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Original Article

FORMULATION, EVALUATION AND SOLID-STATE THERMOGRAPHIC CHARACTERIZATION OF CFC FREE BECLOMETASONE DIPROPIONATE PRESSURIZED METERED DOSE INHALATION

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ABSTRACT

Objective: In view of the aim, the objective of the present work was to develop a solution based pulmonary drug delivery system containing Beclometasone dipropionate 250mcg using a Hydrofluoroalkane (HFA 134a and HFA 227ea), a non-chlorofluorocarbon, as propellant to be administered as pressurized metered dose inhalation (pMDI) for direct and targeted delivery of the drug to the lungs and to evaluate the effect of formulation variables on its efficacy and stability.

Methods: In the formulations, 0.015 % oleic acid and 14% of ethanol were used as surfactant and co-solvent respectively. To assure stability of the formulations various quality control tests were performed. Quantitative estimation was done by HPLC method, In-vitro drug deposition studies was carried out using Anderson cascade impactor and the particle characterization was done by using Twin impinge. Thermographic characterization was done by DSC.

Results: The formulation with HFA 227ea was found to be more stable than the formulation containing HFA 134a in three months study period. DF 316 valves with 50µl spraying capacity having pp actuator systems were found to encompass acceptable results for dosage test or pump delivery and leak test with better fine dose respirable fraction. DSC indicated solid-state property of a net respirable fraction and thermograhic characterization of the particle emitted from optimized formulations.

Conclusion: The formulation may be an attempt to develop pulmonary administrable product of Beclometasone dipropionate in form of pMDI using an eco-friendly chlorine-free propellant HFA 227ea as hydrofluoro alkanes were found to be the stable and robust for obtaining the desired attributes to meet the packaging requirements.

Keywords: Beclometasone dipropionate, Pressurized Metered dose inhaler (pMDI), CFC, Hydrofluoro alkane, HFA 134a, HFA 227ea, HPLC, DSC

INTRODUCTION

Asthma sufferers are more than three hundred million in number globally and near about 8% community suffering from asthma and routinely found to be reported in their immedicable stage after 5% expansion of diagnosis of the disease all over the world. Mortality found to be more than seventeen hundred in every 12 months period in United Kingdom [1]. More than 30 million people suffer from asthma in U.K.[2]and interestingly only 7% of the patients reported in the year 1993 [3]. Children death in Britain were also reported to be seventeen hundred per year due to time honored suffering from asthma [4]. In India more than 20million of patients were reported for suffering from asthma in the year 1998 [5] and 20 to 30% asthmatics were found in high human development country of Latin America [6].

Environment and Gene plays a vital role to develop Chronic Obstructive Pulmonary diseases but several other factors are also responsible for the obstruction of airflow to lungs [7]. Bronchodilator, Corticosteroid and Combinational therapy are mainly used to treat this chronic asthma [8]. Metered Dose Inhalation (MDI) therapy, Aerosol therapy has become a prime therapy from 20th century [9] with an advantage of small pocket sized inhaler which is easy to carry for the patients. The aerosol delivery system depends on individual patient characterization and aerosol properties i.e. distribution of particle, particle size, airway layout nature etc [10]. Beclometasone dipropionate is use to treat asthma and the major advance in this therapy is to target the drug directly at the site of inflammation by the development of inhaler so that the enhancement of therapeutic index may be achieved thereby decreasing unwanted side effects without altering the clinical efficacy [11,12,13]. In the formulation of aerosol, the propellant of choice was chlorofluorocarbon due to slow depletion of ozone layer with formation of active chlorine free radical by solar energy which is stable in stratosphere causes high UV radiation transmission [14, 15]. Hence, the chlorofluorocarbon were substituted with non-chlorofluorocarbons, tetraflouroethane (HFA 134a and HFA 227ea) that contains zero chlorine atom and with advantage of null ozone depletion action. Therefore, the present work was to formulate Beclometasone Dipropionate 250mcg MDI in an objective to develop a chlorine free formulation using HFA 134a and HFA 227ea propellant for treatment of asthma and to study the changing of efficacy and stability of the formulation, on variation of formulation variables and reported herein.

MATERIALS AND METHODS

Micronized Anhydrous Beclometasone Dipropionate Propellant HFA 134a and HFC-227ea was given as free sample from Glenmark, Mumbai. DF 316 valves of 50 µl spraying capacity were provided by Valois Ltd, India.

Preparation of Beclometasone dipropionate Pressurized Metered Dose Inhaler

Beclometasone dipropionate 250mcg Pressurized Metered Dose Inhaler (pMDI) was formulated using Modified Pressure Filling Technique [16]. Co-solvent (Oleic acid and ethanol) was weighed (Table 1) and filtered through 0.22µ filter then transfer into closed mixing vessel maintained in ice bath. It was homogenized at 300-400RPM. Then weighed Surfactant was added into mixing vessel with continuous stirring. Anhydrous Beclometasone Dipropionate was accurately weighed, transferred into mixing vessel and stirred at 600-800RPM. And then pour to the container and brassomatic aerosol Crimping Machine used to compress immediately that filled.

The valve of Inverted cylinders of propellant was unlocked and let that flow up to the mark to cylinder to be stored which linked to aerosol filling machine. Then the valve was locked and the valve of the cylinder contained Nitrogen was unlocked to get 15lbs/kg cm^2 pressure. Then that valve was locked and formulation was sonicated for 30 min to get a stable homogeneous aerosol solution.

The temperature was made constant 20^{0}C with humidity bellow 40% for the entire formulation period.

Characterization of Formulations

Spray Pattern

From Pressurized MDI Container the formulation was sprayed on a glass slide which contained mixture dye of activated silica gel. Then spray patterns were observed for shape and the dimension under long UV light [17].

Number of Delivery per Inhaler

From the inhaler canister discharge the content with an interval for more than 5 second by pressing the valve and record the discharge.

Net Content

The container filled with formulated aerosol was weight. Then discharged the whole aerosol by actuating the valve and weigh again. The net content of the aerosol in container was calculated by taking the difference in mg.

Valve Delivery

Clean actuator and formulation filled aerosol container was weighed and actuated to deliver the dose then again weighed and commit to paper. This total method was repeatedly done for 12 containers and average was taken as Valve Delivery [18,19].

Particle Size Distribution

Particle size distribution plays an important role in *in-vitro* performance of the formulation and less than 5µm particle size shows optimum therapeutic activity [20,21]. To determine the particle size formulation were actuated on a glass plate and that evaluated under 100X magnification binocular microscope and size of the particle were determined.

Flame Stretching Test

From 18 centimeter of distance the formulation from pMDI actuated on a flame of a candle over 18-20 seconds and the stretching distance was measured with the help of a ruler [22].

Leak Test

Indiscriminately twelve canisters were selected and date and time was recorded by rounding to the nearest half hour. Every canister was weighed in milligram and noted as W_1. Then canister was kept in invert position in room temperature for 3 days and weighed again and recorded as W_2 after recording of date and time by rounding to the nearest half hour. Time was determined as T hours. According to specification the average leakage rate should not be more than 525mg/year and for a single canister should be less than 750mg/year in case of 15gram net weight of canister. But for a single canister if the leakage is more than 750mg/year and lesser equal to 1.1gram/year, the test should be repeat for another 24 container in addition with the previous 12 container and again to pass this test less or equal to 2 containers may leak 750mg/year out of 36 and not even a single should leak more than 1.1gram/year.

The following expression was used to calculate leakage rate (mg/year)

$$\text{Leakage rate}\left(\frac{\text{mg}}{\text{year}}\right) = \frac{(W1-W2)\times 365 \times 24}{T}$$

Vapour Pressure

The vapour pressure of aerosol in canister was determined by Comes' Pressure Gauge by calculating the average of six reading.

Water Content

The volumetric water content was determined by means of Metrohm Karl Fischer Colorimeter by injecting 2 to 3 actuations of finished product through the gas inlet tube. Colorimetric titration took place at the electrometric end point and the total water content was determined.

Quantitative determination of Beclometasone Dipropionate

Drug content of Beclometasone Dipropionate MDI was investigated by HPLC (Jasco, Japan) with the help of Chrompass software using HiQ Sil C18HSm (150 mm X 4.6 mm, particle size of 5 µm) reverse phase chromatographic column. The MDI was inverted and kept in a glass beaker which contained Acetonitrile (HPLC grade) and water (Mili Q or equivalent) in 60:40 ratios, after shaking the MDI for 30 min. A quantity of spray equivalent to 2.5mg of Beclomethaone dipropionate (10sprays at 15 sec intervals) was dissolved in the mobile phase and dilution was done to 250 ml using the mobile phase then passed through 0.45µm nylon filter and applied with a floe rate of 1 ml/ min for 10 min. Absorbance was measured at 254 nm.

The amount of Beclometasone dipropionate was calculated and in % by using following formula.

$$\%\frac{w}{w}(mcg) = \frac{\text{Area of Sample}}{\text{Area of std.}} \times \frac{\text{Wt of Std.(mg)}}{100} \times \frac{5}{20} \times \frac{250}{10} \times \frac{\%\text{ Potency of Std.}}{100} \times 1000$$

$$\%\ \text{Assay} = \frac{\%\ w/w}{\text{Label Claim}} \times 100$$

Deposition of the Emitted Fine Particulate Dose of Beclometasone Dipropionate

Determination of emitting Fine Particulate dose and Mass Median Aerodynamic Diameter of Beclometasone dipropionate MDI was done according to Indian Pharmacopoeia, 2007 by delivering 10 sprays into the Apparatus after primping 6 sprays in to the waste. At first each stage was washed with 10 ml of Methanol with mobile phase then filtered through 0.45micron filter and injected to analyze using HPLC technique.

Calculate the % of emitted dose of Beclometasone Dipropionate by using formula.

$$\% = \frac{\text{Area of Beclo in Spl.}}{\text{Area of Beclo Std.}} \times \frac{\text{Wt. of Std. in mg}}{100} \times \frac{1}{20} \times \frac{5}{10} \times \frac{100}{10} \times \frac{\%\text{ Potency of Std.}}{100} \times \frac{1000}{250} \times 100$$

Characterization of Particles Emitted from Beclometasone Dipropionate MDI

Beclometasone dipropionate is having typically poor pulmonary targeting characteristics. The solubility of Beclometasone dipropionate was found less than 0.1µg/ml in aqueous solution [23]. Emitted Beclometasone dipropionate droplet size was found significantly lesser than the size of the particle in traditionally formulated chlorofluorocarbon (CFC) MDI and results in high grater lower airway deposition. So, DSC was used to characterize the solid-state characteristics of emitted fine droplet of Beclometasone dipropionate. In this method the modification of 2nd stage of Twin Stage Impinger was done to allow the direct collection of emitted substance on the dose collector which was lined with aluminum foil. The formulation was actuated into Twin Stage Impinger then the collector was removed after dissembling of Twin Stage Impinger and consolidated. Then slowly sample was heated to 350^{0}C in DSC using a DSC 1500 (Mettler Toledo, USA). To prevent thermally induced oxidation, the purge gas (Oxygen free Nitrogen) was injected at an flow rate of 110 ml/min. Indium was used as reference standard to calibrate the temperature and heat flow of the DSC as per manual using aluminum crucible and the graph were coupled with STAR^e software.

Uniformity of Delivered Dose

Determination of uniformity of the dose emitted was done by Indian Pharmacopoeial, 2007 specification. Emitted dose was collected by actuating the valve several time to get the require quantity of 100 ml in a volumetric flask after agitating the MDI vigorously for more than 2 min. then sonicated for 5 min and then filtered by using 0.45µm filter and that sample was analyzed with HPLC technique.

Stability Study

Stability study was done as per ICH Q1A (R2) guidelines by withdrawing the sample initially, after 1 month, 2 month, and 3 months and the MDI formulation were analyzed for spray pattern,

number of delivery per inhaler, net content, fine particle dose, mass median aerodynamic Diameter, water content, deposition of emitted dose and was assayed by HPLC method described previously.

RESULTS AND DISCUSSION

Beclometasone dipropionate metered dose inhaler were formulated using 0.015% of Oleic acid as surfactant and 14% ethanol was used as co-solvent in chlorine free, non-CFC propellant Hydrofluroalkanes i.e. HFA 134a and HFA 227ea (Table.1).

Table 1: **It shows the compositions of various formulations**

Ingredients	F_1	F_2
Anhydrous Beclometasone dipropionate	0.043 %	0.043 %
Ethanol	14 %	14 %
Oleic Acid	0.015 %	0.015 %
Propellant HFA 134 a	85.50 %	-
Propellant HFA 227 ea	-	85.65 %

Spray pattern, an important parameter to evaluate valve and actuator performance depending on droplets distribution.

The average diameters of the spot were 1.48 ± 0.04 cm for the formulation F_1 and 1.47 ± 0.02 cm for the formulation F_2 indicating similar valve and actuator performance for both the formulations and under UV for both the formulations particle was found round to oval spot with violet centre (Table.2).

The total number of deliveries discharged from the inhaler for both the formulations found was not less than 200 deliveries and the net content was found in between 13.6-14.0 g (Table.2).

Therapeutic action of MDI is administered by the deposition of emitted dose in terms of particle size distribution. From the analysis of data, Fine Particle Dose was found 74.9020mcg for formulation F_2 which was higher than F_2 i.e. 72.3600mcg and similarly the Fine Particle Fraction was found higher in F_2 (39.64%) than F_1 (35.16%).

Mass Median Aerodynamic Diameter of particle F_2 formulation was found little higher (1.9346µm) than F_1 (1.9054µm).In both the formulations Geometric Standard Diameter of particle was found bellow 3µm in diameter but F_2 formulation Geometric Standard Diameter of particle was found little higher (2.1280µm) than F_1 (2.0839µm) (Table 3, Figure 1, Figure 2, Figure3). So, formulation F_2 would provide better pulmonary deposition as compared to F_2.

Table 2: **It shows the evaluations of various formulations**

Formulation	Spray pattern		Total no. of delivery ±S.D.	Net content (g) ±S.D.	Valve delivery (mg) ±S.D.	Flame extension test	Leakage rate	Vapour Pressure (psi)	Water content (ppm)	Content per actuation ±S.D.
	Avg. diameter ±S.D.	Description								
F_1	1.48 ± 0.04	Round to oval spot with violet centre	203 ±0.76	13.63 ±0.43	62 ±3	FQ	NCW	80-83	NMT 2500	102.21 ±0.5
F_2	1.47 ± 0.02	Round to oval spot with violet centre	206 ±0.92	14.05 ± 0.71	64±2	FQ	NCW	83-85	NMT 2500	103.16 ±0.32

S.D.: standard deviation, FQ: Flame Quenched, NCW: No Change in Weight, Valve delivery for the F_2 (64 ± 2 mg) was found higher than the F_1 (62 ± 2 mg) that was due to the high internal pressure of the HFA 227 ea (Table.2).

Table 3: It shows the Fine Particle Dose, Mass Median Aerodynamic Diameter and Geometric Standard Diameter Analysis

Analysis Setting	Value	
	F_1	F_2
Fine Particle Fraction Summation group	Throat to Filter	Throat to Filter
Fine Particle Dose (FPD) (mcg)	72.3600	74.9020
Fine Particle Fraction (%)	35.16	39.64
Mass Median Aerodynamic Diameter (µm)	1.9054	1.9346
Geometric Standard Diameter (µm)	2.0839	2.1280

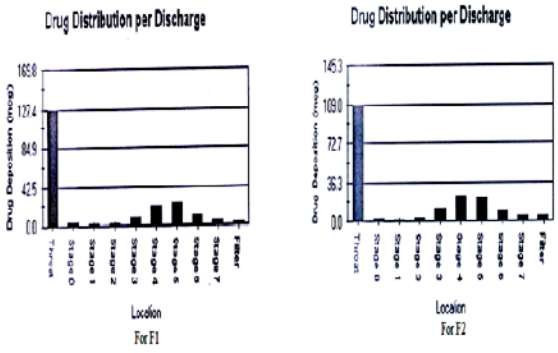

Fig. 1: It shows the drug deposition v/s location plot

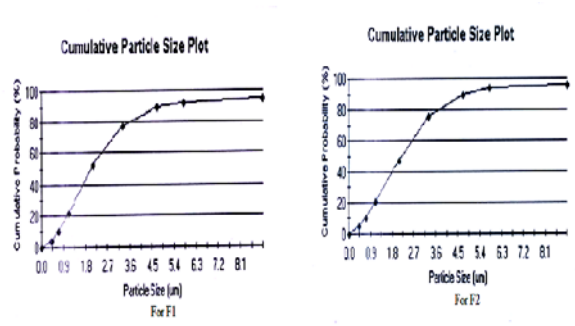

Fig. 2: It shows the cumulative probability v/s particle size plot

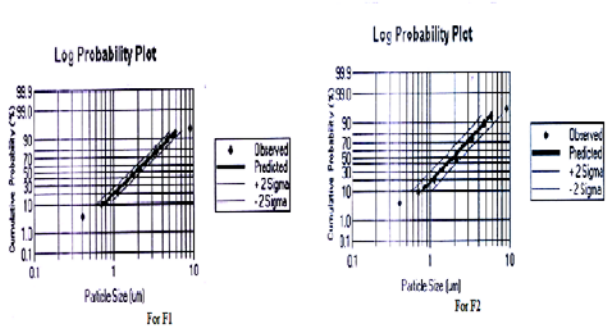

Fig. 3: It shows the cumulative probability v/s particle size log probability

The Flame Expansion test proved the formulation as Non-Inflammable. The Leak test data also demonstrate that both formulations were leak proof as there were no changes in the weight. Internal pressure governs the emitting of proper dose in form of fine spray from the valve and the internal pressure was found a little higher for the formulation content HFA 227 ea as propellant (F_2) ie 83-85 psi than formulation content HFA 134 a as propellant (F2) i.e. 80-83 psi (Table.2).

So, DF 316 valves of 50 µl spraying capacity having pp actuator systems were found to encompass acceptable results for dosage test or pump delivery and leak test.

The expected water content in inhalation was in ppm level. So to determine this low water content (0.1% to 0.0001%), volumetric titration method was applied and water content for the both formulations were found NMT 2500 ppm (Table 2). Thus, both the formulations would suppose to provide better pulmonary deposition and stabilization of formulations.

The drug content released in each actuation from the value were found within the limit specified in Indian Pharmacopoeia, 2007 (Table 2) for the both formulations and chromatograph (Figure 4) demonstrate the drug content 97.6% in F_1 and 101.3% in F_2.

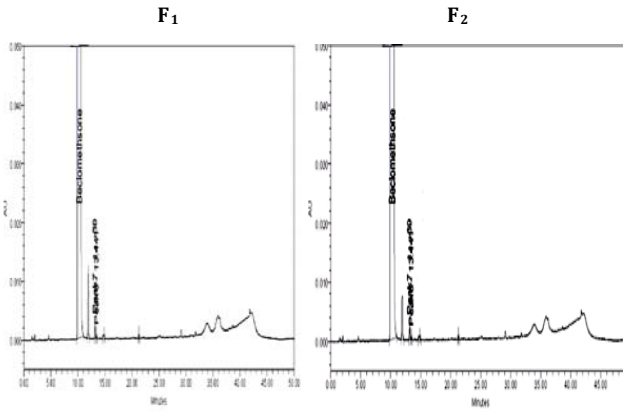

Fig. 4: It shows the chromatogram of assay

In-vitro drug availability of aerosolized formulations in terms of therapeutic efficacy supposes to be monitored by a critical factor, The Deposition of Emitted Dose. Drug deposition results of both formulations were shown in Figure 5. The net Respirable Fraction of the formulation contained HFA 227 ea as propellant (F_2) was 35 ± 0.34% and for formulation contained HFA 134a as propellant (F_1) was 33 ± 0.28%, by Cascade impactor, indicating higher pulmonary deposition for formulation F_2. So, the performance of the formulation content HFA 227ea as propellent was found better as compared to the formulation content HFA 134a as propellent.

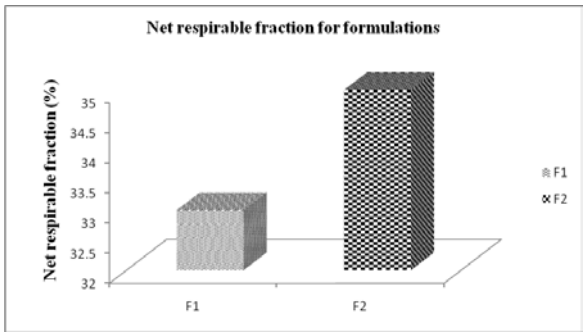

Fig. 5: It shows the drug deposition studies for developed formulations

Single endothermic peak was found at an onset of 211^{0}C that corresponded to the Melting Transition (T_m) of reference anhydrous Beclometasone dipropionate powder (Figure 6).

No glass transition (T_g) or exothermic re-crystallization transition on thermograph implied that principle constituent of the material was crystalline in nature and no or little amorphous in nature.

Under slow DSC heating the collected material from meter dose inhaler (Beclometasone dipropionate) was found an endothermic transition region at starting of 208^{0}C (F_1) and 209^{0}C (F_2) which was equivalent to the melting point of corresponding crystalline anhydrous Beclometasone dipropionate. With the increased number of spray from 60 to 100 by actuating the valve into the twin stage impinger collection unit, the exothermic peak and increased intensity of melting endothermic peak appeared in thermograph due to concurrent desolvation of the solvate followed by re-crystallization to an anhydrous crystalline lattice. In hyper DSC, the thermograph of Beclometasone dipropionate metered dose inhaler resembled the Desolvation- Recrystallization hypothesis as a single endothermic peak was found at an onset of 208^{0}C (Figure 7) in the thermal profile of reference anhydrous Beclometasone dipropionate.

The material collected from the formulated both Beclometasone dipropionate metered dose inhalers was not found any thermal transition in comparison to the reference melt endothem but a large endothermic peak was found at an onset of 150^{0}C (F_1) and 151^{0}C (F_2) with no exothermic peak. No melt endothermic peak signified that no crystalline amorphous form was found when sample was collected from Beclometasone dipropionate MDI.

The results of solid-state characterization by DSC of the sample emitted from the both formulated Beclometasone dipropionate metered dose inhalers showed that the anhydrous Beclometasone dipropionate was having a transition solvate particulate property (Figure 6 & Figure 7).

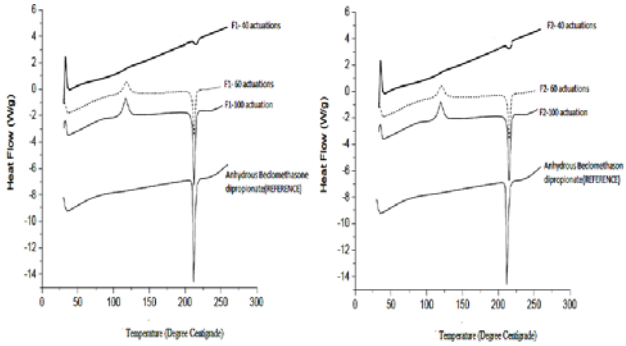

Fig. 6: It shows the DSC thermogram of sample emitted from formulated Beclometasone dipropionate metered dose inhalers

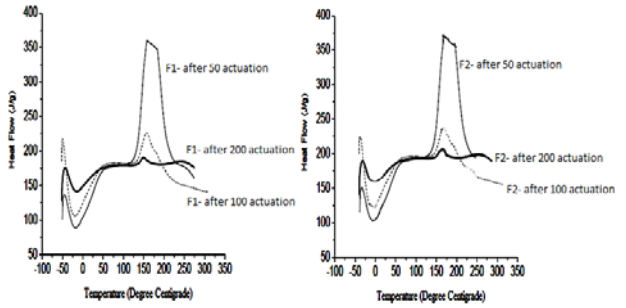

Fig. 7: It shows the hyper DSC thermogram of sample emitted from formulated Beclometasone dipropionate metered dose inhalers

The content of the both formulations were uniform till the last dose and the results are depicted in the table (Table 4). From the stability studies for 3months as per ICH guidelines proved that there was no significance change in dissolution profile and other parameter of the optimized formulations. So, F_1 and F_2 formulations were found to be in an acceptable limit (Table 4).

Table 4: It shows the stability study

Formulation	Stage	Spray pattern		Total no. of delivery ±S.D.	Assay	Fine Particle dose (mcg)	Mass Median Aerodynamic Diameter (µm)	Moisture Content (ppm)	Deposition of emitted dose (%)	Uniformity of content (%)
		Avg. diameter ±S.D.	Description							
F_1	Initial	1.48 ± 0.040	Round to oval spot with violet centre	203 ±0.76	97.6	72.3600	1.9054	NMT 2500	33 ± 0.28	91.5 ± 0.21
	1 M	1.48 ± 0.024	Round to oval spot with violet centre	203 ±0.98	99.5	72.5972	1.4078	NMT 2500	32 ± 0.31	89.3 ± 0.29
	2 M	1.48 ± 0.041	Round to oval spot with violet centre	203 ±0.71	100.7	71.9810	1.5313	NMT 2500	33 ± 0.22	88.5 ± 0.73
	3M	1.48 ± 0.032	Round to oval spot with violet centre	203 ±0.73	100.9	72.5600	1.6547	NMT 2500	32 ± 0.19	90.5 ± 0.30
F_2	Initial	1.47 ± 0.022	Round to oval spot with violet centre	206 ±0.92	101.3	74.9020	1.5833	NMT 2500	35 ± 0.34	94.26 ± 0.64
	1 M	1.47 ± 0.022	Round to oval spot with violet centre	206 ±0.55	103.3	74.8051	1.8415	NMT 2500	34 ± 0.21	90.5 ± 0.51
	2 M	1.47 ± 0.012	Round to oval spot with violet centre	206 ±0.45	102.9	74.7653	1.9131	NMT 2500	34 ± 0.46	91.8 ± 0.32
	3M	1.47 ± 0.036	Round to oval spot with violet centre	206 ±0.76	104.9	73.981	1.9981	NMT 2500	35 ± 0.33	90.3 ± 0.22

CONCLUSION

Hence it was concluded that pressurized based MDI formulation in an attempt to develop pulmonary administrable product of Beclometasone dipropionate was found to be stable and robust with desired attributes.

CONFLICT OF INTERESTS

Declared None

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