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In Vitro Aerosol Measurements of the Novel Single Dose Reusable Dry Power Inhaler with Combination of Long-Acting Bronchodilator and Inhalated Corticosteroid

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Abstract: ***A novel dry powder inhaler, was developed to overcome the variability of inhaler performance on patient inspiratory effort when deaggregating the active medicament from lactose carrier during inhalation for the treatment of asthma. Herein the in vitro fine particle distribution of formoterol fumarate-budesonide combination delivered via newly developeddry powder inhaler was determined by Anderson Cascade Impactor at flow rates of 28.3 l/min, 54.8 l/min, and 90 l/minusing a an aluminum idealized mouth-throat geometry (Alberta idealized throat model), Grgic et al. 2004). Uniformity of delivered dose at 28.3 l/min using the USP-DUSA collecting tube was also generated. The percentage fine particle fractions (FPF) for formoterol fumarate and budesonide were (mean ±SD) 22.49 ± 1.58 and 23.53 ± 1.55 at 28.3 l/min and 25.72 ± 4.72and 25.75 ± 0.36 at 54.8 l/min respectively. As a percent of label claim, budesonide delivery distal to the mouth-throat was found to be 22.5±1.1% at 28.3 l/min, 23.6±0.7% at 60 l/min, and 28.6±0.6% at 90 l/min, while for formoterol fumarate these values were 18.9±0.8%, 19.4±0.8% and 22.8±0.8%. Flow rate differences between 28.3 l/min, 60 l/min and 90 l/min are not significant (p>0.05). From these in vitro measurements, the developed dry powder inhaler demonstrated consistent fine particle fraction, low device retention, and consistent uniformity of delivered dose in range of 85.0% to 115% (SD: ±5%).***

Keywords: "Dry powder inhaler device", "Dry powder inhalation", "Alberta Idealized throat deposition", "Anderson Cascade impactor", "DUSA (Dose Uniformity Sampling Apparatus)"

1. Introduction

Drug delivery for the treatment of asthmatics and COPD commonly involves delivery of the medicament to the respiratory tract. Pulmonary drug delivery via the inhalation route is more localized and targeted compared to delivery to the systemic circulation. The airways leading to the lungs, including the throat and the oropharyngeal region, act as an effective filter, and drug particles must penetrate these regions in order to deposit in the distal regions. The fraction that is responsible for efficacy is normally considered to be comprised of particles with diameters of 5 microns.[1], [2] Drug particles immediately on either side of this range namely, coarse and ultrafine particles, are normally not delivered with enough efficiency to the lungs to impact significantly on efficacy.[3]

Dry powder inhalers (DPIs) are breath-actuated devices that deliver powder medicament to the lungs. DPI devices operate on the basis of patient's inspired air and do not use propellant to deliver the medicament as in the case of pressurized metered dose inhalers (pMDIs).[4] DPIs may be either single dose (based on capsules) or multi-dose where the powder medicaments are mostly in an inhalation based lactose carrier is stored in the device.[5]

Inhaled corticosteroids (ICS) alone and in with long acting β-agonists are considered standard treatment for asthma and COPD (chronic obstructive pulmonary disorder).[6]Device development of dry powder inhalers containing combinations of beta agonist and corticosteroids has seen considerable activity in recent years. Passive DPIs rely on a patient's inspiratory efforts to aerosolize the drug particles to be inhaled and deposited in to the airways of the lungs. "Active" inhalers use an external form of energy that either pre-aerosolizes the powder, or enhances the aerosolization, to aid with powder deaggregation upon inhalation. [7] Active devices can potentially enhance the efficacy and also potentially reduce drug loading in the powder formulation.[8]

A novel passive dry powder inhaler has been developed with a view to enhance the efficiency of the delivered dose through control of the air flow pattern within the device. [9] The device consists of six components, namely the mouthpiece, bottom housing assembly, top housing assembly, mesh, an impinger/plunger, and a spring (Fig. 1). The novel dry powder inhaler is designed to load a specific size capsule with a specific locking length, which ensures zero defect and provides a failure proof mechanism. A unique design feature of the Developed dry powder inhaler is its 90° angle between the air inlet and inhalation port. This results in a unique tangential air flow pattern that causes turbulence to aid the dispersion of the powder. The parabolic shape of the bottom chamber where the active ingredient is dispersed and its small volume further enhances

the turbulence generated upon inspiration. Unlike some other commercial passive inhalers, the entire chamber is exposed to the mouthpiece, and the chamber and mouthpiece are co-linear. The mouthpiece has a tapered structure to provide a funnel effect to the air flow upon inhalation. The position of the inlet air holes and their diameter has been carefully designed based on design excellence tests. The materials science aspects of the components used for construction are chosen based on compatibility studies. The objective of this study was to establish the performance of developed dry powder inhaler using in vitro studies, namely Anderson Cascade Impactor, uniformity of emitted and delivered dose, device performance on drug delivery, and lung dose estimation using the amount of drug delivered distal to the mouth-throat with the Albertathroat geometry.

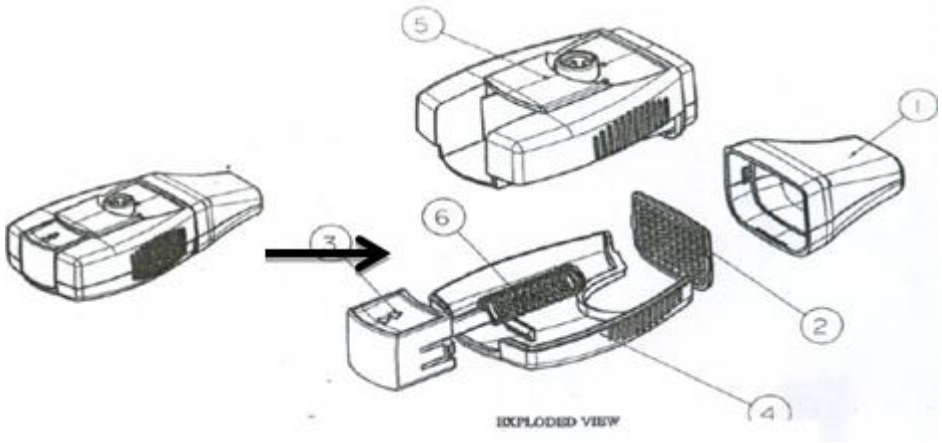

Figure 1: CAD drawing of the developed dry powder inhaler Device with full view and the components separated out (1-mouth piece, 2-mesh/filter, 3: plunger/impinge, 4: bottom housing assembly, 5: Top housing assembly with capsule insertion hole and powder dispersion chamber, 6: Spring

2. Materials and Methods

2.1 Measurement of Air Resistance

In order to quantify air resistance of the device, air flow rates from the developed dry powder inhaler at different pressure drops between 1 and 6 kPa were determined by attaching the developed dry powder inhaler devices to a dose uniformity sampling unit (DUSA, Copley, U.K.) that had a manometer (RS components, U.K.) attached to its port to measure pressure drop. After the pressure drop was adjusted to a pre-determined value, the flow rate across the DUSA was measured by a digital flow meter (4143 series, TSI). A total of 10 devices were tested and the mean flow rate at each pressure drop was utilized to calculate the air resistance of the device. The specific resistance (R) was then calculated from the flow (Q) and the pressure drop (dp) using the equation

$$R = (dp)^{0.5}/Q.$$

2.2 Formulations

A combination of formoterol fumarate (6µg) and budesonide (100 µg) Redicaps-Combihale FB100® (Dr. Reddy's Laboratories Ltd, India) were used for characterization of developed dry powder inhaler devices. The powder formulation contained formoterol fumarate (6 µg) and budesonide (100 µg) blended in lactose monohydrate (25mg) contained in hard gelatin size '3' capsules (Associated Group Capsules Ltd, India). The content uniformity of the lactose based formulation was determined using HPLC and was found to be 101.5 ± 1.43% and 99.87 ± 2.45 % for formoterol fumarate and budesonide respectively (n=10).

2.3 Uniformity of Delivered Dose Studies

The emitted dose was determined using the DUSA (Copley instruments, UK). The flow was adjusted to 54.8 l/min (the flow rate that generated a pressure drop of 4 kPa across the device), and the actuation time was set to 4.4 sec. After 4 litres of air had been drawn through the device, the emitted dose was analyzed using a validated HPLC method. The collecting DUSA tube, filter, and adapter were rinsed with 20 ml acetonitrile and phosphate buffer pH 3.0 (35:65) to dissolve the drugs4. The filter was placed in an ultrasonic bath for a further 5 min. About 1.5 ml of each of the individual, thoroughly mixed samples were finally transferred to glass HPLC vialsto quantitative analysis by HPLC. Formoterol fumarate and budesonide content were assayed by HPLC using UV detection at 220 nm. A 100-µl aliquot was injected into a Waters (Milford, MA) HPLC system consisting of a 515 pump, a 2487 dual wavelength UV-VIS detector and a 717 auto-injector. The stationary phase was C18 (150 x 4.6 mm, 5 µm, Kromasil®, Sweden) and the mobile phase was a mixture of acetonitrile and phosphate buffer pH 3.0 (35:65) using a flow rate of 2.0 ml/min. Data analysis was carried out using Waters Empower software (version 2.04).

2.4 Particle Size Distribution Studies by Andersen Cascade Impactor

The in-vitro pulmonary deposition profile of the dry powder inhaler was investigated using an 8-stage Andersen cascade impactor (Copley Instruments, UK) with a USP Throat under controlled relative humidity (40-50%) [10]. Hard gelatin capsules (size '3') with 25 mg formulations, Redicaps-Combihale FB100® were loaded into developed dry powder inhaler devices. The powder was dispersed into an Andersen Cascade Impactor (ACI) from the developed dry powder inhaler for 8s at an air flow rate of 28.3 l/min,

4.0 s at 60 l/min and 90 l/min at 2.7s . Deposition observed as per table:1.

Table 1: The effective cut-off diameter of each stages at volumetric airflow rate of 28.3 l/min, 60 l/min and 90 l/min

Stages	*Cut-off diameter in µm*		
	28.3 l/min	*60 l/min*	*90 l/min*
Stage-2	NA	NA	8.0
Stage-1	NA	8.6	6.5
Stage-0	NA	6.5	5.2
Stage 0	9.0	NA	NA
Stage 1	5.8	4.4	3.5
Stage 2	4.7	3.2	2.6
Stage 3	3.3	1.9	1.7
Stage 4	2.1	1.2	1
Stage 5	1.1	0.55	0.22
Stage 6	0.7	0.26	NA
Stage 7	0.4	NA	NA

The impaction plates were coated with a 1% (v/v) solution of Tween 20 (S.D. Fine Ltd, India) in methanol to prevent particle bounce and re-entrainment. A critical flow controller (TPK -2000, Copley Instruments, UK) was used to adjust the flow rate and measure the pressure ratio and time of the actuation. Ten capsules were used in sequence for each impactor test run. To determine the active ingredient distribution, the individual impactor components and the inhalation device, including the mouth piece adapter, were rinsed quantitatively with a mixture of acetonitrile and phosphate buffer pH 3.0 (35:65; 50ml). In each case, the individual, thoroughly mixed samples were then transferred to glass vials, for quantitative analysis of formoterol fumarate and budesonide by HPLC. The emitted dose was defined as the percent of total powder mass exiting the inhaler. The fine particle fraction (FPF) defined as the fraction of API particles emitted from the inhaler with an aerodynamic size ≤ 5µm was calculated. The mass median aerodynamic diameter, MMAD, was obtained by a linear fit of a plot of the cumulative mass plotted as a function of the logarithm of the effective cut-off diameter, and recording the diameter at the midpoint of the curve fit. The ratio between 84% undersize and 50% size (MMAD) represented the GSD.

2.5 Measurement of Powder Deposition in an Alberta Idealized -Throat

Dose delivery distal to the mouth-throat with developed dry powder inhaler (6 µg formoterol fumarate/ 100 µg budesonide) was measured in vitro using an aluminum idealized throat geometry. [11],[12] It has been previously reported that the Alberta mouth-throat model is an excellent predictor of average in vivo mouth-throat deposition.[13], [14]. Each inhaler was mounted onto the Alberta mouth-throat using a purpose-built mouthpiece adaptor that allows an air-tight connection to the mouth-throat cast. The outlet from the mouth-throat cast was attached directly to a Respirgard low resistance filter (catalogue # 303, Vital Signs Inc. 20 Campus Road, Totowa, NJ, 07512). The emitted dose depositing on this filter is a measure of in vitro drug delivery distal to the mouth-throat i.e. lung delivery. Flow rates of 28.3 l/min, 60 l/min and 90 l/min, calibrated using a mass flow meter (4143 series, TSI), were achieved using a vacuum pump. The inhalation time in each case was 4 s, although pilot tests at 28.3 l/min for 9 sec and 90 lm/in for 3 sec gave the same results. For each flow rate, three sets of measurements were performed. Each measurement consisted of five runs where one capsule was used in each run, i.e. for one measurement, five capsules were required. For each measurement a different developed dry powder inhaler was used. Before each run, the Alberta mouth-throat was washed and sprayed twice at 15 minute intervals with silicone grease (Molykote 316 Silicone Release Spray, USA, Dow Corning Corporation, Midland, MI – 48686-0994). Each Respirgard 303 filter was used to collect drug from five capsules.

2.6 Stability studies

Extensive stability studies are being performed on these products under International Conference on Harmonisation (ICH) conditions. For the present study, devices and formulations were stored at 25oC/60% RH and 40oC/75% RH for 6 months. The performance of these devices with stored formulation were carried out as part of ongoing work to support a shelf life of at least 18 months for inhalers (Device and Capsules) packed in moisture-proof PVC pouches and in-use life of at least 2 months after packaging has been removed.

2.7 Statistical Analysis

SPSS for windows version 10.0 and CITDAS were used for calculating means and inhalation parameters respectively. For the Alberta mouth-throat data, means were compared by ANOVA Tukey H.S.D. tests with Systat 12 (Systat Software, Chicago, IL) and probability value (P) of less than 0.05 was considered significant.

2.8 Robustness of the Device

The choice of spring was selected using a poke yoke exercise in terms of failure mode and was determined to be rigid for 1 million cycles before replacement. A drop test from 6' height has been performed for the device and it withstands any sudden shock resulting from a free fall.[15] The microbial test has been performed for dry powder inhaler device to measure total aerobic microbial count (TAMC) and total combined moulds and yeast (TCMY) were measured as per the method described in Indian Pharmacopeia, 2007. The device has not shown any microbial growth for 15 days of study periods and TAMC value were 18 CFU/ml (Limit: NMT 100 CFU/ml) and TCMY value was 4 CFU/ml (Limit: NMT 10 CFU/ml).

3. Results and Discussion

3.1 Measurement of Air Resistance

Air resistance is an important characteristic for DPIs since it determines the inspiratory flow rate achievable by patients. [16], [17] The air flow rate also governs drug delivery from the device to the lung. It is therefore essential to control device air resistance so as to produce adequate air flowrate by a majority of patients in various age groups after inhalation with ''comfortable'' effort normally

corresponding to a pressure drop of 4 kPa (40.8cmH2O) across the device. [17], [18]

Plotting the square root of pressure drop (dp) against volumetric flow rate (Q) as shown in Fig. 2 resulted in a straight line (with intercept of 0, and a slope of 0.087 (cm H2O)0.5/lpm (Between 0.06 (cm H2O)0.5/lpm to 0.09 (cm H2O)0.5/lpm) as the air flow resistance.[19]Which is in the range that was found to be preferred by patients. [17]

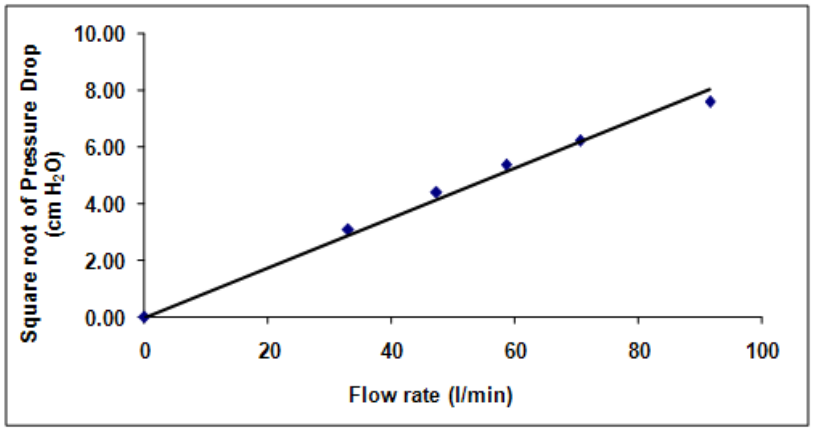

Figure 2: Relationship between flow rate (Q) and the square root of pressure drop (dp)

3.2 Uniformity of Delivered Dose Studies

The uniformity of delivered dose from developed dry powder inhaler for Redicaps-CombihaleFB100® is presented in Fig. 3. The overall mean (±SD) of 6µg formoterol fumarate was 99.9 ± 5.60 % of label claim (n=10) and the overall mean (±SD) of 100µg budesonide was 96.77±5.03 % of label claim (n=10). Each individual device produced mean formoterol fumarate and - budesonide within 85-115% of label claim. The delivered dose from the developed dry powder inhaler found in this study is thus found to be consistent at flow rate of 54.8 l/min (the flow rate that generated a pressure drop of 4 kPa across the device) for the combination product, Redicaps-CombihaleFB100®.

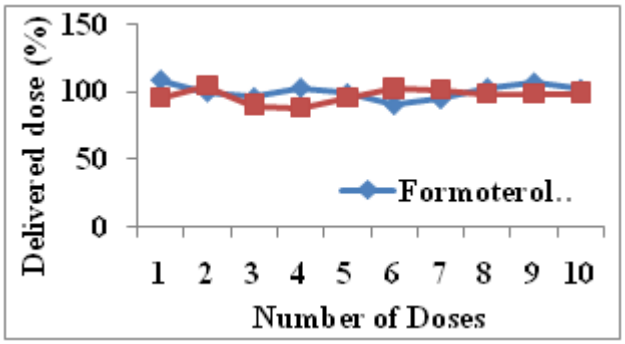

Figure 3: Uniformity of delivered dose (%) by developed dry powder inhaler for Redicaps-CombihaleFB100 formulation

3.3 Particle Size Distribution Studies By Andersen Cascade Impactor

In vitro aerodynamic particle size characterization data is shown in Table 2. Statistical analysis between the three flow rates revealed no significant differences indicating that the developed dry powder inhaler performance is consistent over this range of flow rates. The results reveal that for the combination product (Redicaps-CombihaleFB$_{100}$), flow rate does not significantly alter the dose or particle size properties of this inhaler. In addition, amounts of budesonide and formoterol fumarate emitted as fine particles from the same dose were similar.

Table 2: *In vitro* aerodynamic particle size characterization using ACI (n= 3 inhalers) for one determination from each inhaler using 10 doses at each flow rate. Data are expressed as mean (± SD).

In vitro Parameter	*28.3 l/min*		*60 l/min*		*90 l/min*	
	Formoterol	*Budesonide*	*Formoterol*	*Budesonide*	*Formoterol*	*Budesonide*
$FPD_{\leq 5\ \mu m}$ (µg)	1.261 (±0.44)	21.56 (±2.28)	1.26 (±0.32)	23.42 (±3.58)	1.38 (±2.89)	27.21 (±3.17)
FPF (%)	22.49 (±1.58)	23.53 (±1.55)	25.72 (±4.72)	25.75 (±0.36)	27.8 (±2.41)	29.73 (±3.02)
MMAD (µm)	5.06 (±0.47)	5.13 (±0.59)	5.54 (±0.77)	5.08 (±0.65)	4.82(±0.71)	4.98 (±0.87)
GSD	1.60 (±0.05)	1.79 (±0.06)	1.69 (±0.11)	1.75 (±0.18)	1.65(±0.13)	1.74 (±0.23)

Fig. 4, Fig. 5 and Fig. 6 show the particle size distribution of formoterol and budesonide on different stages of the -ACI at 28.3 l/min, 60 l/min and 90 l/min respectively. The device retention for formoterol fumarate and budesonide were 14.38 (± 0.93) % and 13.55 (± 1.23)%at 28.3 l/min; 8.37 (±2.55)% and 7.71 (± 2.13) % at 60 l/min; and 8.9 (± 1.88)% and 7.46 (± 0.53) % at 90 l/min respectively. These data indicate that the developed dry powder inhaler has low device retention which may be attributed to the unique parabolic shape of the dose dispersion chamber. At 28.3 l/min, the majority of the emitted dose was deposited in the throat and the preseparator, which for formoterol fumarate and budesonide was 56.75 (±2.66)% and 56.59 (±2.05)% of the total dose emitted, respectively. At higher flow rate 60 l/min and 90 l/min, there was significantly more emitted dose deposited in the throat and preseparator for both formoterol fumarate and budesonide compared to 28.3 l/min (Fig. 5 and Fig. 6).

Based on the delivered dose data and emmited dose data the developed dry powder inhaler demonstrated reproducible mass delivery of the blend, leading to consistent delivery of the drug. Formoterol fumarate used in the combination product would be expected to be a difficult molecule to disperse due to the small dose compared to the mass of lactose carrier. For a low dose component (formoterol fumarate, 6µg) and a high dose component (budesonide, 100µg), the fine-particle fraction (< 5 µm) was measured to be over 20%. suggesting that developed dry powder inhaler is able to generate deeply respirable drug particles.

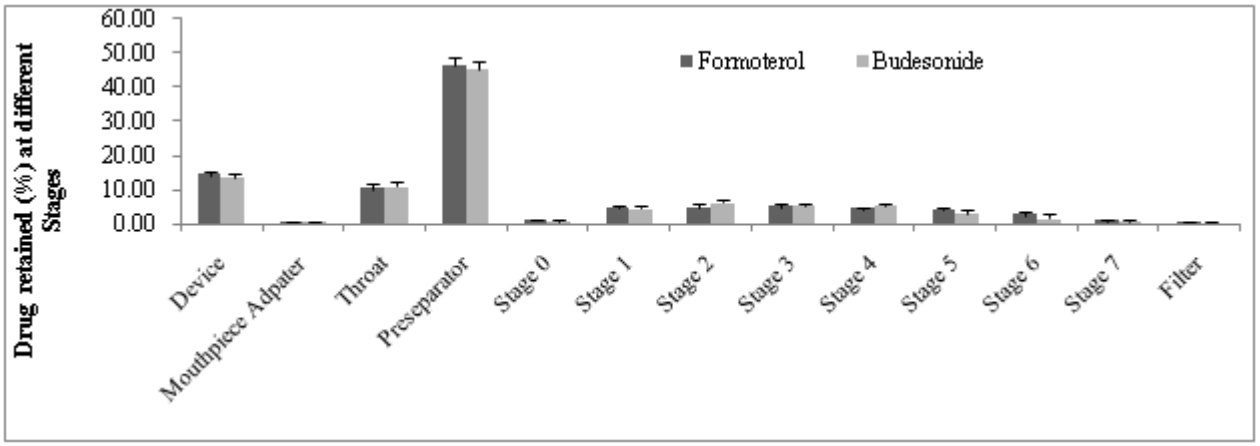

Figure 4: Aerodynamic Particle size distribution of formoterol fumarate and budesonide at different stages of ACI at 28.3l/min for 8 seconds. Data are expressed as mean ± SD (n=3, 10 capsules per ACI)

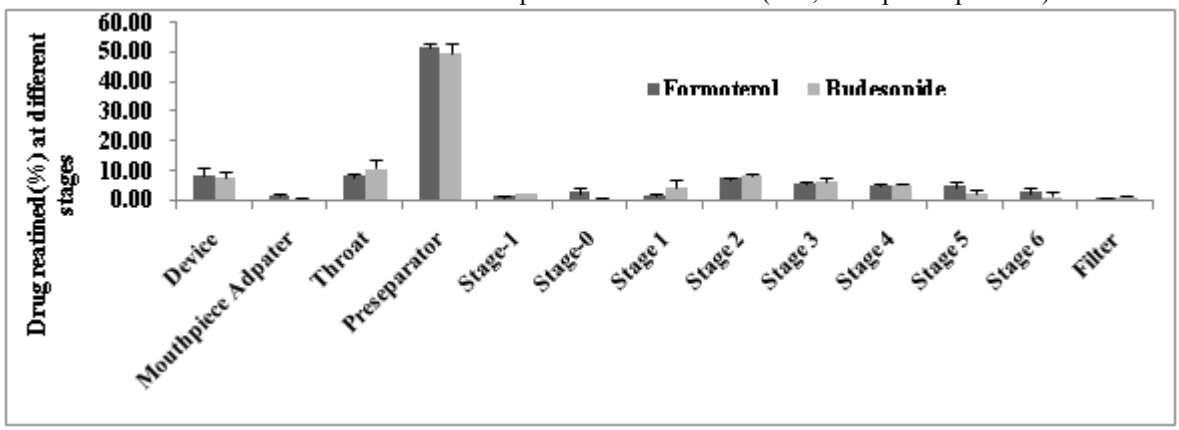

Figure 5: Aerodynamic Particle size distribution of formoterol fumarate and budesonide at different stages of ACI at 60l/min for 4 seconds. Data are expressed as mean ± SD (n=3, 10 capsules per ACI)

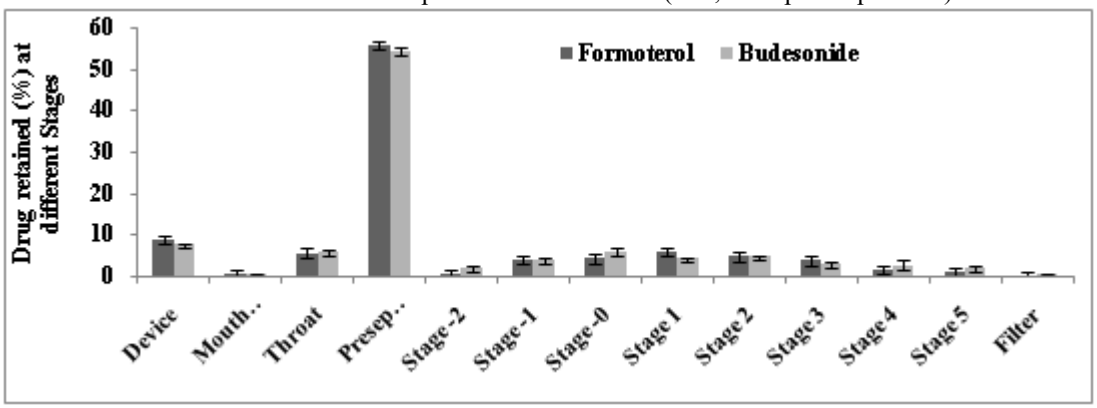

Figure 6: Aerodynamic Particle size distribution of formoterol fumarate and budesonide at different stages of ACI at 90 l/min for 2.7 seconds. Data are expressed as mean ± SD (n=3, 10 capsules per ACI)

3.4 Measurement of Powder Deposition with an Idealized Mouth-Throat model

To more accurately replicate mouth-throat deposition, the Alberta mouth-throat, which has been found to be an excellent predictor of in vivo mouth-throat deposition was used. [13], [14]The amounts of drug delivered distal to the in vitro mouth-throat were measured by collection on a filter downstream of the Alberta idealized throat. Deposition is as per Table-3

Table 3: Average Delivery of the content distal to Alberta throat deposition

Flow rate	*Budesonide*	*Formoterol Fumarate*
	Mean ± SD	*Mean ± SD*
28.8 l/min	22.5±1.1%	18.9±0.8%
60 l/min	23.6±0.7%	19.4±0.8%
90 l/min	28.6±0.6%	22.8±0.8%

There is no significant difference in drug delivery distal to the mouth-throat between 28.3 and 60 l/min ($p>0.05$), while drug delivery at 90 l/min is higher and this difference is significant ($p<0.05$). However, compared to 28.3 l/min, the increase in delivery at 90 l/min is 6.1% of the label claim for budesonide and 3.9% for formoterol. Such differences are relatively small and it can be speculated that they would not be clinically significant, although in vivo tests would be needed to confirm this speculation.

3.5 Stability studies

Particle size distribution by ACI at 28.3 l/min and uniformity of delivered dose at 54.8 l/min were measured for the stability samples. As shown in Table 3, FPD, FPF and uniformity of delivered dose were not affected when samples were stored at 25°C/60%RH and 40°C/75%RH for 6 months, indicating stability of both device and formulation i.e. drug delivery and the performance from developed dry powder inhaler is maintained.

Table 4: Aerosol performance with stability samples at 25°C/60%RH and 40°C/75%RH for 6 months. Data are expressed as mean ± SD (n=3, 10 capsules per ACI and n=10 capsules for uniformity of delivered dose)

In vitro Parameter	25°C/60%RH		40°C/75%RH	
	Formoterol	Budesonide	Formoterol	Budesonide
FPD (µg)	1.13±0.25	16.80 ± 2.21	1.45 ± 0.35	20.80±1.45
FPF (%TRD)	19.4± 2.68	19.3±1.63	24.9 ± 3.56	20.3± 2.30
Delivered Dose (%)	94.6 ± 3.68	98.6 ± 3.87	92.8 ± 3.12	.9 ± 4.15

4. Conclusions

In vitro fine particle distribution of formoterol fumarate-budesonide combination delivered via - developed dry powder inhalerdemonstrated consistent fine particle fraction, low device retention, and uniformity of delivered dose. The delivery of corticosteroid and beta agonist by - developed drypowder inhaler showed only weak flow-rate variation in dose delivery distal to the mouth-throat as determined using the Alberta throat model.

References

[1] Byron PR, *et al*. Prediction of drug resistance times in regions of human respiratory tract following aerosol inhalation, *J. Pharm. Sci.* 75, 433-438 (1986).
[2] Ganderton D, Kassem NM, *et al*. Saccharides as aerosol carriers, US Patent, 5376386, (1994).
[3] Tarsin W, Assi K, and Chrystyn H, *et al*. *In-Vitro* Intra- and Inter-Inhaler Flow Rate–Dependent Dosage Emission from a Combination of Budesonide and formoterol in a Dry Powder Inhaler,*J. Aerosol Med.*, Volume 17, Number 1, Pp. 25–32 (2004).
[4] Throsson L, Edsbäcker S, Källén A, and Löfdahl CG, *et al*. Pharmacokinetics and systemic activity of fluticasone via Diskus® and pMDI, and of budesonide via Turbuhaler®. *Br. J. Clin. Pharmacol.* 52:529–538 (2001).
[5] Hugh DC, Smyth and Hickey AJ, *et al.* Carriers in Drug Powder Delivery Implications for Inhalation System Design, *Am J Drug Deliv.* 3 (2): 117-132 (2005).
[6] Barnes P. Mechanisms of action of glucocorticoids in asthma, *et al*, . *Am. J. Respir. Crit. Care Med.*154, S21-27 (1996).
[7] Virchow JC, Crompton GK, Dal Negro R, Pedersen S, Magnan A, Seidenberg J and Barnes PJ, *et al*. Importance of inhaler devices in the management of airway disease, Respiratory Medicine 102, 10–19 (2008).
[8] Borgström L, Bondesson E, Morén F, Trofast E, and Newman S, *et al,* Lung deposition of budesonide inhaled via Turbuhaler®: A comparison with terbutaline sulphate in normal subjects. *Eur. Respir. J.* 7:69–73, (1994).
[9] Naidu PS, Subramony JA and Gautama Buddha T, *et al*. Device for inhaling powdered medicaments**,** Indian Patent Application no: 124/CHE/2008**,** US Patent Application no:61/046,828.
[10] Nichols SC, *et al*. Calibration and mensuration issues for the standard and modified Andersen cascade impactor. Pharmacopeial Forum 26:1466–1469.
[11] Grgic B, Finlay WH and Heenan AF, *et al*. Regional Aerosol Deposition and Flow Measurements in an Idealized Mouth and Throat. *J. Aerosol Science*, 35: 21-32 (2004).
[12] Stapleton KW, Guentsch E, Hoskinson MK and Finlay WH, *et al*. "On the suitability of k-ε turbulence modelling for aerosol deposition in the mouth and throat: a comparison with experiment", J. Aerosol Sci. 31:739-749.
[13] Grgic B, Heenan AF, Burnell PKP, and Finlay WH, *et al*. *In Vitro* Inter subject and Intra subject Deposition Measurements in Realistic Mouth-Throat Geometries. J. Aerosol Sci. 35:1025-1040 (2004b).
[14] Chew NYK, Chan HK, *et al. In vitro* aerosol performance and dose uniformity between Foradile Aerolizer and the Oxis Turbuhaler. *Journal of Aerosol Med.*, vol.14 (4), 495-501, (2001).
[15] ASTM International. "Standard Test Method for Drop Test of Loaded Containers". ASTM Standards D5276-98 (2004).
[16] Srichana T, Martin GP and Marriott C, *et al*. Dry powder inhalers: The influence of device resistance and powder formulation on drug and lactose deposition *in*

vitro, *European Journal of Pharmaceutical Sciences* 7, 73 –80 (1998).

[17] Clark AR, Hollingworth AM, *et al*. The relationship between powder inhaler resistance and peak inspiratory conditions in healthyvolunteers: implications for in vitro testing. *J Aerosol Med.* 6: 99–110 (*1993)*.

[18] Labiris NR, and Dolovich MB, *et al*. Pulmonary drug delivery. Part I: Physiological factors affecting therapeutic effectiveness of aerosolized medications. *Br. J. Clin. Pharmacol.* 56:588–599 (2003).

[19] Ower E, Pankhurst RC, *et al*. The Measurement of Air flow, 5th edn. Oxford Diagnostic, Oxford: Pergamon Press, (1977).