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Original Article

Evaluation of Respirable Fraction by Next Generation Impactor for Beclomethasone and Formoterol Fumarate Dihydrate Metered Dose Inhaler

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ABSTRACT

Introduction: Asthma and Chronic Obstructive Pulmonary Disease are treated with a combination pharmaceutical aerosol (Pressurized Metered Dose Inhaler) dose form, such as Formoterol fumarate dihydrate and Beclomethasone dipropionate pressurized inhalation solution, contains 6 µg and 100 µg, respectively. **Aim:** The *in vitro* aerodynamic characteristic-respirable fraction using cascade impactors simulate drug particle deposition on patient's lungs. The current work aimed to evaluate respirable fraction as aerodynamic characteristics of the delivered dose using Next Generation Impactor and a sensitive high pressure liquid chromatography approach. **Materials and Methods:** The samples are subjected to Next Generation Impactor and analyzed on a ODS Hypersil (250 mm×4.6 mm), 5 µm with 40°C column oven, volume of injection 100 µL and 1.2 mL per min flow rate using a mobile phase that is a 35:65% v/v combination of ammonium phosphate buffer and acetonitrile in high pressure liquid chromatography. The designed approach was validated for its anticipated function and determined respirable fraction of delivered dose. **Results:** Respirable fraction (fine particle dose) in µg for Formoterol fumarate dihydrate and Beclomethasone dipropionate were observed 2.6 and 44.1, respectively. Fine particle fraction in percentage for Formoterol fumarate dihydrate and Beclomethasone dipropionate were observed 51.5 and 52.8, respectively. Mass Median aerodynamic diameter in µm for Formoterol fumarate dihydrate and Beclomethasone dipropionate were observed 1.8 and 1.7, respectively. **Conclusion:** The technique produced a trustworthy result and can be used to quantify aerodynamic characteristics-respirable fraction of delivered dose of Formoterol fumarate dihydrate and Beclomethasone dipropionate in inhaler form, are ≤ 5 µm in size and equivalent to lung deposition.

Keywords: Aerosol, Beclomethasone, Formoterol fumarate, HPLC, Inhaler, Pulmonary.

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INTRODUCTION

A kind of obstructive lung illness is Chronic Obstructive Pulmonary Disease (COPD).[1] It is distinguished by chronic respiratory issues and inadequate airflow. Breathlessness and coughing up phlegm are two of the basic signs of COPD. Since COPD is a progressive condition, it usually gets worse with time.[2] A productive cough that lasts at least three months for at least two to three years is also referred to as "chronic bronchitis".[3] Unique pharmaceutical dosage forms metered dose inhalers, nebulizers and dry powder inhalers can be employed to treat COPD. These dosage forms are primarily used to treat asthma and require relatively small doses, with amounts ranging from 5 µg to 400 µg. By delivering an active chemical engineered with hydrofluoroalkane via the pulmonary route, a local or systemic impact can be achieved; such delivery methods designated as Metered Dose Inhalers (MDI).[4] Medication has the greatest lung specificity and a rapid onset of action, making MDIs favourable in reducing undesired systemic effects. Product performance is assessed employing aerodynamic characteristics, providing an extensive view of lung deposition behaviour based on particle aerodynamic size and identify the location of deposition in the respiratory system. Particles smaller than 5 µm, considered ideal for deposition into deep lung tissue, are found in the respirable fraction.[5,6] The pharmaceutical industry has created a new device called the Next Generation Impactor to be utilised in inhaler testing. The seven size-fractionation phases plus a Micro Orifice Collector (MOC) are combined with an induction port to form Next Generation Impactor, collects the small particles. The oropharynx and throat are mimicked by the induction port and different stages simulate a lower region as the deep lungs.[7,8] The

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cut-off stages 1 through 7 are, in order, 11.72 µm, 6.40 µm, 3.99 µm, 2.30 µm, 1.36 µm, 0.83 µm and 0.54 µm at a flow rate of 30 L/min, respectively.[8] Formoterol fumarate dihydrate an official drug in EP[9] and BP,[10] is a long-acting b2-agonist, is employed in the treatment of COPD and/or asthma. Inhaled Formoterol works similarly to other b2-agonists in treating asthma exacerbations by causing bronchodilation by softening the smooth muscle in the airway.[11,12] Beclomethasone dipropionate an official drug in European Pharmacopoeia (EP) and British Pharmacopoeia (BP), is glucocorticoid steroid's anti-inflammatory[13] actions in asthma include modification of cytokine and chemokine production, suppression of eicosanoid synthesis, significant reduction of basophil, eosinophil and other leukocyte accumulation in lung tissue and decreased vascular permeability.[14]

A review of the literature reveals that for BDP and FFD as individual[15] and drug product combination,[16] analytical methods are available for its quantification by UV Spectrophotometry and HPLC. Some of the procedures are also available with other medications for the quantification of BDP and FFD.[17-22] However; there is no technique available for the combination of FFD and BDP to analyze aerodynamic characteristics (Figures 1 and 2).

The current work aimed to characterize the aerodynamic characteristics of the delivered dose using Next Generation Impactor (NGI) and a sensitive high pressure liquid chromatography approach, for the evaluation of *in vitro* respirable fraction as a part of aerodynamic characteristics using cascade impactors simulate drug particle deposition on patient's lungs.

MATERIALS AND METHODS

Chemicals and materials

Sykem Pharma (Dhule, Maharashtra, India) supplied Formoterol fumarate dihydrate (99.6%) and Beclomethasone dipropionate (99.8%). The Niveoli Inhaler, produced by Cipla Ltd, was obtained from a local pharmacy. Finar Limited (Ahmedabad, India) provided analytical reagent grade ammonium dihydrogen phosphate, acetonitrile and phosphoric acid. The method was developed and validated using Water's HPLC system equipped with a quaternary pump. Chromatographic inspection is performed on the ODS Hypersil (250 mm × 4.6 mm), 5 µm column. The aerodynamic properties were investigated using NGI (Make: Copley, UK) at stipulated 30 LPM flow rate.

Preparation of the Mobile Phase and diluent

Ammonium dihydrogen phosphate (1.15 g) was dissolved in 1 L of HPLC water containing 0.1 mL orthophosphoric acid 85%. This buffer solution is mixed with Acetonitrile in a 35:65 %v/v ratio and degassed by sonication. As a diluent, 50:50 v/v mixture of acetonitrile and water was used.

Preparation of FFD standard stock solutions

Weighed precisely 20.5 mg of FFD, sonicated to dissolve in the presence of diluent, using same diluent diluted to 100 mL.

Preparation of BDP standard stock solutions

Weighed precisely 42.2 mg of BDP, sonicated to dissolve in the presence of diluent, using same diluent diluted to 100 mL.

Preparation of standard solution

Both standard stock solutions were diluted to accomplish the concentration of 0.2 µg/mL of FFD and 4.2 µg/mL of BDP.

Sample preparation for assay

Primed the canister with one actuation. Removed the pressurized canister from its actuator, clean the valve with diluent, air dried the valve completely and performed the delivered dose. Placed base plate in a 100 mL beaker containing 50 mL diluent. Placed canister in inverted position in beaker through base plate, actuated immediately first spray below the surface of the 50 mL diluent and rested the canister in the same inverted position for 15 sec. Repeated this procedure for further nine actuations (Total 10 actuations). Mixed the solution in 100 mL beaker for 2 min and shift the contents to a 250 mL flask. Rinsed the beaker through 50 mL of diluent and shift the contents to a same 250 mL flask. Repeated this procedure for one more time. Finally made up to the volume up to 250 mL mark with diluent and mixed.

Sample preparation for aerodynamic particle size distribution of delivered dose through NGI (collection of respirable fraction using NGI)

The NGI imitates drug particle deposition on human lungs based on the particle size of active ingredients.[23,24] Aerodynamic particle size distribution was determined using the NGI (Copley, UK). Following impaction, the aerosol is classified into seven stages based on its aerodynamic diameter. NGI (Copley, UK) was configured with a pump and flow rate was adjusted to 30 L per min using a flow meter (Copley, UK). Before using the Niveoli Inhaler, it was primed with one spray into the air. In the upright position, insert the inhaler into the mouthpiece adaptor. To discharge one spray, press hard on the canister and hold for 1 second. The sequentially second spray was actuated in the same way. Similarly, a total of ten sprays were actuated. After 10 sprays, switch off the vacuum pump, disassemble the NGI and recover the therapeutic ingredient by washing each stage and accessory of the NGI with diluent. Inhaler actuator and valve-stem was rinsed with 50 mL and 25 mL of diluent, respectively. Induction port and each collection cup of NGI were rinsed with 100 mL and 20 mL of diluent, respectively.

HPLC Method development and optimization

During method optimization, several buffer mixtures, mobile phases and stationary phases were looked at while taking into consideration different chemical and physical properties of FFD and BDP. The buffer solution used during process optimization is composed of formic acid, sodium dihydrogen phosphate monohydrate and ammonium dihydrogen phosphate. The mixture of mentioned buffer solutions and organic solvents used as mobile phases. The columns used were YMC triart C18 (150 mm × 4.6 mm, 3 µm), X-bridge C18 (150 mm × 4.6 mm, 5 µm), Kromasil C18 (150 mm × 4.6 mm, 5 µm) and Hypersil ODS C18 (250 mm × 4.6 mm, 5 µm). The wavelength was selected at 220 nm.

Method validation

For the determination of aerodynamic properties for the combination of FFD and BDP, a simultaneous HPLC procedure validated in accordance with the ICH guideline Q2 (R1)[25-30] and outcome presented in results section.

RESULTS

System suitability Precision

Six injections of standard solution followed by diluent injections were used to determine the system's appropriateness. The developed approach seemed to be selective and in line for FFD and BDP, with retention periods of 4.44 and 6.25 min, respectively. System suitability seemed for FFD and BDP, with low %RSD 0.58 and 0.40, respectively.

Specificity

Injections of standard solution followed by diluent and mimic of formulation except active ingredient (placebo) were used to determine the specificity. There was no interference from diluent and placebo at the retention time of FFD and BDP was proven by specificity study. The representative chromatogram of standard solution is displayed in Figure 3.

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

By testing LOQ and LOD on lower concentrations of standard dilutions using a visual methodology, the method was determined to be sensitive and signal to noise ratios found more than 3 and more than 10 for LOD and LOQ respectively.[28] The calculated concentrations of LOD and LOQ with % RSD were disclosed in Table 1.

Linearity and range

The chromatographic conditions specified in the analytical technique were followed in the preparation and analysis of the linearity solutions by diluting FFD and BDP stock solutions. For the peak area response to its concentration, the linearity curve is plotted. The established range of the analytical method was determined to be between LOQ (0.0051 µg/mL) to 0.5617 µg/mL for FFD and LOQ (0.0563 µg/mL) to 10.4291 µg/mL for BDP. The observed correlation values (NLT 0.999) for FFD and BDP were 0.9999 and 0.9999, respectively. For FFD and BDP, the observed values of Y-Intercept bias at 100% level (limit±2) were 0.7 and 0.4, respectively. The linearity was deemed acceptable and meets the requirements. Figures 4 and 5 represent the linearity charts for FFD and BDP, respectively.

Accuracy (Recovery)

By spiking FFD and BDP in the placebo matrix, the analytical method's accuracy was confirmed. The recovery samples were created in three copies and subjected to the prescribed analytical process. The collective average recovery was found 99.9% (±1.2) and 99.5% (±0.9) for FFD and BDP, respectively. The findings of the analytical approach demonstrate that it is evidently reliable. Table 2 shows the outcomes of recovery at each level of recovery.

Stability of Analytical Solutions

The stability of analytical solution was tested at room temperature. Standard and sample solutions created and subjected to regular time interval analyses in accordance with the analytical process to ascertain the stability of the analytical solutions. Up to 36 hr, standard and sample solutions were discovered to be stable. Table 3 shows the outcomes of solution stability at room temperature.

Table 1: Result of LOD and LOQ.

Parameters	Observed values		Acceptance criteria
	FFD	BDP	
LOD (ng/mL)	1.7	18.8	--
% RSD (for LOD)	9.90	3.18	NMT 30%
LOQ (ng/mL)	5.1	56.3	--
% RSD (for LOQ)	8.66	5.24	NMT 10%
s/n ratio (in LOQ)	49	79	NLT 10

NMT: Not More Than; NLT: Not Less Than.

Table 2: Recovery results for FFD and BDP.

Drug	Spiked level (%)	Amount added (µg/mL)	Amount found (µg/mL)*	% Recovery*	Average Recovery
FFD	LOQ	0.0051	0.0051 (±1.7)	100.3 (±1.7)	99.9 (±1.2)
	50%	0.1123	0.1123 (±1.1)	99.9 (±1.1)	
	100%	0.2247	0.2264 (±0.8)	100.8 (±0.8)	
	150%	0.3370	0.3357 (±1.0)	99.6 (±1.0)	
	250%	0.5617	0.5545 (±1.1)	98.7 (±1.1)	
BDP	LOQ	0.0563	0.0566 (±1.3)	100.4 (±1.3)	99.5 (±0.9)
	50%	2.0858	2.0924 (±0.4)	100.3 (±0.4)	
	100%	4.1716	4.1397 (±0.5)	99.2 (±0.5)	
	150%	6.2575	6.2043 (±0.2)	99.2 (±0.2)	
	250%	10.4291	10.2808 (±0.1)	98.6 (±0.1)	

* mean value of n=3.

Table 3: Results for Solution Stability study.

Drug	Time difference in hour	Standard Solution		Sample Solution	
		Peak area	% Difference	% Assay	% Difference
FFD	Initial	72638	--	99.0	--
	24 hr	72873	-0.3	98.6	0.4
	30 hr	72365	0.4	99.3	-0.3
	36 hr	72984	-0.5	99.0	0.0
BDP	Initial	284301	--	101.4	--
	24 hr	284634	-0.1	101.4	0.0
	30 hr	284287	0.0	101.4	0.0
	36 hr	283982	0.1	101.7	-0.3

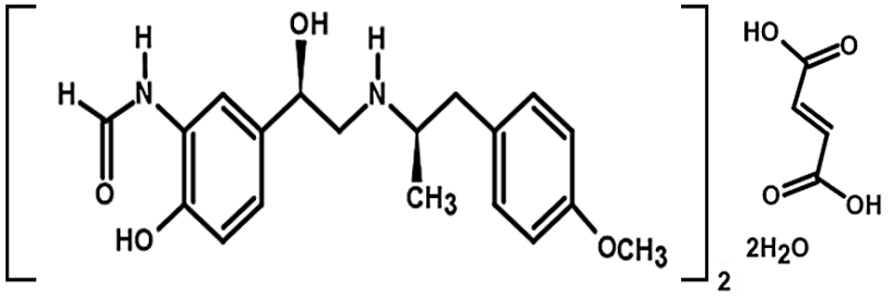

Figure 1: Chemical structure of Formoterol Fumarate Dihydrate (FFD).

Precision

The evaluation of (i) System precision (repeatability of standard injections: measured response of analyte peak in standard and %RSD), (ii) Method precision (repeatability of sample analysis: measure % assay results and its %RSD) and (iii) Intermediate precision (repeatability of method: measured response of analyte peak in standard, % assay results and %RSD) determines the precision of analytical methods. The standard deviation of a set of measurements is typically used to express the precision of a method. The outcomes prove the precision of analytical method. Tables 4 and 5 suggest a summary of the outcomes.

Robustness

Variations in the column oven temperature, mobile phase composition and flow rate are purposefully introduced to put an analytical procedure to the test. FFD and BDP system suitability characteristics had no significant influence. The outcomes of changed circumstances are equivalent to those of unchanged situations. According to the robustness results (Table

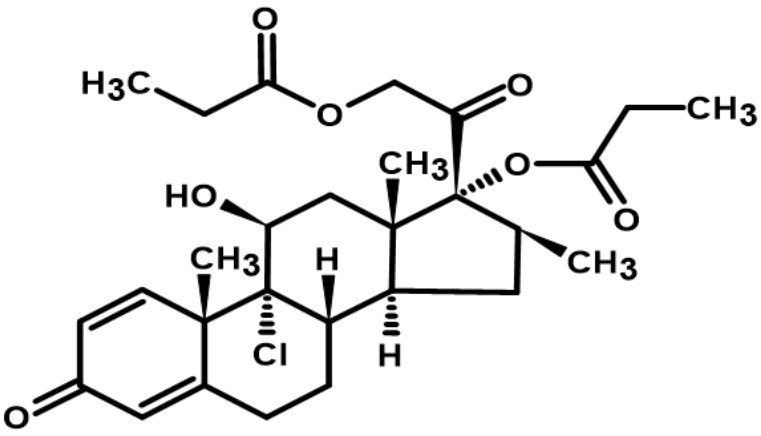

Figure 2: Chemical structure of Beclomethasone dipropionate (BDP).

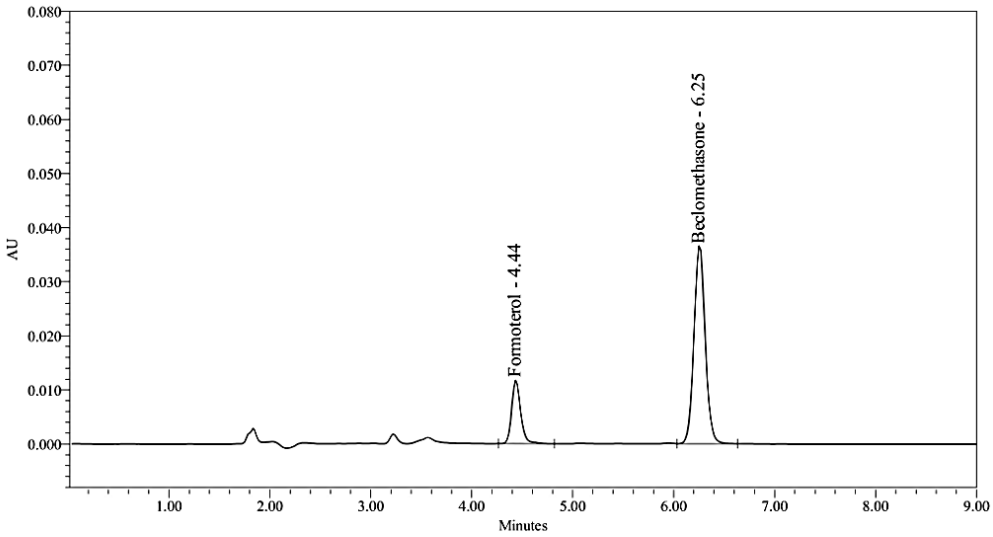

Figure 3: Chromatogram of Standard.

Table 4: Results for precision study.

System precision			Method precision (% Assay)		
Injection No.	**FFD**	**BDP**	**Preparation No.**	**FFD**	**BDP**
Injection-1	72638	284301	Preparation-1	99.0	101.4
Injection-2	72306	280872	Preparation-2	99.2	100.9
Injection-3	71766	281847	Preparation-3	98.5	101.3
Injection-4	71879	282659	Preparation-4	98.0	101.4
Injection-5	71506	282487	Preparation-5	98.5	100.4
Injection-6	71737	282798	Preparation-6	98.0	100.4
Average	71972	282494	Average	98.5	101.0
% RSD (NMT 2%)	0.6	0.4	% RSD (NMT 2%)	0.5	0.5

NMT: Not More Than; RSD: Relative Standard Deviation.

6), the analytical process is robust and it has no effect on the chromatographic system or the results under varied test settings.

Aerodynamic Particle Size Distribution (collection of respirable fraction) by Next Generation Impactor

Aerodynamic particle size distribution was determined using the NGI (Copley, UK). NGI was configured followed by pump; flow rate was adjusted using a flow meter (Copley, UK) and total 10 sprays actuated at constant flow rate of 30 LPM. Disassemble the NGI and recover the therapeutic ingredient by washing each stage and accessory of the NGI with diluent. Deposition of % FFD and % BDP on different stages represented in graphical manner (Figure 6). Table 7 outlines the findings of aerodynamic characteristics including respirable fraction using CITDAS software.

DISCUSSION

The suggested approach has additional advantages, such as a simple, quick and reproducible RP-HPLC method for simultaneous assessment of aerosol properties of FFD and BDP in combination inhalation formulation. There is no published technique for determining the aerosol properties of FFD and BDP.

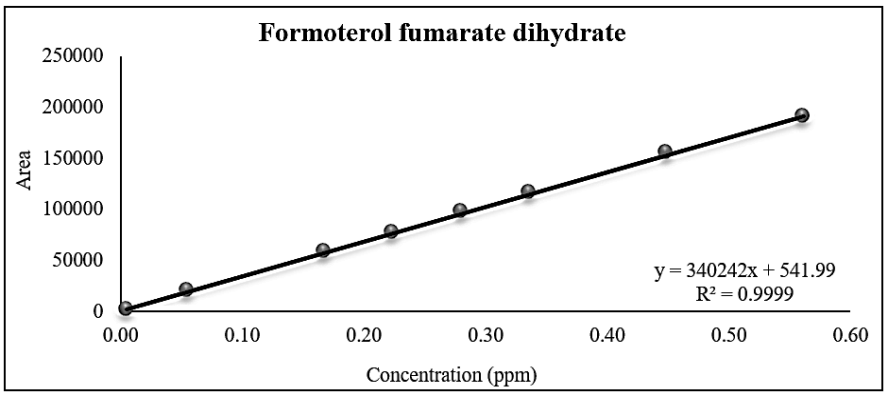

Figure 4: Linearity plot for FFD.

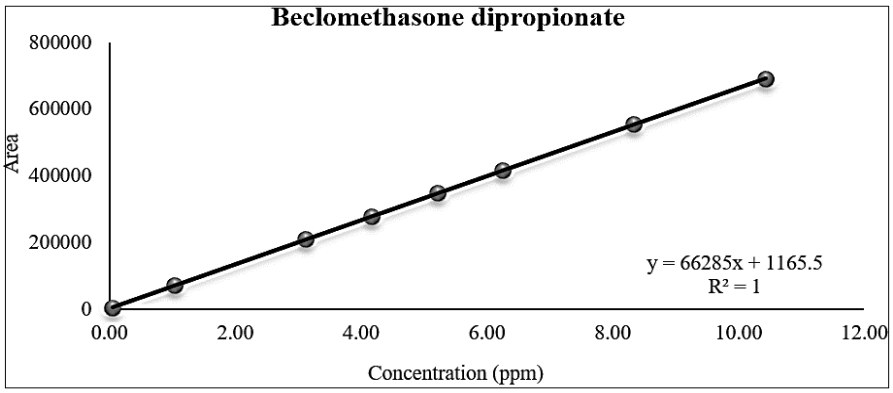

Figure 5: Linearity plot for BDP.

Table 5: Results for method and intermediate precision.

Sr. No.	FFD peak area in Standard		BDP peak area in Standard		% Assay of FFD		% Assay of BDP	
	MP	IP	MP	IP	MP	IP	MP	IP
1	72638	70462	284301	285673	99.0	99.1	101.4	101.9
2	72306	70353	280872	286002	99.2	99.1	100.9	100.7
3	71766	69936	281847	284800	98.5	100.3	101.3	100.2
4	71879	70328	282659	285493	98.0	98.8	101.4	99.9
5	71506	69036	282487	285033	98.5	98.7	100.4	100.6
6	71737	69133	282798	283009	98.0	99.1	100.4	100.0
Average	71972	69875	282494	285002	98.5	99.2	101.0	100.6
% RSD	1.7		0.6		0.6		0.6	

MP: Method Precision, IP: Intermediate Precision.

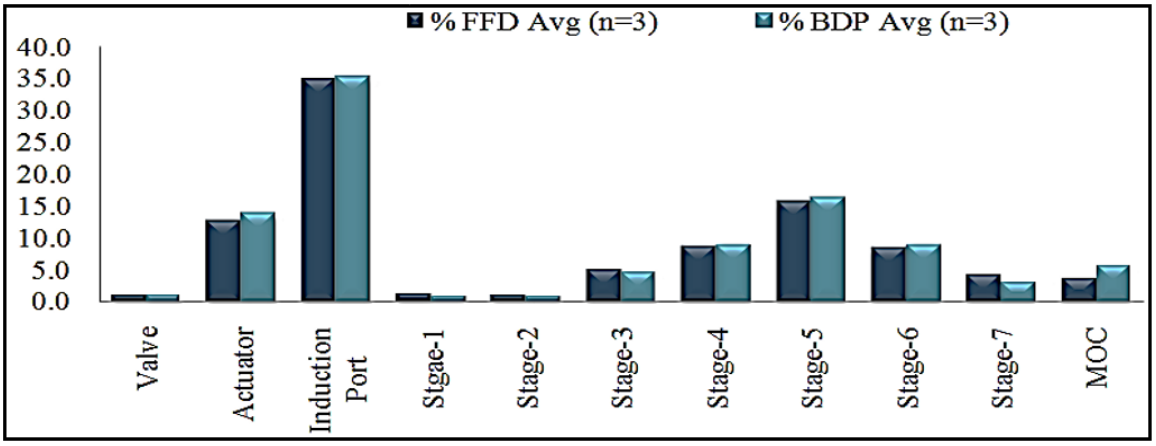

Figure 6: Graphical presentation of deposition of % FFD and % BDP on each stage.

Table 6: Results of robustness study.

Conditions		FFD			BDP		
		Standard		% Assay in sample	Standard		% Assay in sample
		Peak area	% RSD		Peak area	% RSD	
Normal		71972	0.58	99.0	282494	0.40	101.4
Flow rate (mL/min)	1.1	80705	0.34	98.5	309198	0.34	101.6
	1.3	74477	0.83	98.8	263575	0.81	100.5
Column oven temperature (°C)	35	72250	1.35	98.8	285840	0.46	101.5
	45	75794	0.16	97.3	286055	0.20	100.4
Organic (Acetonitrile) composition in mobile phase	- 10%	69964	0.03	98.6	284553	0.26	100.8
	+ 10%	71005	0.96	97.5	294656	0.64	101.8

RSD: Relative Standard Deviation.

Table 7: Results for aerodynamic characteristics by NGI.

Drug	Particulars	Experiment-1	Experiment-2	Experiment-3	Average
Formoterol fumarate dihydrate	M.B.%	93.3	97.4	97.9	96.2 (±2.6)
	FPD (µg)	2.522	2.57	2.566	2.552 (±1.0)
	FPF (%)	52.218	52.059	50.345	51.541 (±2.0)
	MMAD (mm)	1.785	1.785	1.795	1.788 (±0.3)
	GSD	2.134	2.125	2.194	2.151 (±1.7)
Beclomethasone dipropionate	M.B.%	98.3	98	99	98.4 (±0.5)
	FPD (µg)	44.455	43.546	44.434	44.145 (±1.2)
	FPF (%)	53.017	52.815	52.677	52.836 (±0.3)
	MMAD (mm)	1.738	1.747	1.72	1.735 (±0.8)
	GSD	2.141	2.144	2.13	2.138 (±0.3)

FPD: Respirable Fraction (particle size < 5µm) equivalent to lungs deposition; M.B: Mass Balance; FPD: Fine Particle Dose; MMAD: Mass Median aerodynamic diameter; GSD: Geometric Standard Deviation.

Over the stated approaches, there is more selectivity, sensitivity, shorter analysis time, improved accuracy and precision.

CONCLUSION

For the analysis of Formoterol fumarate dihydrate and Beclomethasone dipropionate in Niveoli Inhaler, a novel reverse phase liquid chromatography method that is straightforward, isocratic and sensitive was created. For each of the validated method's assessed validation parameters, the findings were satisfactory. For the *in vitro* Aerodynamic Particle Size Distribution (APSD) analysis of Formoterol fumarate dihydrate and Beclomethasone dipropionate in the commercially available inhaler dosage form, Niveoli Inhaler, the devised method was effectively used. The technique produced a trustworthy result and can be used to quantify APSD, content per actuation and delivered dose of Formoterol fumarate dihydrate and Beclomethasone dipropionate in inhaler form.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FFD: Formoterol fumarate dihydrate; **BDP:** Beclomethasone dipropionate; **NGI:** Next generation impactor; **COPD:** Chronic obstructive pulmonary disease; **MDI:** Metered-dose inhalers; **EP:** European pharmacopoeia; **BP:** British pharmacopoeia; **LOQ:** Limit of quantitation; **LOD:** Limit of detection; **RSD:** Relative standard deviation; **ppm:** Parts per million; **MP:** Method precision; **IP:** Intermediate precision; **LPM:** Liter per minute; **APSD:** Aerodynamic particle size distribution; **MB:** Mass balance; **FPD:** Fine particle Dose; **FPF:** Fine particle fraction; **MMAD:** Mass median aerodynamic diameter; **GSD:** Geometric standard deviation.

CONTRIBUTION OF AUTHORS

Mr. Chirag Chilka (First author) has performed all experiments, prepared the manuscript. Dr. Khushal Kapadiya and Dr. Darshan Jani have interpreted chromatograms and prepared calculation sheet. Dr. Jayesh Dhalani (Corresponding author) has designed and reviewed all data of study including manuscript.

SUMMARY

The suggested approach has the added benefit to study important behaviour of aerodynamic particle size assessment as respirable fraction which can be correlated to the drug deposition into the human lungs. The HPLC method used for the sample analysis is sensitive, fast, simple, accurate and repeatable for determining the concentration of Beclomethasone and Formoterol fumarate dihydrate in inhalation dosage form, any other tablet dosage and in API as well. The shorter runtime benefits the method for its use for single drug without combination. The method having advantage of lower LOD and LOQ than previously reported. Overall, the proposed method having importance to analyse single and combination analyte.

REFERENCES

1. Lin H, Wang C, Yu H, Liu Y, Tan L, He S, *et al.* Protective effect of total Saponins from American ginseng against cigarette smoke-induced COPD in mice based on integrated metabolomics and network pharmacology. Biomed Pharmacother. 2022;149(1):1-20. doi: 10.1016/j.biopha.2022.112823, PMID 35334426.
2. Zayed S, Fouad F, Belal F. A simple and economic chromatographic method for simultaneous determination of six bronchodilator drugs in pharmaceutical dosage forms. J Iran Chem Soc. 2021;18(6):1251-59. doi: 10.1007/s13738-020-02107-6.
3. Alghamdi AF. Square wave-adsorptive stripping voltammetric technique development and validation for the determination of budesonide in pharmaceuticals and biological fluids using graphite working electrode. J Taibah Univ Sci. 2022;16(1):372-80. doi: 10.1080/16583655.2022.2062883.
4. Kumar R, Mehta P, Shankar KR, Rajora MAK, Mishra YK, Mostafavi E, *et al.* Nanotechnology-Assisted Metered-Dose Inhalers (MDIs) for High-Performance Pulmonary Drug Delivery Applications. Pharm Res. 2022;39(11):2831-55. doi: 10.1007/s11095-022-03286-y, PMID 35552983.
5. Gondhale PS, Deshmukh MJ, Omase RV, Arbune AN, Kolsure P, Bhope SG, *et al.* Comparative Evaluation of Fine Particle Fraction and T2 Fraction by Using Next Generation Impactor (NGI) and Glass Twin Impinger (GTI) on Formoterol Fumarate and Tiotropium Dry Powder Inhaler (DPI) Marketed Products. Pharm Chem J. 2022;56(5):720-26, doi: 10.1007/s11094-022-02700-4.
6. United States Pharmacopoeia; 2021. USP 44 and NF 39.
7. Omer HK, Husein NR, Hamadameen HA. Comparison between the next generation impactor and the twin glass impinge as model pulmonary drug delivery devices. Zanco J. Med. Sci. 2019;23(1):74-80. doi: 10.15218/zjms.2019.010.
8. COPLEY. Driving results in inhaler testing. 2021 edition.
9. European pharmacopoeia; 2017. ed 9.1 suppl. 8.
10. British Pharmacopoeia; 2020. v 1.
11. Usmani OS, Baldi S, Warren S, Panni I, Girardello L, Rony F, *et al.* Lung Deposition of Inhaled extrafine Beclomethasone dipropionate, Formoterol Fumarate, Glycopyrronium Bromide in Healthy Volunteers and Asthma: The STORM Study. J Aerosol Med Pulm Drug Deliv. 2022;35(4):179-85. doi: 10.1089/jamp.2021.0046, PMID 35128939.
12. Salem YA, Shaldam MA, El-Sherbiny DT, El-Wasseef DR, El-Ashry SM. Simultaneous Determination of Formoterol Fumarate and Budesonide Epimers in Metered Dose Inhaler Using Ion-Pair Chromatography. J Chromatogr Sci. 2017;55(10):1013-20. doi: 10.1093/chromsci/bmx067, PMID 28977479.
13. Wu HT, Chuang YH, Lin HC, Hu TC, Tu YJ, Chien LJ. Immediate Release Formulation of Inhaled Beclomethasone Dipropionate-Hydroxypropyl-Beta-Cyclodextrin Composite Particles Produced Using Supercritical Assisted Atomization. Polymers (Basel). 2022;14(10). doi: 10.3390/polym14102114, PMID 35631996.
14. Salhi M, Lahmar O, Salah MO, Banić I, Binghao B, Malik W, *et al.* GLCCI1 and STIP1 variants are associated with asthma susceptibility and inhaled corticosteroid response in a Tunisian population. J Asthma. 2021;58(2):197-206. doi: 10.1080/02770903.2019.1666867, PMID 31516081.
15. Sambandan E, Kathavarayan T, Sellappan S, Shiea J, Ponnusamy VK. Identification and characterization of unknown degradation impurities in beclomethasone dipropionate cream formulation using HPLC, ESI-MS and NMR. J Pharm Biomed Anal. 2019;167:123-31. doi: 10.1016/j.jpba.2019.02.013, PMID 30771645.
16. Chassot JM, Ferreira LM, Gomes FP, Cruz L, Tasso L. Stability-indicating RP-HPLC method for determination of beclomethasone dipropionate in nanocapsule suspensions. Brazilian J Pharm Sci. 2015;51(4):803-10. doi: 10.1590/S1984-82502015000400006.
17. Kanaka PK, Abimanyu S. Environmental benign AQbD based estimation of ketoconazole and beclomethasone by RP-HPLC and multi-analytical UV spectrophotometric method. Microchem J. 2022;172(A). doi: 10.1016/j.microc.2021.106968.

18. Anandan K, Xavier Rajarathinam SR, Sarvanan C. Simultaneous Quantification of Formoterol Fumarate and Glycopyrrolate Using Reverse Phase High Performance Liquid Chromatography. Asian J Chem. 2018;30(11):2389-93. Doi: 10.14233/ajchem .2018.21370.
19. Merey HA, El-Mosallamy SS, Hassan NY, El-Zeany BA. Validated chromatographic methods for the simultaneous determination of Mometasone furoate and Formoterol fumarate dihydrate in a combined dosage form. Bull Fac Pharmacy, Cairo Univ. 2016;54(1):99-106. doi: 10.1016/j.bfopcu.2016.02.001.
20. Mamillapalli SP, Prasanna GL, Subbaiah BV, Annapurna N. Formoterol Fumarate Dihydrate and Mometasone Furoate in Metered Dose Inhalation Aerosol. Asian J Chem. 2021;33(11):2723-28. doi: 10.14233/ajchem.2021.23415
21. El-Bagary RI, Fouad MA, El-Shal MA, Tolba EH. Forced degradation of mometasone furoate and development of two RP-HPLC methods for its determination with formoterol fumarate or salicylic acid. Arab J Chem. 2016;9(3):493-505. doi: 10.1016 /j.arabjc.2015.05.005.
22. Elkady EF, Tammam MH, Elmaaty AA. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Tiotropium Bromide, Formoterol Fumarate and Olodaterol HCl in Bulk and Metered Dose Aerosols: Application to Olodaterol HCl Forced Degradation Study and Degradation Kinetics. Chromatographia. 2017;80(12):1749-60. doi: 10.1007/s10337-017-3413-0.
23. Sahib MN, Darwis Y, Khiang PK, Tan YTF. Aerodynamic characterization of beclomethasone dipropionate from Beclate-50 Inhaler® by HPLC-UV. J Liq Chromatogr Relat Technol. 2011;34(8):613-21. doi: 10.1080/10826076.2011.557464.
24. Patil AT, Patil SD, Shaikh KA. Sensitive LC method for simultaneous determination of ciclesonide and formoterol fumarate in dry powder inhaler. J Liq Chromatogr Relat Technol. 2011;34(15):1568-77. doi: 10.1080/10826076.2011.575980.
25. Oliphant EA, Purohit TJ, Alsweiler JM, McKinlay CJD, Hanning SM. Validation and application of a simple and rapid stability-indicating liquid chromatographic assay for the quantification of caffeine from human saliva. J Liq Chromatogr Relat Technol. 2022;45(1-4):10-17. doi:10.1080/10826076.2022.2095402.
26. Panchal J, Dhalani J. Novel Ultra-Fast Liquid Chromatography Method for Simultaneous Quantification of Metformin Hydrochloride and Remogliflozin Etabonate. Asian J of chem. 2023;35(9):2055-60. https://doi.org/10.14233/ajchem.2 023.27910
27. Panchal JH, Dhaduk BB, Dhalani JM. Stability Indicating Isocratic Rp-Hplc and Second Derivative Uv Spectroscopic Methods for Simultaneous Determination of Remogliflozin Etabonate and Vildagliptin Hydrochloride. Rasayan J Chem. 2023;16(2):579-87. doi: 10.31788/RJC.2023.1628212.
28. ICH. International conference on harmonisation, validation of analytical procedure: Text and methodology. Vol. Q2; 2005.
29. Shah T, Kapadiya K, Dhalani J. Isolation, Characterization and Cumulative Quantification approach for nine organic impurities in empagliflozin and linagliptin drugs. Indian J of chem tech. 2024;31(3):470-82. DOI: 10.56042/ijct.v31i3.4234.
30. Singh R, Dhaduk B, Dhalani J. A rapid quantification method for simultaneous determination of pendimethalin and metribuzin contents in suspoemulsion formulation. Results in Chem. 2023;5. doi: 10.1016/j.rechem.2023.100779.

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