mL (designated as solution-A) and Formoterol Fumarate (FF) at a concentration of 120 µg/mL (designated as solution-B) were prepared. Additionally, a recovery stock solution (solution C) containing both BDP and FF was prepared as a mixture. The recovery studies involved adding a known quantity of the standard recovery solution containing BDP and FF to a placebo solution.

The resulting solutions were then analyzed in triplicate for each level using the proposed analytical method. The total amount of drug detected, and the percentage recovery were subsequently calculated. To ensure the reliability of the method, the % Relative Standard Deviation (%RSD) of the standard absorbance was required to be less than 2.0%. Moreover, the percentage recovery was expected to fall within the range of 98.0% to 102.0%. These criteria ensured the accuracy and precision of the analytical method in quantifying BDP and FF in the tested samples (Table II).

Table II. Dilutions for each accuracy level in triplicate

Accuracy level	*Solution to be taken from Solution(C)'ml*	*Placebo Taken (No of sprays)*	*Final Volume with diluent*	*Amt. of BDP added (µg/ml)*	*Amt. of FF added µg/ml)*
Accuracy 50.0%-1	5.0	10	100	10.00	0.30
Accuracy 50.0%-2	5.0	10	100	10.00	0.30
Accuracy 50.0%-3	5.0	10	100	10.00	0.30
Accuracy 100.0%-1	10.0	10	100	20.00	0.60
Accuracy 100.0%-2	10.0	10	100	20.00	0.60
Accuracy 100.0%-3	10.0	10	100	20.00	0.60
Accuracy 150.0%-1	15.0	10	100	30.00	0.90
Accuracy 150.0%-2	15.0	10	100	30.00	0.90
Accuracy 150.0%-3	15.0	10	100	30.00	0.90

2.6.5 ***System / method precision***

The precision of the spectroscopic method was evaluated through two aspects: repeatability and system/method precision. For repeatability assessment, the absorbance of standard solutions containing Beclomethasone Dipropionate (BDP) and Formoterol Fumarate (FF) at specified concentrations (µg/mL) was measured six times. The % Relative Standard Deviation (%RSD) was then calculated to gauge the variability within the measurements.

In the case of system/method precision, six replicates of both standard and sample solutions were analyzed for each of the initial, middle, and end stage sprays. The %RSD for standard absorbance was expected to be below 2.0%, ensuring consistency and reliability of the method. Additionally, for sample absorbance, the %RSD was required to be within 5.0%, indicating acceptable variability within the samples. These precision evaluations ensure the robustness and reliability of the spectroscopic method for

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quantifying BDP and FF, enabling accurate and consistent measurement of these compounds in various formulations and conditions.

2.7 *Analysis of Content of FF and BDP in pMDI.*

The % assay of pressurized metered dose inhaler (pMDI) samples at the initial, middle, and end stages of the canister were analyzed using the developed and validated analytical method to detect the active contents of Formoterol Fumarate and Beclomethasone Dipropionate. This analysis aimed to assess the concentration of the active ingredients in the pMDI samples at different stages of canister usage, ensuring consistent and accurate delivery of the intended doses throughout the product's lifecycle. By utilizing the established analytical method, any variations in the content of Formoterol Fumarate and Beclomethasone Dipropionate across the canister's lifespan could be effectively monitored and controlled, thereby ensuring the efficacy and safety of the medication for patients.

3.0 RESULTS AND DISCUSSION

The solutions containing Beclometasone Dipropionate (BDP) and Formoterol Fumarate (FF) were subjected to UV scanning in the spectrum mode over the wavelength range of 200-400 nm, with data recorded at 1 nm intervals. The resulting UV absorbance versus wavelength curve is depicted in Figure 3. Maximum absorbance peaks were observed at 240.0 nm for Anhydrous Beclometasone Dipropionate (BDP) and at 216.0 nm for Formoterol Fumarate (FF). These specific wavelengths were further employed for the determination of BDP and FF in combination pharmaceutical dosage forms, specifically pressurized metered dose inhalers (pMDIs). The developed analytical method underwent rigorous validation for various parameters including specificity, linearity, precision, and accuracy, in accordance with the guidelines outlined in the International Conference on Harmonisation (ICH) Q2 (R1). This comprehensive validation ensures the reliability and robustness of the analytical method for quantifying BDP and FF in pMDIs, thereby facilitating accurate and precise pharmaceutical analysis. [21-23]

3.1 *Linearity and Range*

A linear correlation was established between the absorbance at λmax and the concentrations of Beclometasone Dipropionate (BDP) and Formoterol Fumarate (FF). These correlations were determined within the selected concentration ranges of 0.3-0.9 µg/ml for FF and 10-30 µg/ml for BDP.

The correlation coefficient for both BDP and FF was found to be 0.9999, indicating an exceptionally strong linear relationship between absorbance and standard drug concentration. (Table. III).

Table III. Results of Linearity and Range

Linearity Level	*Solution to be taken from 'Solution(C)'ml*	*Final Volume (ml)*	*Conc. (µg/ml)*		*Absorbance*	
			BDP	*FF*	*BDP*	*FF*
50%	5	100	10	0.3	0.1523	0.3079
80%	8	100	16	0.48	0.2391	0.4936
90%	9	100	18	0.54	0.2667	0.5509
100%	10	100	20	0.6	0.2962	0.6096
110%	11	100	22	0.66	0.3266	0.6770
120%	12	100	24	0.72	0.3537	0.7330
150%	15	100	30	0.9	0.4379	0.9150
				Average	0.2960±0.09	0.6124±0.19

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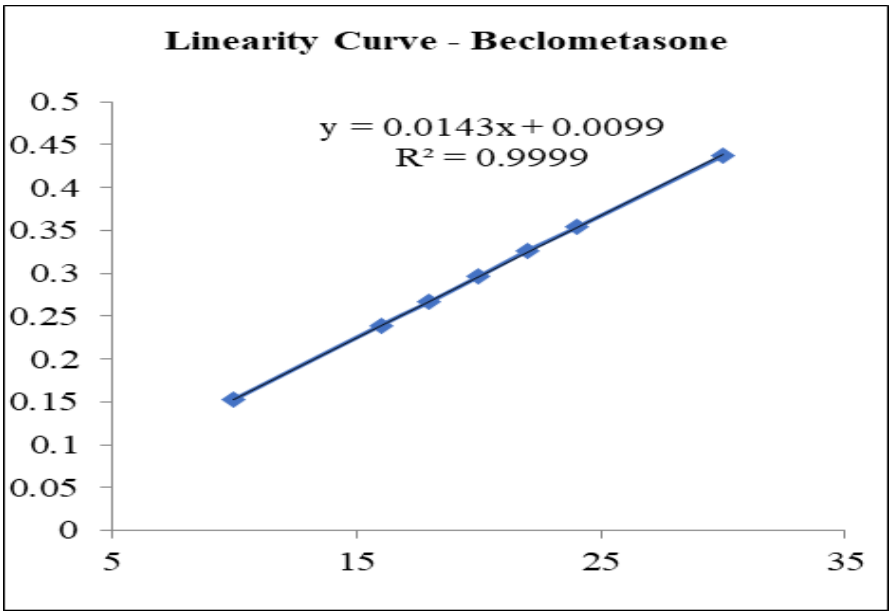

Fig. 4. Linearity Curve of Beclometasone Dipropionate at 240 nm

Figures 4 and 5 depict the calibration and linearity curves for BDP and FF, respectively. These curves visually demonstrate the linear correlation between absorbance and drug concentration within the specified ranges. Table III provides additional details regarding the calibration and linearity parameters for both BDP and FF.

Overall, these findings underscore the robustness and accuracy of the developed analytical method in quantifying BDP and FF within the defined concentration ranges, ensuring reliable and precise pharmaceutical analysis in pressurized metered dose inhalers (pMDIs).

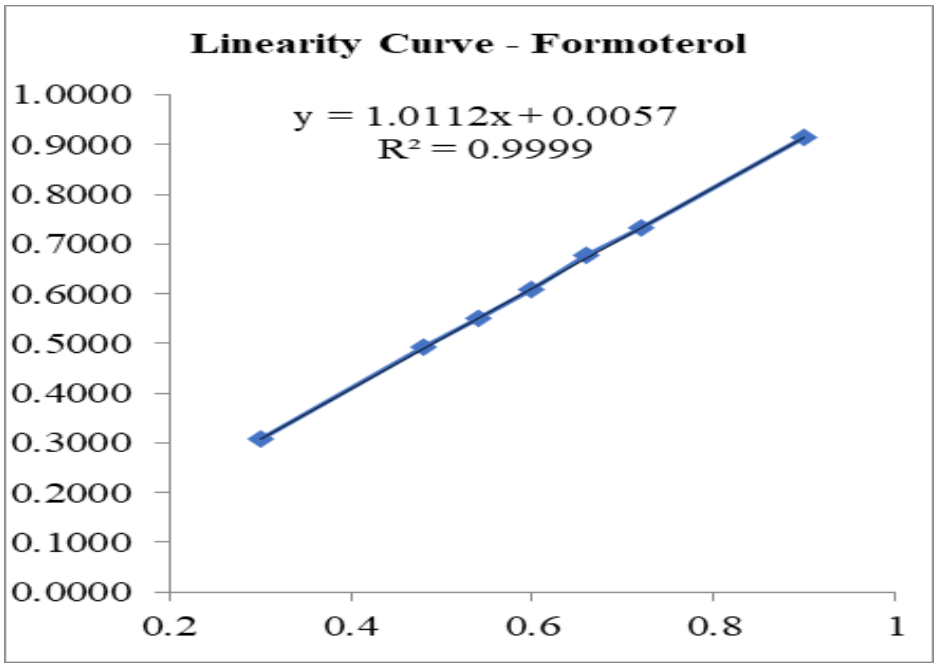

Fig. 5. Linearity Curve of Formoterol Fumarate at 216 nm

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3.2 *Detection and Quantification Limits*

The limit of detection (LOD) and limit of quantification (LOQ) for Formoterol Fumarate (FF) were determined to be 0.0082 µg/ml and 0.0247 µg/ml, respectively. Similarly, for Beclometasone Dipropionate (BDP), the LOD and LOQ were found to be 18.4154 µg/ml and 55.8042 µg/ml, respectively. These values indicate that the proposed UV method exhibits high sensitivity for detecting both FF and BDP in pharmaceutical formulations. For detailed regression analysis data of the developed method, please refer to Table IV. This high sensitivity enables accurate and reliable detection of FF and BDP even at very low concentrations, making the UV method well-suited for pharmaceutical analysis and quality control purposes.

Table IV. Regression analysis data of developed method

Parameters	***Formoterol***	***Beclometasone***
Wavelength (nm)	216.00	240.00
Linearity (µg/ml)	0.3-0.9	10-30
Regression Equ. (y=mx+c)	y = 1.0112x + 0.0057	y = 0.0143x + 0.0099
Slope = m	1.0112	0.0143
Intercept = C	0.0057	0.0093
Corr. Coef. (R^2)	0.9999	0.9999
STD. DEV. of Intercept	0.191880	0.090505
STEYX	0.0025	0.0798
LOD (µg/ml)	0.0082	18.4154
LOQ (µg/ml)	0.0247	55.8042

3.3 *Specificity, and System Suitability*

The blank and placebo solutions demonstrated no interference at the wavelengths corresponding to Beclometasone Dipropionate (BDP) and Formoterol Fumarate (FF), indicating non-interference by the inactive ingredients added to prepare the placebo solution. The % Relative Standard Deviation (%RSD) of the absorbance of standard solutions was monitored and found to be well within the specified limit of not more than 2.0%.

The system suitability criteria were met in each sequence and scan of the validation parameters. Additionally, the testing method using UV spectrophotometry was found to be specific, demonstrating its ability to measure BDP accurately and selectively and FF in the presence of other components. Overall, these findings confirm the reliability and robustness of the developed UV spectrophotometric method for quantifying BDP and FF in pharmaceutical formulations, ensuring accurate and precise analysis for quality control purposes.

3.4 *Accuracy*

The accuracy of an analytical method reflects the degree of closeness between test results and the true value. In this study, the accuracy of the method was assessed using the standard addition method at three concentration levels. Standard quantities equivalent to 50%, 100%, and 150% of the expected concentration were added to the placebo sample solutions. The % Relative Standard Deviation (%RSD) of the absorbance of standard solutions was found to be less than 2.0%, indicating good precision within the method. Furthermore, the % recovery fell within the range of 98.0% to 102.0%, confirming the accuracy of the developed analytical method. This range of % recovery also suggests non-interference with the excipients present in the samples. Hence, based on these results presented in Table V and VI, it can be concluded that the analytical method exhibits accuracy and reliability in quantifying the target analytes, Beclometasone Dipropionate (BDP) and Formoterol Fumarate (FF), in the tested samples.

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Table V. Results of % Recovery of Formoterol Fumarate

Accuracy Level %	*Drug added (µg/mL)*	*Amount recovered (µg/mL)*	*% Recovery (n=3)*	*Mean± SD*	*% RSD*
	0.300	0.303	101.04		
50.0%	0.300	0.304	101.52	101.79±0.92	0.90
	0.300	0.308	102.82		
	0.599	0.605	100.87		
100.0%	0.599	0.609	101.65	101.63±0.75	0.74
	0.599	0.614	102.37		
	0.899	0.918	102.06		
150.0%	0.899	0.908	101.01	100.60±1.70	1.69
	0.899	0.888	98.73		

Table VI. Results of % Recovery of Beclometasone Dipropionate

Accuracy Level %	*Drug added (µg/mL)*	*Amount recovered (µg/mL)*	*% Recovery (n=3)*	*Mean ± SD*	*% RSD*
	9.898	9.828	99.28		
50.0%	9.898	9.903	100.05	99.82± 0.46	0.45
	9.898	9.910	100.12		
	19.796	19.843	100.23		
100.0%	19.796	19.810	100.07	100.26±0.21	0.21
	19.796	19.895	100.50		
	29.694	30.104	101.38		
150.0%	29.694	29.933	100.80	100.89±0.44	0.44
	29.694	29.841	100.49		

3.5 *System / method precision*

The % Relative Standard Deviation (%RSD) of standard absorbance under system precision was determined to be 0.06% for Beclometasone Dipropionate (BDP) and 0.10% for Formoterol Fumarate (FF). These values are well below the specified limit of not more than 2.0%, indicating excellent precision within the system. Regarding the % RSD of six replicates absorbance for the initial sample of Formoterol, it was found to be 2.49%, and for Beclometasone, it was 1.11%. For the middle sample, the % RSD was 0.36% for Formoterol and 0.13% for Beclometasone, while for the end sample, it was 0.21% for both Formoterol and Beclometasone. Please refer to Table VII and VIII for detailed data. These results demonstrate good precision and consistency in the measurement of both BDP and FF across different stages of sample analysis, ensuring reliable and accurate quantification of these compounds in pharmaceutical formulations.

Table VII. Results of Standard Sample set System Precision

Std. Samples Set	*FF*	*BDP*	*Acceptance Criteria*
Std. Solution 1	0.2925	0.6025	% RSD of Standard absorbance should not be more than 2.0%.
Std. Solution 2	0.2919	0.6019	
Std. Solution 3	0.2919	0.6020	
Std. Solution 4	0.2919	0.6017	

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Std. Solution 5	0.2916	0.6023
Std. Solution 6	0.2917	0.6014
Mean	0.2919±0.00	0.6019±0.00
%RSD	0.10	0.06

Table VIII. Results of Method Precision

Samples Set	*Initial*		*Middle*		*End*		*Acceptance Criteria*
	FF	*BDP*	*FF*	*BDP*	*FF*	*BDP*	
Sample_1	0.6569	1.0924	1.2010	2.2047	1.2018	2.2052	% RSD of Six replicate samples absorbance should NMT 5.0%
Sample_2	0.6673	1.1021	1.1998	2.2055	1.2039	2.1994	
Sample_3	0.7044	1.1265	1.2080	2.2059	1.2048	2.2060	
Sample_4	0.6678	1.0997	1.1995	2.1998	1.2017	2.1998	
Sample_5	0.6649	1.0967	1.2096	2.2059	1.2083	2.2121	
Sample_6	0.6659	1.0967	1.2058	2.2001	1.2066	2.2062	
Mean	0.6712 ±0.01	1.1024 ±0.01	1.2040 ±0.00	2.2037 ±0.00	1.2045 ±0.00	2.2048 ±0.00	
%RSD	2.49	1.11	0.36	0.13	0.21	0.21	

3.6 *Assay results of pMDI Samples*

The developed and validated analytical method was employed to determine the % Assay of Formoterol Fumarate (FF) and Beclometasone Dipropionate (BDP) in pressurized metered dose inhaler (pMDI) samples at the initial, middle, and end stages of the canister. The results obtained were well within the acceptance criteria of 80-120%, as depicted in Table IX.

Table IX. % Assay for the developed pMDI with Proposed Analytical Method

Sample No	*% Assay of Formoterol Fumarate*			*% Assay of Beclomethasone Dipropionate*		
	Drug added (µg/mL)	*Amount Found (µg/mL)*	*% Label Claim*	*Drug added (µg/mL)*	*Amount Found (µg/mL)*	*% Label Claim*
1	0.599	0.605	100.87	19.796	19.843	100.23
2	0.599	0.609	101.65	19.796	19.81	100.07
3	0.599	0.614	102.37	19.796	19.895	100.5
Mean	0.60	0.61	101.63	19.80	19.85	100.27
SD	±0.000	±0.005	±0.750	±0.000	±0.043	±0.217
%RSD	0.00	0.74	0.74	0.00	0.22	0.22

This finding indicates that the % Assay of FF and BDP in the pMDI samples met the established acceptance criteria, signifying the accuracy and reliability of the analytical method in quantifying the active ingredients within the pharmaceutical formulation. These results validate the suitability of the developed method for routine quality control analysis of pMDI samples, ensuring consistent and accurate assessment of FF and BDP content throughout the canister's usage lifespan.

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CONCLUSION

The assay analytical test method for determining the concentration of Beclometasone and Formoterol in the 6+200 mcg/Spray inhaler formulation was found to exhibit desirable characteristics of linearity, precision, and accuracy. Through rigorous validation, this developed method was confirmed to be specific, as there was no interference observed from any excipients present in the drug product formulation during the analysis of Formoterol and Beclometasone.

The results obtained, along with the statistical parameters, underscored the simplicity, rapidity, reliability, accuracy, and precision of the proposed UV Spectrophotometric method. The method demonstrated robustness and reproducibility in analysing metered dose inhaler samples, ensuring consistency in the Assay test parameters for Formoterol Fumarate (FF) and Beclometasone Dipropionate (BDP). Notably, the assayed samples showed no interference in the determination of Formoterol and Beclometasone, further validating the method's specificity. In light of these findings, the developed method holds promise for routine use in the determination of Formoterol Fumarate and Beclometasone Dipropionate in combination formulations of pressurized metered dose inhalers (pMDIs). Its reliability and reproducibility make it a valuable tool for pharmaceutical quality control, ensuring the potency and consistency of these critical active ingredients in inhaler products used for the management of respiratory condition.

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