Research Paper

Comparison of Aerosol Formulations of Formoterol Fumarate and Budesonide

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The aerodynamic diameter of pharmaceutical aerosols is the main factor governing their deposition in the human respiratory tract. Particle size of the pharmaceutical aerosols is characterized by liquid impingers and Andersen Cascade Impactors. The present study was aimed at comparing two metered dose inhaler formulation containing formoterol fumarate (6 µg) and budesonide (200 µg). These two formulations were evaluated by using Twin Stage Impinger and Andersen Cascade Impactor. Study revealed that developed metered dose inhaler I formulation of the formoterol fumarate and budesonide had lower mass median aerodynamic diameter and higher fine particle fraction than marketed formulation.

Key words: ACI, budesonide, formoterol fumarate, MDIs, TSI

Inhalation route is been successfully used to treat asthma and chronic obstructive pulmonary disease (COPD) since active substances, such as beta agonists, anticholinergics, corticosteroids and mast cell inhibitors are delivered directly at the target cells in the lungs[1].

Formoterol fumarate (FF, (*RS*,*SR*)-*N*-[2-hydroxy-5-[1-hydroxy-2-[1-(4-methoxyphenyl) propan-2-ylamino]ethyl] phenyl]formamide) also known as formoterol is a long-acting β_2-agonist used in the management of asthma and/or chronic obstructive pulmonary disease (COPD)[2]. Budesonide (BUD, (16,17-(butylidenebis(oxy))-11,21-dihydroxy-(11-β,16-α)-pregna-1,4-diene-3,20-dione) is a glucocorticoid steroid for the treatment of asthma, non-infectious rhinitis (including hay fever and other allergies), and for treatment and prevention of nasal polyposis[3].

Dose availability from an inhalation device is a result of metering and dispensing in coordination with the patient's inspiratory cycle. Thus, deposition of drug is not only affected by metering, but also by its simplicity of use and affability to the patient. Metered dose inhaler (MDI) is the widely used inhalation delivery system principally due to its portability, durability, reliability, shelf life, microbial robustness[4], cost effectiveness and ease of use especially in critical situations. However, despite these advantages, there are some weaknesses like variation in dosing dependent on shaking, priming, actuation time and canister content[5-7]. The move to replace CFC propellant with hydrofluroalkane (HFA), Geneva protocol (1989) provided opportunity for redevelopment of MDIs. HFA powered MDIs require intricate re-formulation and the use of new valve-types, actuators and mouthpieces[6].

MDIs are pressurised delivery systems which can be manufactured either in solution or suspension form. Formulation of MDI contains critical components like propellant, excipients viz, co-solvents and surfactants. The type of dosage form can influence stability and performance of MDI, where in an active pharmaceutical ingredient is either in the suspension or solution form. Solution formulations of metered dose inhalers of salbutamol sulphate and triamcinolone acetonide performed better and gave lower mass median aerodynamic diameter (MMAD) values as compared to their suspension formulation[8].

Vapour pressure and density of propellants are employed to assist in formulation. A higher vapor pressure usually results in a finer aerosol with a

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greater initial forward velocity causing a higher oropharyngeal deposition. This can result in an increased deposition to the whole lung, seen mainly in the central airways[9]. Smaller metering volumes may also give a finer aerosol with higher respirable fractions and more peripheral lung deposition. The particle size of pharmaceutical aerosols is the main factor governing their deposition in the human respiratory tract.

Inertial methods, which can mimic *in vivo* conditions, give the most representative results of aerosol performance[10]. They are the only methods available in the pharmacopoeia, which are accepted for particle size characterization for aerosols. Apparatus working on this principle have been included in the United State Pharmacopoeia (Apparatus 1), British Pharmacopoeia, (Apparatus D) and European Pharmacoepiea (Apparatus D). The present study aims at comparison of in-house developed MDI with marketed formulation by using Twin Stage Impinger (TSI) and Andersen Cascade Impactor (ACI).

.MATERIALS AND METHODS

Working standards of FF and BUD were gift from Glenmark Pharmaceuticals Ltd. MDI I formulation of formoterol fumarate and budesonide was free sample from Glenmark Pharmaceuticals Ltd. MDI II , marketed formulation containing same quantity of FF and BUD, was obtained as gift sample from Glenmark Pharmaceuticals Ltd. Acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 μm filtered water used for HPLC analysis and preparation of buffer. Buffers and all other chemicals were analytical grade.

In MDI I different excipients like surfactant, polymer and micronized drug were suspended in HFA 134a (Table 1) and filled in previously crimped 14 ml standard aluminium canisters by single stage filling process.

Characterisation:

Homogeneity of the suspension was evaluated by visually inspecting formulation I when filled into glass bottles. Particle size distribution of MDI I formulation was determined by ocular microscopy. The canister was sprayed on clean glass slide. Particles were observed under 40X magnification, by using calibrated ocular micrometer and particles were measured as per IP 2007[11]. Dose uniformity was determined over entire content (initial, middle and end actuations) as per the official method described in USP[12]. Both MDI formulations were characterized for in *vitro* pulmonary deposition by TSI and ACI. Stability of MDI I formulation was evaluated at 40°/75% RH for three months, wherein assay and fine particulate fraction (FPF) were determined at the end of every month.

HPLC analysis method:

The chromatographic system consisted of the following components from Jasco Corporation (Tokyo, Japan): A Jasco PU 2080 plus Intelligent HPLC pump solvent delivery system. A UV detector (UV 2075 plus) covering the range of 200–400 nm and interfaced to a computer for data acquisition and a recorder model Star 800 interface module. A rheodyne, with 50 μl loop injector. BDS Hypersil column C18 (150×4.6 mm, 5 μm) was used as stationary phase (Thermo Electron Corporation). The mobile phase consists of Acetonitrile and phosphate buffer pH 3.1 using gradient program given in Table 2. Flow rate was 1.5 ml/min. The column was maintained at 30°. Detector was programmed at 214 nm for detection of FF for 10 min and 247 nm for detection of BUD up to 30 min.

Twin stage impinger:

The impinger, fabricated as per specification given in IP 2007[13] was attached to a vacuum pump that was

TABLE 1: COMPOSITION OF MDI I FORMULATION

Ingredients	Qty. per Actuation (mg)
Formoterol fumarate dihydrate IP Eq. to formoterol fumarate (Micronised)	0.006
Budesonide IP (Micronised)	0.200
Polymer	0.01-5.0
Surfactant	0.01-5.0
Propellant 1,1,1,2- tetrafluoroethane (HFA 134a)	q.s.to 60

TABLE 2: HPLC GRADIENT FLOW

Time in min.	A (%)	B (%)
0-0.01	80	20
0.01-5.0	80	20
5.0-6.0	68	32
6.0-20.0	68	32
20.0-21.0	80	32
21.0-30.0	80	32
30.0	End of run	

A is Phosphate buffer pH 3.1 containing 0.025M sodium dihydrogen orthophosphate dihydrate and B is acetonitrile

set at a continuous air flow of 60±5 l/min. The upper stage of the impinger was filled with 7 ml of solvent and 30 ml were filled in the lower stage. First three deliveries of the MDIs were discharged to waste. After firing 10 puffs into the apparatus, throat and the impinger stages were rinsed with solvent. Two solutions were obtained: The first was from rinsing the throat and stage 1, second solution was from stage 2 of the impinger. Stage 1 washings included those from the throat and from the stage 1 inlet tube. Stage 2 washings included those from the inside and outside of the stage 2 inlet tube and the jet spacer. Total respirable fractions from both the MDI products were compared using student's *t* test.

***In vitro* deposition study using Andersen Cascade Impactor:**

Andersen Cascade Impactor (Copley Scientific, UK) was assembled with glass fiber filter paper in place on filter stage. The ACI was attached to suitable vacuum pump, set at 28.3 l/min (±5%) flow rate. The procedure was performed as per the Indian pharmacopoeia 2007 guidance. The cutoff diameters for all the eight stages were: 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7 and 0.4 µm, respectively. From the size distribution, a log-probability plot was constructed and characteristics of the aerosol determined, such as the amount of drug contained in particles less than 5 µm, the MMAD (*i.e.* the aerodynamic size of a particle, such that half of the drug is in larger and half in smaller particles), and the geometric standard deviation (GSD) a measure of the heterogeneity of the aerosol particle size, was determined by CITDAS software (Copley Scientific, UK)[14]. Mean recovery of FF and BUD from the two formulations was compared using a two-tailed t-test.

RESULT AND DISCUSSION

MDI I formulation was observed to be a homogenous fine suspension. The suspension was found to be stable for two minutes after shaking (fig. 1). Dose uniformity was found to be 97.1±4.8% for FF and 97.5±5.0% for BUD. Microscopic observation under 40X revealed 96% particles below 5 µ (fig. 2). Aerosol performance of the MDIs is affected by addition of excipients to suspension formulation of MDIs. In present study**,** we evaluated performance of MDI formulation of FF and BUD (6 µg+200 µg, formulation I) and marketed formulation of FF and BUD (6 µg+200 µg, formulation II) aerosol. The MDI formulations were evaluated using TSI (apparatus A Phr. Eur., BP) and ACI (apparatus D Phr. Eur., BP, apparatus 1 USP).

The analytical method was developed for simultaneous estimation of both the drugs by using gradient flow method (fig. 3). Seven point standard curves ranging from 0.05 to 10 ppm of FF and BUD was constructed in triplicate. Calibration curves (y= ax), represented by the plots of the peak-area of the analyte versus the nominal concentration (x) of the calibration standards, were generated using linear least square regression. The equation for standard curve obtained for HPLC method for FF was as follows, Y= 50753.46832X+625.21612, R^2= 0.99997, where Y is the area and X is the concentration in ppm of FF. The R^2 value of 0.99997 indicated good correlation. Budesonide is mixture of two epimeric forms, epimer A (BUD A) and epimer B (BUD B)[15], combined area of BUD A and BUD B was considered for analysis. The equation for standard curve obtained for HPLC method for BUD was as follows, Y= 90197.33000X-2144.29357 with R^2= 0.99997, where Y is the area

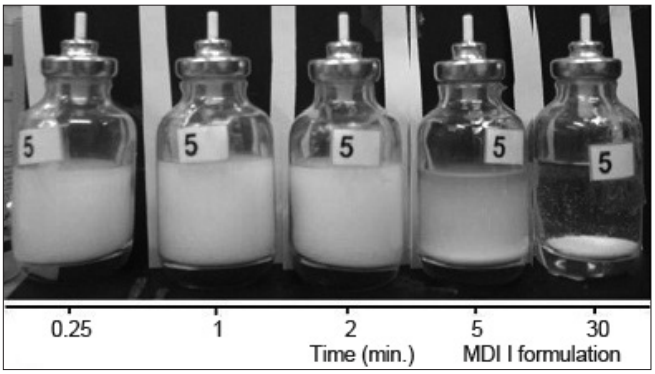

Fig. 1: Visual observation of MDI I formulation in glass vial

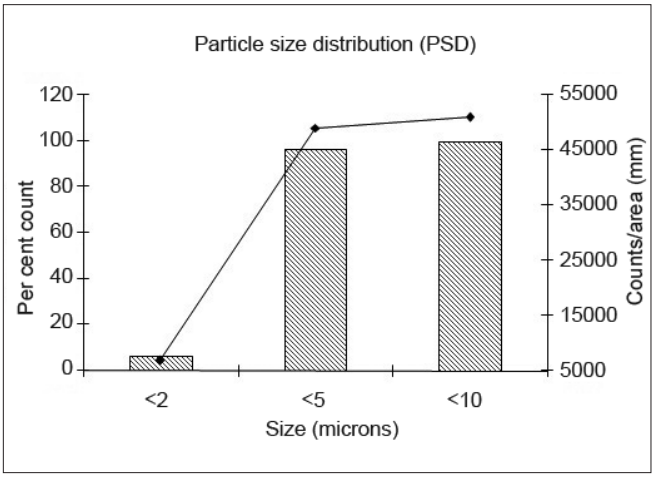

Fig. 2: Particle size distribution of MDI I Formulation

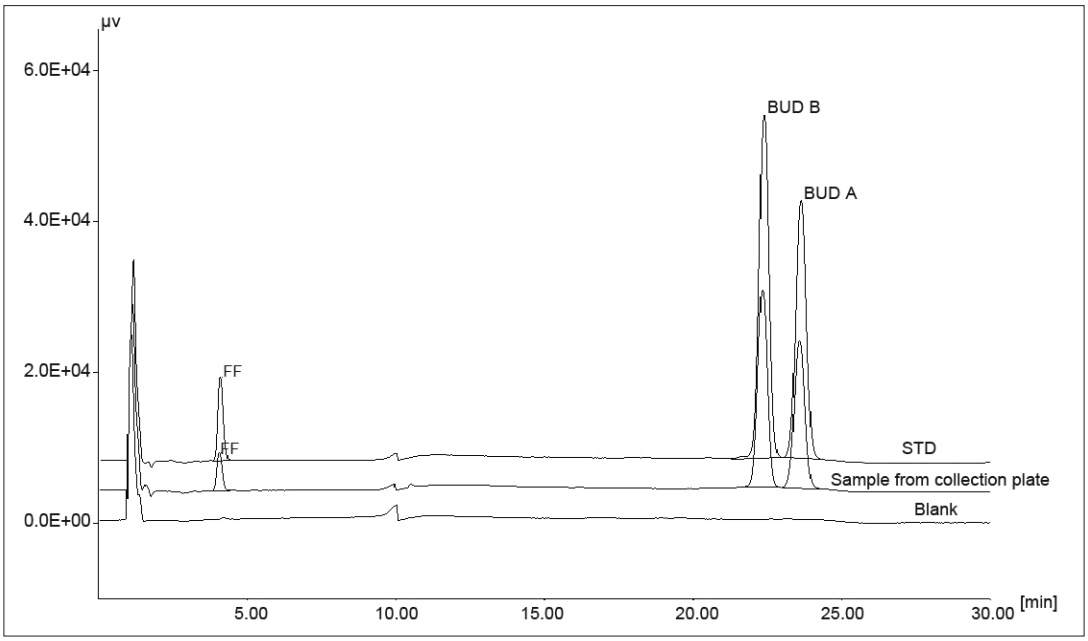

Fig. 3: Overlay Chromatogram of sample from Anderson Cascade Impactor with standard formoterol fumarate and budesonide

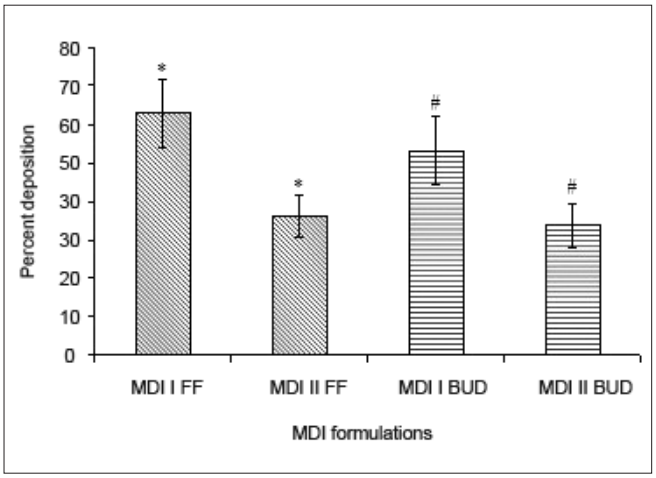

Fig. 4: Percent respirable fraction from TSI study of two MDI devices Error bars represents standard deviation (n=3) and (*,# *P* <0.05).

and X is the concentration in ppm of BUD. The R^2 value of 0.99997 indicated good correlation.

The total dose recovered from all the stages of TSI from MDI formulation I for drug FF was found to 76.12±0.23% and MDI formulation II was 63.59±3.06%. The total recovered dose of BUD from formulation I was 64.44±5.40% and formulation II was 59.44±6.43%. Total respirable fraction from the MDI formulation I was statistically significant from that of MDI formulation II fig. 4. The study reveals no statistical difference in total dose per shot between two MDI formulations. Statistically significant difference was observed between MDI formulation I and MDI formulation II for fine particle dose and fine particle fraction Table 3 and 4. MMAD for FF was found to be 3.03±0.058 for MDI formulation I and 3.86±0.058 for formulation II. MMAD for BUD was found to be 3.53±0.06 for MDI formulation I and 4.10±0.10 for MDI formulation II. Improvement in aerosol performance of MDI formulation I could be attributed to optimised levels of polymers and surfactants, which were incorporated in sufficient amount to enhance the physical stability of suspension and to reduce the drugs adherence with canisters and valves during storage.

Li and Seville recently demonstrated that fine particle fraction of spray dried bovine serum albumin (BSA) along with sodium carboxy methyl cellulose, showed statistically significant fine particle fraction over the period of storage time compared to standalone spray dried powder of BSA[16]. In this study authors suggested that the use of sodium CMC either prevented adsorption of BSA to the canister walls or prevented degradation of BSA over the storage period. Another study conducted by Young *et al*. showed addition of fines in increasing concentration significantly lowers the fine particle fraction of HFA containing MDI formulation. The lowering of the fine particle fraction depends on the excipients used in the formulation. Fine lactose containing formulation showed significantly lower fine particle fraction than Mannitol containing MDI formulation[17]. These reports confirm that selection of excipients is critical to performance of MDI formulations. Stability studies

TABLE 3: RESULTS OF ACI STUDY FOR FORMOTEROL FUMERATE

Characterisation	MDI formulation I Mean* (±SD)	MDI formulation II Mean*(±SD)	*P* value
Total dose per shot (µg)	4.61±0.097	4.45±0.062	
Fine particle dose (µg)	2.23±0.111	1.38±0.112	0.00073
Fine particle fraction (%)	48.48±1.955	31.18±2.959	0.00108
MMAD# (µm)	3.03±0.058	3.86±0.058	0.00006
GSD†	1.73±0.058	2.03±0.153	

The *P* values are obtained by comparing MDI formulation I with II by Students "t" test. *Results are mean of 3 experiments #Mass median aerodynamic diameter †Geometric standard deviation.

TABLE 4: RESULTS OF ACI STUDY FOR BUDESONIDE

Characterisation	MDI formulation I Mean*(±SD)	MDI formulation II Mean*(±SD)	*P* value
Delivered dose per shot (µg)	152.18±4.47	152.49±3.4	
Fine particle dose (µg)	68.01±1.22	42.18±2.49	0.00009
Fine particle fraction (%)	44.73±2.03	27.70±1.93	0.00046
MMAD# (µm)	3.53±0.06	4.10±0.10	0.00105
GSD†	1.57±0.06	1.9± 1.11	

The *P* values are obtained by comparing MDI formulation I with II by Students "t" test. *Results are mean of 3 experiments #Mass median aerodynamic diameter †Geometric standard deviation.

of MDI I formulation conducted at 40º/75% RH revealed no change in the assay of the drug and no significant change in the FPF of both FF and BUD.

The study presents the comparison of *in vitro* lung deposition of two MDI formulations. TSI and Cascade impaction are an established *in vitro* method for the characterisation of pharmaceutical aerosols. Screening of MDI formulations using TSI and ACI equipment will not only assist the production of required regulatory data, but also improve the efficiency of pMDI formulation development.

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