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A New Validated Method for Simultaneous Determination of Aerodynamic Particle Size Distribution of Drug Particles of Mometasone Furoate and Formoterol Fumarate Dihydrate in 200 mcg/5 mcg Inhalation Aerosol

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Simultaneous determination of aerodynamic particle size distribution (APSD) of drug particles of mometasone furoate and formoterol fumarate dihydrate – 200 mcg/5 mcg inhalation aerosol (MDI) has been developed and reported in this work. The method was developed in consistent with USP <601> inhalation and nasal drug products *e.g.* aerosols, sprays and powders. The next generation impactor (NGI) was used and operated at 30 ± 5% L/min. The proposed method was demonstrated for sensitivity, minimum number of actuations, diluent volume optimization, mass balance and precision. The proposed method was found to be robust, accurate, linear and precise.

Keywords: Mometasone furoate, Formoterol fumarate, Aerodynamic particle size, Fine particle distribution, Impactor sized mass.

INTRODUCTION

Metered dose inhaler dosage forms (MDIs) were developed and targeted mainly for chronic obstructive pulmonary disease (COPD) patients as well as for disease conditions which were not adequately controlled by a long-term asthma medication such as an inhaled corticosteroid (ICS) or long-acting β_2-adrenergic agonist (LABA) [1,2]. Metered-dose inhalers (MDIs) have distinct advantage over other conventional dosage forms in delivering fixed therapeutic dose directly to the lungs, surpassing first pass metabolism, with enhanced efficacy and ease of administration [3]. Subsequently MDIs are portable, easy to use, provides immediate relief, prefilled multidose delivery actuations and low risk of bacterial contamination. Since a decade, demand for MDIs is increasing due to rise in pollution across the world and climatic changes collectively leading to COPD, bronchial and other related respiratory issues. To meet the global demand, pharmaceutical companies have to equip a distinct portfolio of inhalations, starting from research and development, clinical studies, chemistry, manufacturing and controls throughout the lifecycle which were driven by stringent regulatory guidelines.

To evaluate quality and quantity of drugs in MDIs, few analytical methods are available in public domain as well as pharmacopial forums. Literature reveals that the various analytical methods have been available for the separation and quantification of formoterol fumarate and mometasone furoate either alone or in combination with other drugs using instruments such as HPLC, GC, UV spectrophotometry and supercritical fluid chromatography [4-12]. However, there is no method reported for determination of aerodynamic particle size distribution for both the lower and higher strengths.

Aerodynamic particle size (APSD) is considered as one of the critical quality attribute for *in vitro* charcatrizating of aerosols as the test reveals where the aerosol cloud particles are deposited after inhalation [13]. APSD analysis is effective to assess *in vitro* behaviour of respiratory products for which the API particles size is in the range of 1-5 µ. For MDIs, the particles of interest is mostly between 1 to 5 µ and APSD analysis by next generation impactor (NGI) will be very critical assessment [14]. Next generation impactor (NGI) is designed with seven stages and each stages has specified cut off diameters in the range of 0.5 to 5 µ depending on the flow rate selected. The NGI work as the air flow passes through the impactor in

a saw tooth pattern. Particle separation and sizing is achieved by successively increasing the velocity of the airstream as it passes through each by forcing it through a series of nozzles. Therefore, efforts were made to develop and suitable method for quantification of aerodynamic particle size distribution of both the analyte in inhalation aerosol dosage form.

Formoterol fumarate dihydrate (FD) is a β_2-agonist by pharmacological action and is very effective bronchodilating agent. Chemically named as (±)-2′-hydroxy-5′-[(R*)-1-hydroxy-2-[[(R*)-*p*-methoxy-α-methylphenethyl]amino]ethyl] formanilide fumarate (2:1) (salt), dihydrate (Fig. 1a) and is a white crystalline powder, soluble in ethanol and methanol, slightly soluble in water, practically insoluble in acetonitrile. Mometasone furoate (MT) is a topical corticosteroid having anti-inflammatory, anti-pruritic and vasoconstrictive properties. Chemically named as (11β,16α)-9,21-dichloro-11β-hydroxy-16-methyl-3,20-dioxopregna-1,4-dien-17-yl-2-furoate (Fig. 1b) and is a white crystalline powder, soluble in acetone and dichloromethane and slightly soluble in ethanol, practically insoluble in water. The combination of these two drugs are effective in the management of asthma in patients of 12 years and older by acting on the lungs locally by bronchodilating and relaxing the airway muscles for improved breathing [15]. The orally administered MDI aerosol drug product is available in pressurized multidose canister containing formoterol fumarate dihydrate and mometasone furoate as 5/100 µg and 5/200 µg per actuation and HFA227 as a propellant.

EXPERIMENTAL

Qualified working standards (purity > 98%) and respective impurities for formoterol fumarate dihydrate and mometasone furoate were procured from API vendors and qualified by Dr. Reddy's Labs. Hyderabad, India. Orthophosphoric acid 88%, hydrogen peroxide HPLC-Gradient grade acetonitrile and methanol were procured from Merck, Germany for mobile phase preparation and diluent preparation.A 0.45 µ filtered deionized water was obtained from the Milli-Q system, Millipore, USA.

Equipments: Next generation impactor (NGI) (Make: Copley) equipped with critical flow controller (Model: TPK 2100, make Copley), vacuum pump (Model: HCP-5, Make: Copley), NGI cooler (Make: Copley), flow meter (Make: Copley), HPLC system (Model: 1200 series, Make: Agilent) equipped with quaternary pump (G1311A), UV-visible detector (G1314B), injector (G1328B) with (100 µL) injector loop and degasser (G1322A). The output signal was monitored and processed using Empower-3 software. The sonicator (Power sonic 420), centrifuge (Thermo electron GmbH, Germany) was used during the preparation of solutions.

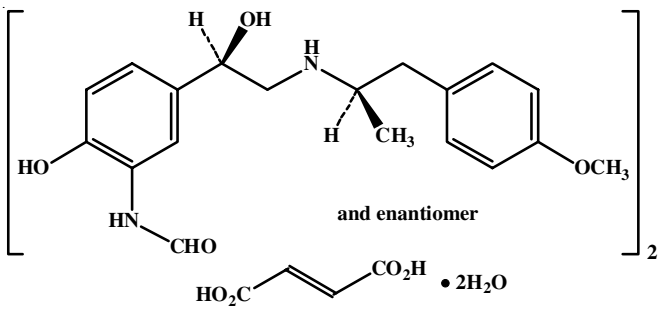
Chromatographic conditions: The chromatographic separation and quantitation was achieved on Hypersil ODS C_{18} column (250 × 4.6 mm, 3 µm). The mobile phase comprising of solution-A [10 mM of sodium dodecyl sulphate in 25 mM of phosphate buffer pH 3.0 and acetonitrile (70:30%v/v)]: Solution-B (acetonitrile) mixed in the ratio of 70:30%v/v. The chromatographic system was operated at a flow rate of 1.2 mL/min, column temperature of 50 ºC and a detection wavelength of 215 nm. Impurity mix, Standard and samples were analyzed using HPLC system with 100 µL injection volume. The diluent was used as methanol:water in the ratio of 60:40 v/v, respectively.

Standard solution preparation: Weighed and diluted formoterol fumarate dihydrate and mometasone furoate working standards in diluent to make a concentration of about 1 µg/mL and about 28 µg/mL, respectively for 100 mcg/5 mcg strength and about 1 µg/mL and about 56 µg/mL, respectively for 200 mcg/5 mcg strength.

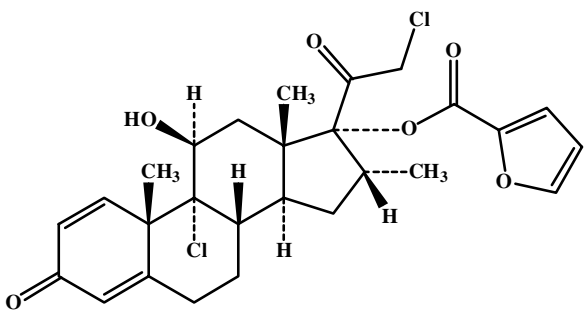
Test preparation and procedure: The procedure for the sample preparation can be sub-sectioned as NGI setup, priming of MDI canister, NGI collection and sample recovery.

NGI setup: The targeted MDI drug product is available in two different strengths, 100/5 µg per actuation and 200/5 µg per actuation. As a worst case scenario, considering drug to placebo ratio, 200/5 µg per actuation of formoterol fumarate dihydrate and mometasone furoate was chosen for the method validation studies in comparison to other available strengths. Attached the induction port to the NGI. Turn on the vacuum pump and allow it to equilibrate for atleast 3 min. Attached flow meter to the inlet of the induction port and adjusted the flow rate on critical flow controller to 30.0 ± 0.7 L/min.

Priming/seating of inhalation aerosol canister: Priming procedure to saturate the pump/device and to deliver right dose to the patient. Priming shall be performed for a new pack or if the canister is not used for more than 5 days. It must be primed/seated for four times with the actuator manually. Placed the inhalation aerosol canister into the actuator. Shaken the canister with actuator for 5 s in an actuating position of valve downside. Actuated the inhalation aerosol canister, into a waste shot collector inside a fume hood, by depressing the canister into the



(a) Formoterol fumarate dihydrate (FFD)



(b) Mometasone furoate (MF)

Fig. 1. Structure of (a) formoterol and (b) mometasone and its impurities

actuator and holding the canister down in the actuation for few seconds before releasing finger pressure. Wait for about 30 s for the aerosol cloud to disperse. Repeated the steps until a total of four actuations have been performed.

NGI collection: Attached the NGI adapter to the mouth of the actuator of the inhalation aerosol canister. Removed the actuator from the mouth piece adapter and place the adapter on the throat of NGI. Shaken the canister with actuator vertically in an actuating position of valve downside. After completing the shaking, attached the inhalation aerosol canister to the mouthpiece adapter of the NGI and immediately actuated the inhalation aerosol canister by depressing the canister into the actuator and holding the canister down for few seconds before releasing finger pressure. Hold the canister for about 30 s for the aerosol cloud to disperse before removing the actuator from the mouth piece adapter. Repeated above steps to deliver nine additional actuations for a total of ten shots dispensed into the NGI.

Recovery of sample: Removed adapter from the induction port, rinsed it with 10 mL of diluent. Rinsed both the inner and outer surfaces and collected it in to a 10 mL volumetric flask and made up the volume. Detached the induction port from the NGI, closed one end with the cap and transfer 20 mL of diluent slowly by rinsing the walls of the induction port into it, once after complete transfer of diluent and close the other end with another cap. Hold both the ends tightly to avoid spillage and shake the induction port for 1 min for proper recovery of the drug and collected the sample. For NGI stages 1 to 7 and the micro-orifice collector (MOC), added diluent directly to the cups of NGI. After adding the specified volume of diluent, shaken the tray gently using NGI rocker such that the diluent is spread to whole of the cup and side walls. Analyzed samples with chromatographic method and calculated mass median aerodynamic distribution, geometric standard deviation, fine particle distribution, total mass, impactor sized mass (ISM) and fine particle mass < 5 μ value from the CITDAS software by entering the values of formoterol/mometasone delivered in mcg/actuation value.

RESULTS AND DISCUSSION

Method development

Selectivity and recovery: To evaluate selectivity, verified interference from each individual excipient components like oleic acid and ethanol at the concentration greater than expected from the drug product. Accuracy or recovery of active components in presence of active and inactive matrix plays a critical role to undoubtedly assess the level and extent of recovery and extraction efficiency of the method. Inadequacy of recovery leads to non-reproducible and unreliable results, which in turn impacts the quality of product. To assess recovery strength of the method, spiked both target components of mometasone furoate and formoterol fumarate dihydrate at theoretical target concentrations. The spiked component was then recovered using the diluent and determined recovery of both active components. From the observation, there is no interference from excipients and recovery of product observed within acceptable limits.

Minimum number of actuations: Minimum number of actuations is the key factor to determine sample concentration for desired sensitivity of method. As per the inhalation guidance, the maximum allowed actuations are not more than 10. The sample concentration shall be optimized using not more than 10 actuations from the device. Samples prepared with 5, 8 and 10 actuations and verified the response of target active components to attain desired response with respect to formoterol. Formoterol component response was selected as it is the lowest active component in the product. Data collected with different actuations and results are tabulated in Table-1.

TABLE-1
RECOVERY WITH DIFFERENT NUMBER OF ACTUATIONS

Preparation	Collection		
	1	2	3
Number of actuations	5	8	10
Ex actuator FD recovery	86.7	91.5	95.4
Ex actuator MT recovery	88.5	92.3	96.7

Optimization of diluent ratio: Extraction solvent plays a critical role to assess potency of the active components in drug product. Extraction solvent shall be selected based on the solubility of active components and based on formulation matrix. The solvents shall be efficient to extract from formulation matrix and soluble the active components. Based on the nature of excipients using in formulation, the extraction solvent shall be could be with combination of aqueous and organic solvents. Based on the solubility of active and excipients, methanol and water are selected for diluent selection. Multiple combination of water and methanol are tested with formulation and checked recovery factor and peak shape of target analyte peaks. Based on the data observed, selected methanol:water:: 60:40 v/v, respectively as extraction solvent and the results are given in Table-2.

TABLE-2
OPTIMIZATION DATA OF DILUENT RATIO

Preparation	Methanol:water (v/v)		
	60:40	50:50	40:60
Ex actuator FD recovery	100.8	98.2	95.6
Ex actuator MT recovery	99.5	94.3	94.8
Average of 3 preparations for 10 actuations			

Mass balance: Mass balance is the sum of active components deposited at different stages of NGI instrument. As the sample is collected from different stages of NGI, it is important to extract components without losing or sticking to the surfaces. The mass balance also reveals the interstage losses in the next generation impactor. Mass balance shall be calculated as sum of drug collected from all NGI stages, device, induction port and adopters. The sum of drug shall be compared against the label claim of sum of actuations. Mass balance was calculated using the optimized diluent, for both active components, for both ex-valve and ex-actuator. The results observed are within acceptable limit and tabulated in Tables 3 and 4.

Reproducibility: Reproducibility of the analysis is critical parameter to assess consistency of results produced. Verified

TABLE-3
RECOVERY AND MASS BALANCE RESULTS FOR FD IN TEST PRODUCT PREPARATION FROM Ex ACTUATOR AND Ex VALVE

Preparation	FD-Ex actuator			FD-Ex valve		
	Recovery at 50% level	Recovery at 100% level	Recovery at 120% level	Recovery at 50% level	Recovery at 100% level	Recovery at 120% level
Prep-1	98.5	92.3	94.2	100.8	101.3	98.5
Prep-2	97.1	94.8	98.3	97.6	97.9	97.3
Prep-3	95.5	93.5	96.2	94.3	97.2	96.5
Mean recovery	97.0	93.5	96.2	97.6	98.8	97.4

TABLE-4
RECOVERY RESULTS AND MASS BALANCE FOR MT IN TEST PRODUCT PREPARATION FROM Ex ACTUATOR AND Ex VALVE

Preparation	MT-Ex actuator			MT-Ex valve		
	Recovery at 50% level	Recovery at 100% level	Recovery at 120% level	Recovery at 50% level	Recovery at 100% level	Recovery at 120% level
Prep-1	99.3	102.5	100.2	100.2	103.8	97.8
Prep-2	95.4	97.7	96.4	99.4	100.6	99.2
Prep-3	96.8	101.9	98.4	97.2	104.6	96.5
Mean recovery	97.2	100.7	98.3	98.9	103.0	97.8

method reproducibility using three replicate preparations and calculated the individual stage deposition, fine particle dose, fine particle mass and mass balance. Used 3 different canisters for inter canisters, collected data from 2 preparation from each 3 canisters resulting total 6 preparations. The average results from grouping are tabulated in Tables 5 and 6.

Conclusion

The proposed method for quantification of aerodynamic particle size distribution (APSD) for the formoterol fumarate dihydrate and mometasone furoate in inhalation aerosol (MDI) found to be precise, specific and accurate. Trials related to the selection of best chromatographic conditions to achieve consistent and accurate method were discussed. Optimization studies revealed the sensitivity and ability of method to determine APSD of target analyte components. The method can be used for the development lab scale batches as well as for characterization of drug product.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

TABLE-5
REPRODUCIBILITY FROM 3 CANISTERS WITH MASS BALANCE FOR Ex-VALVE AND Ex-ACTUATOR, % FINE PARTICLE DOSE FOR FD

	Delivered label claim: 5.0 mcg/actuation, data for FD					
	Prep-1	Prep-2	Prep-3	Average	SD	%RSD
Valve-stem	0.185	0.181	0.142	0.169	0.024	14.029
Actuator	0.786	0.905	0.720	0.804	0.094	11.666
Induction port	1.988	2.019	2.099	2.035	0.057	2.814
Stage-1	0.079	0.082	0.072	0.078	0.005	6.607
Stage-2	0.201	0.191	0.184	0.192	0.009	4.450
Stage-3	0.769	0.759	0.707	0.745	0.033	4.468
Stage-4	1.031	0.945	0.947	0.974	0.049	5.038
Stage-5	0.328	0.385	0.305	0.339	0.041	12.137
Stage-6	0.108	0.107	0.103	0.106	0.003	2.496
Stage-7	0.046	0.046	0.039	0.044	0.004	9.255
MOC	0.022	0.018	0.019	0.020	0.002	10.585
ISM	2.505	2.451	2.304	2.420	0.104	4.299
Mass balance delivered (mcg)	4.572	4.552	4.475	4.533	0.051	1.130
Mass balance % delivered	91.440	91.040	89.500	90.660	1.024	1.130
Mass balance metered (mcg)	5.543	5.638	5.337	5.506	0.154	2.795
Mass balance % metered	101.000	103.000	97.000	100.333	3.055	3.045
%FPF (5 µm)	43.114	42.442	40.543	42.033	1.333	3.172
MMAD	3.539	3.509	3.535	3.528	0.016	0.462
GSD	1.616	1.629	1.616	1.620	0.008	0.463

TABLE-6
REPRODUCIBILITY FROM 3 CANISTERS WITH MASS BALANCE FOR
Ex-VALVE AND Ex-ACTUATOR, % FINE PARTICLE DOSE FOR MT

	Delivered label claim: 200 mcg/actuation, data for MT					
	Prep-1	Prep-2	Prep-3	Average	SD	%RSD
Valve - Stem	7.222	7.112	6.912	7.08	0.16	2.22
Actuator	28.950	28.740	28.790	28.83	0.11	0.38
Induction port	88.178	88.295	81.097	85.86	4.12	4.80
Stage-1	3.676	6.087	3.706	4.49	1.38	30.81
Stage-2	7.621	7.702	7.575	7.63	0.06	0.84
Stage-3	29.003	28.883	29.806	29.23	0.50	1.72
Stage-4	45.441	48.822	46.877	47.05	1.70	3.61
Stage-5	16.116	16.970	15.269	16.12	0.85	5.28
Stage-6	3.415	3.788	3.538	3.58	0.19	5.31
Stage-7	1.234	1.189	0.931	1.12	0.16	14.62
MOC	0.930	1.054	0.950	0.98	0.07	6.81
ISM	103.76	108.41	104.95	105.70	2.42	2.28
Mass balance delivered (mcg)	195.61	202.79	189.75	196.05	6.53	3.33
Mass balance % Delivered	97.81	101.40	94.87	98.03	3.27	3.33
Mass balance metered (mcg)	231.79	238.64	225.45	231.96	6.60	2.84
Mass balance % Metered	107.81	111.00	104.86	107.89	3.07	2.84
%FPF (5 µm)	42.73	43.34	44.54	43.54	0.92	2.11
MMAD	3.43	3.42	3.45	3.43	0.02	0.51
GSD	1.64	0.70	1.63	1.32	0.54	40.85

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