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Metered Dose Inhalation Formulations of Salbutamol Sulphate Using Non-CFC Propellant Tetrafluoroethane

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ABSTRACT
Salbutamol sulphate metered dose inhaler formulations were developed with chlorine free, non-CFC propellant Tetrafluoroethane. Suspension formulations were prepared with 4 mg of oleic acid as the surfactant and varying proportions of ethanol as the co-solvent. Developed formulations were subjected to various qualitative and quantitative tests to ensure proper stability of the final package. In-vitro drug deposition studies using Twin impinger and Anderson cascade impactor indicated a net respirable fraction of 34-36.7% for the optimized formulation. Stability studies showed that the formulation was stable for a period of three months under all storage conditions. Effects of different adaptors and metering valve designs on the performance of the formulation were also checked. Comparison of optimized formulation with commercial CFC and non-CFC formulations exhibited equivalent performance characteristics. Thus, Tetrafluoroethane proved to be an environment friendly substitute for CFC propellants in metered dose inhalation formulations.

Keywords: Asthma, Salbutamol sulphate, Tetrafluoroethane.

INTRODUCTION

A wide variety of population around the globe is affected by Asthma, a chronic respiratory disease of lungs. Although the therapeutic agent for asthma could be delivered by oral and injectable routes, faster action is achieved via the pulmonary route. The pulmonary route of treatment offers many advantages like the vast permeability of lungs and profuse vasculature of airways. The convenience of self-administering the dose of drug accurately and quickly to the pulmonary region has made pressurized metered dose inhalers the treatment of choice for asthma. [1]

Drugs in aerosol formulations are either dissolved or suspended in the propellant for delivery to the lungs. [2] Most of the metered dose inhalers are marketed as suspensions due to their wide applicability and stability. Moreover, the number of drugs that are freely soluble in the propellant and co-solvent mixtures are limited. Typically, suspension formulations contain a mixture of micronized drug, a surfactant, a co-solvent and propellant. In suspension formulations, particle size of the drug is critical because it influences pulmonary deposition and also affects suspension stability. Optimum particle size for deposition into the pulmonary system is less than or equal to 5 µm and not more than 10 µm. [3]

Salbutamol Sulphate, a β-2 receptor agonist has been administered by oral and inhalation routes for asthma treatment. Standard dose of the drug is 2 to 4 mg and given to the patient 3 to 4 times a day. [4] The inhalable dose of Salbutamol Sulphate is found to be lower than the oral dose because of evasion of presystemic metabolism and direct delivery to lungs.

Chlorofluorocarbons were the propellants of choice for marketed inhalation aerosols. Although CFC's were stable in lower atmosphere of earth, in the stratosphere, slow degradation by solar radiation resulted in damage to ozone layer by formation of free radicals of chlorine. [5] Destruction of ozone layer allowed increased transmission of ultraviolet radiation to earth's surface. Hence, the use of Chlorofluorocarbon propellants was halted as per the Montreal protocol, 1987. [6] Non-chlorofluorocarbons like Tetrafluoroethane (HFA-134a) with the absence of chlorine atoms and zero ozone depleting potential can effectively substitute the conventional CFC propellants. Hence, the objective of present work was to develop Salbutamol sulphate metered dose inhalation suspension formulations using non-CFC propellant Tetrafluoroethane for treatment of asthma.

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MATERIALS AND METHODS

Materials

Micronized Salbutamol Sulphate was obtained from Cipla Ltd, Mumbai. Anodized aluminium Canisters of 20 ml capacity were procured from Midas Health care, Mumbai, India. Metered Dose valves of 63 µl capacity were provided by Valois Ltd, India. Propellant HFC-134a was obtained as a gift sample from Stallion enterprises, Mumbai, India. All other reagents and chemicals used in the study were of analytical grade.

Preparation of Salbutamol sulphate metered dose inhalers using Tetrafluoroethane

Salbutamol sulphate MDI formulations were prepared using pressure filling technique. The required quantity of Oleic acid and 95% ethanol were mixed properly in an enclosed vessel and maintained in an ice-bath. Salbutamol sulphate was added into each canister and they were immediately crimped using Brassomatic aerosol crimping machine with 20 mm neck diameter crimping collet. Tetrafluoroethane (HFC-134a) cylinder was kept in an inverted position and connected to Brassomatic aerosol filling machine. The valve was opened and propellant was allowed to flow into the storage cylinder upto the desired level. The valve was then closed tightly. Nitrogen cylinder valve was opened and pressure was built upto 15 lbs/kg cm^2. The valve was closed after achieving the desired pressure. The canisters were kept in sonicator for half an hour to achieve a uniform, well dispersed suspension. The whole operation was carried out at a temperature maintained to about 20-21°C due to the ease in propellant handling and humidity below 40% RH to avoid breakdown of propellant.

Quality control tests on Metered dose inhalers

Qualitative tests based on aerosol performance

- **Spray pattern:** The spray delivered from the formulations was impinged onto a glass plate containing activated silica gel-dye mixture. The MDI was held at a distance of 3 cm from the plate. The spots thus formed as a result of spray testing were observed under long wave U.V light for their characteristics. [7]
- **Particle size distribution:** Binocular Labomed vision 2000 TM microscope was used for particle size determination. Metered dose inhalers under evaluation were sprayed on a glass slide. The slide was rinsed with CCl_4 to prevent the excipient particles from interfering with the measurement of drug particles. After sufficient rinsings with CCl_4, the slide was placed under the microscope and particles were measured by using 100X magnification with oil immersion method. At least 100 particles in 25 different fields were measured. Results were reported as number of particles equal to 3 µm and those greater than and in between 3 to 5 µm.
- **Flame extension test:** Metered dose inhalers were held at a distance of 18 cm from the flame of candle. They were sprayed for 15-20 seconds and flame extension was measured with help of a ruler. [8]
- **Leak test:** Metered dose inhaler containers were weighed and inserted in water bath, maintained at 50ºC. After equilibration, containers were checked for presence of leaks in the form of air bubbles arising from orifice or valve crimp. These containers were wiped clean with a tissue paper and their weights were recorded (W1). The containers were kept in upright position for 3 days and were weighed at the end of third day (W2). Leakage rate was calculated as:

  Leakage rate: 365*24/ T (W1-W2), Where T= Time in hrs
- **Vapour pressure:** Vapour pressure was measured by means of 'Comes Pressure gauge' and average of six readings was recorded.

Quantitative tests for metered dose inhalers

- **Average weight per metered dose:** Canisters were first detached from the adaptor body and their weights were determined. The first five sprays were fired in air and were referred to as 'Test Firing'. After the test fire, the canisters were thoroughly wiped with a tissue paper and their weights were recorded (W1). Five successive deliveries were sprayed from the inhaler after placing the canisters back in their actuators. The canisters were subsequently removed from the adaptor and the valve stem and orifice were wiped clean. The containers were weighed again and their weights were recorded (W2).

  Avg weight per metered dose = (W1-W2)/5
- **Content per spray:** The container was supported inside a beaker containing 75 ml of distilled water. Individually 10 doses were delivered inside distilled water with intermittent shaking for 5 seconds after each actuation. These solutions were introduced into 100 ml volumetric flasks and were diluted to 100 ml with distilled water. 10 ml of above solutions were transferred to 25 ml volumetric flasks to which 1 ml of 4-aminoantipyrine, 2 ml of potassium ferricyanide, 1 ml of sodium carbonate were added and volume was made to 25 ml with distilled water and the absorbance was recorded at 505 nm using V-550 JASCO U.V/Vis spectrophotometer.
- **Retention on adaptor:** Amount retained on the adaptor represented the wasted drug which was not available for inhalation and should be restricted to minimum. Initially 10 deliveries were fired in the exhaust. The components of MDI were separated and washed with the collecting liquid (distilled water). After through rinsing of canister, valve, adaptor, orifice and mouthpiece, the solution was collected in a 100 ml volumetric flask and analyzed for the content of active ingredient retained on the adaptor.
- **Content uniformity:** The uniformity of content of active ingredient in 10 doses was determined using the method similar to that mentioned in the content per spray test for MDI's. In order to check extent of variation, 1^{st}, 25^{th}, 50^{th}, 100^{th}, 150^{th}, 200^{th}, 225^{th} doses sprayed from the device were analyzed for the content of active ingredient in each spray.

In-vitro drug deposition studies

Pulmonary deposition pattern of the developed formulations was determined using two different impactor apparatus i.e. Twin impinger apparatus (Copley Scientific Ltd, U.K) and Multistage Anderson Cascade Impactor (Type ACI-MDI 1000, Erweka)

- **Twin impinger:** The two collecting chambers were filled with required amount of distilled water (7 ml in stage I and 30 ml in stage II). MDI was attached to the device by a rubber collar and 10 sprays were fired into the apparatus. Side arm tube was connected to vacuum pump with flow rate of 60 l/min which mimicked the respiratory flow in normal patients. Gap of 5 seconds was maintained between two successive sprays and MDI was thoroughly shaken before each spray was fired. The reservoirs were rinsed with distilled water and amount of

drug deposited on adaptor, valve and collar (Device, Stage I and stage II) was determined. The deposition of drug at the device represented the non-respirable fraction. [9-10]

- **Anderson cascade impactor:** Eight perforated plates were arranged in ascending order of their diameter with '0^{th}' plate on the top and '7^{th}' plate on the bottom. Cascade impactor plates were placed below each of these and the base plate was connected to vacuum with flow rate of 28.3 l/min. An induction port was connected to the top of the impactor. The metered dose inhaler was attached to the device by means of rubber collar. Exactly 10 sprays were fired in a manner similar to that for twin impinger. Each plate was rinsed with distilled water and amount of drug deposited on each plate was determined. Deposition of drug from stage 2 to filter was termed as "Respirable fraction". [11]

Evaluation of effect of adaptor and metering valve design on performance of the formulation

Adaptor A with orifice diameter of 0.48 mm and adaptor B with orifice diameter 0.33 mm were chosen for the study and drug deposition from the optimized formulation F1 was compared. Metering valves of 63 µl capacity obtained from two companies 'Beespak' and 'Valois' were coded as V1 and V2 respectively. The effect of metering valve was studied using different quality control tests like spray pattern, average wt per metered dose and content per spray.

Stability studies

Formulation F1 was kept at 8°C, room temperature and 40°C for a period of three months. Chemical stability was assessed by monitoring the percent drug remaining in the formulation. Physical stability of the formulation was evaluated based on pH, vapour pressure, particle size distribution, leakage rate, redispersibility and average weight delivered per actuation.

Comparison of CFC and non-CFC formulations

Salbutamol sulphate formulation F1 was compared with marketed CFC (C1) and non-CFC (H1) formulations. The formulations were compared with respect to their spray pattern, particle size distribution, vapour pressure, content uniformity and drug deposition pattern.

Table 1: Composition of developed Salbutamol sulphate formulations

Ingredients (g)	Formulations	
	F1	F2
Salbutamol sulphate equivalent to Salbutamol	0.020	0.020
95% Ethanol	2	4
Oleic acid	0.004	0.004
Tetrafluoroethane (HFA-134a)	15	15

RESULTS AND DISCUSSION

Salbutamol suspension formulations were prepared using 4 mg of oleic acid as the surfactant and 2-4 gm of 95% ethanol as the co-solvent (Table.1). Developed formulations were subjected to various qualitative tests to ensure proper performance of the final package (Table.2). Spray pattern indicated output droplet distribution pattern and was an important parameter for evaluating valve and actuator performance. The average diameter of the spots were found to be 1.5 cm for both F1 and F2 indicating similar output droplet sizes for both the formulations. Particle size distribution governs the deposition of emitted dose from MDI and hence its therapeutic performance. From particle size distribution data, it was seen that for F1 formulation most of the particles i.e 96-97% were less than 3 µm in diameter but in case of formulation F2 only 90% particles were below 3µm. Thus, formulation F1 would provide better pulmonary deposition as compared to F2. The results of flame extension test indicated that the formulations were non-flammable. Results of leak test showed no change in weight indicating that the formulations F1 and F2 were leak proof. Proper development of pressure inside the canister was essential so that desired quantity of the contents are propelled into the metering valve and expelled outside as fine spray. [12] The internal pressure was found to be in suitable range of 70-80 psi for both F1 and F2 formulations.

Several quantitative tests were conducted on the developed formulations (Table 3 and Fig.1). It was observed that the average weight delivered per metered dose was 76 mg for both the formulations indicating effectiveness of the valve system in delivering reproducible amount of formulation per actuation. The content per actuation of the valve for both the formulations was found to be within the pharmacopoeial limits as per the test in I.P 1996 (NLT 80% of the label claim and NMT 120 % of the label claim). Formulation F1 containing 2 ml of ethanol exhibited low standard deviation for most of the quantitative tests indicating better uniformity than formulation F2.

The deposition of emitted dose was a critical factor governing the therapeutic efficacy of the metered dose inhalers. This test was performed to predict the in-vitro availability of active ingredient from the aerosolized product. As seen from Fig. 2, the net respirable fraction for F1 was in the range of 34 to 36.7% while for F2 it was from 20-22%, indicating higher pulmonary deposition for formulation F1.

The results of drug deposition studies from two different adaptors A and B were as shown in Fig. 3. Net respirable fractions for Adaptor A were 35.97% and 36.7% and for Adaptor B were 19.8% and 26.2% by Twin impinger and Cascade impactor respectively. Hence, it was concluded that the formulation performance with Adaptor A was better as compared to adaptor B due to the larger pore size of the former. The results of quality control tests for Valves V1 and V2 were found to be comparable indicating that both the valves V1 and V2 were efficient with equivalent performance characteristics (Table.4).

Formulation F1 was selected for stability studies based on initial results from quality control tests (Table 5). The content per actuation from formulation F1 stored at all temperatures was found to be within the pharmacopoeial limits. Formulation stored at room temperature showed average content per spray of 104.72% while at 8°C and 40°C it showed 104.83% and 104.42 % average content per spray. This indicated there was no degradation of the drug at all a storage conditions. The results of particle size distribution studies indicated no significant change at all storage conditions indicating absence of agglomeration or crystal growth. Optimum vapour pressure was maintained under all conditions. Leakage rate was within the pharmacopoeial standards as per the test in I.P 1996. Average weight delivered per dose was found to be 76.04, 76.01 and 76.05 mg at 8ºC, R.T and 40ºC respectively indicating effectiveness of valve system in delivering reproducible amount of drug content throughout the storage conditions.

Comparative data of formulations F1, H1 and C1 were as shown in Table. 6; Fig. 4 and 5. Particle size distribution and content per spray were comparable for all the formulations.

Table 2: Qualitative tests on Salbutamol sulphate formulations

Formulations	Spray Pattern± S.D		Particle size distribution ± S.D			Flame extension test	Leakage rate	Vapour pressure (psi)
	Avg diameter (cm)	Description	< 3µm	3-5 µm	S.D			
F1	1.5± 0.258	Round spot, distinct violet color	97	3	±0.23	FE	No change in weight	80-85
F2	1.5 ± 0.047	Round to oval spot, violet center	90	10	±2.18	FE	No change in weight	80-85

S.D: Standard deviation, FE: Flame extinguished

Table 3: Quantitative tests for developed formulations

Formulations	Avg weight per metered dose (mg)	Content per spray± S.D	Retention on adaptor (%)± S.D
F1	76.0	103.96±1.49	10.25±0.1008
F2	76.1	101.85± 3.52	11.13±0.6071

S.D: Standard deviation

Table 4: Evaluation of metering valve performance

Metering valves	Spray pattern		Avg wt per metered dose ± S.D	Content per spray ± S.D
	Avg diameter (cm)	Description		
V1	1.5	Round to oval spot with violet centre	76.46 ±0.254	104.64± 0.53
V2	1.5	Round to oval spot with violet centre	76.51± 0.249	104.96± 0.531

S.D: Standard deviation

Table 5: Stability testing

Tests		Stability temperature		
		8ºC	Room temperature	40ºC
% Drug delivered per spray ± S.D		104.83±0.935	104.72±0.872	104.42±0.986
pH range		5-6	5-6	5-6
Vapour pressure (psi)		80-85	80-85	80-85
Particle size distribution	< 3 µm	96.2	96.8	96.6
	3-5 µm	2.8	3.2	3.4
Avg wt per metered dose (mg) ± S.D		76.041±0.051	76.012±0.032	76.051±0.0699
Leakage rate (%)		0.004	0.0039	0.0079

S.D: Standard deviation

Table 6: comparative evaluation of optimized and marketed formulations

Formulations	Spray Pattern± S.D		Particle size distribution ± S.D			Content per spray	Vapour pressure (bar) at 25°C
	Avg diameter (cm)	Description	< 3µm	3-5 µm	S.D		
F1	1.44	Round circular spot, violet color	96	4	0.28	104.60±2.897	5.7
H1	1.5	Round spot, dark violet center	97	3	0.26	105.27±2.309	5.7
C1	1.2	Round uniform spot, dark violet center	96.8	3.2	0.20	104.67±2.268	3.4

S.D: Standard deviation

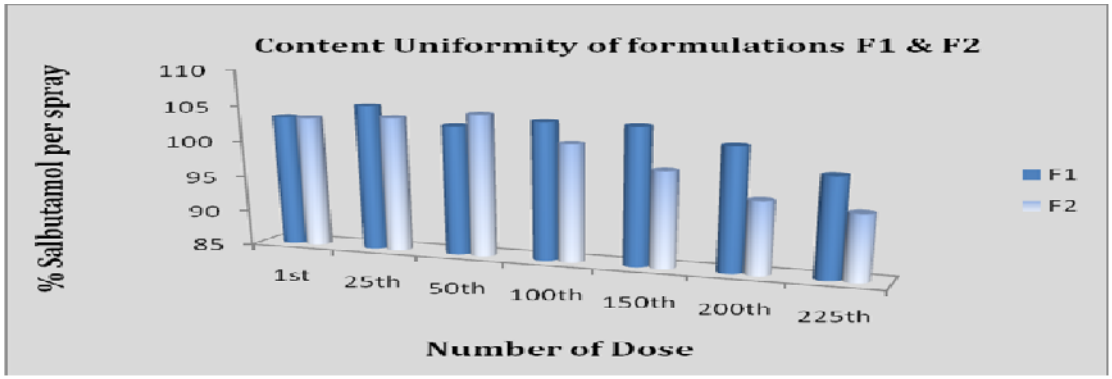

Fig. 1: Content uniformity of Salbutamol sulphate MDI suspensions

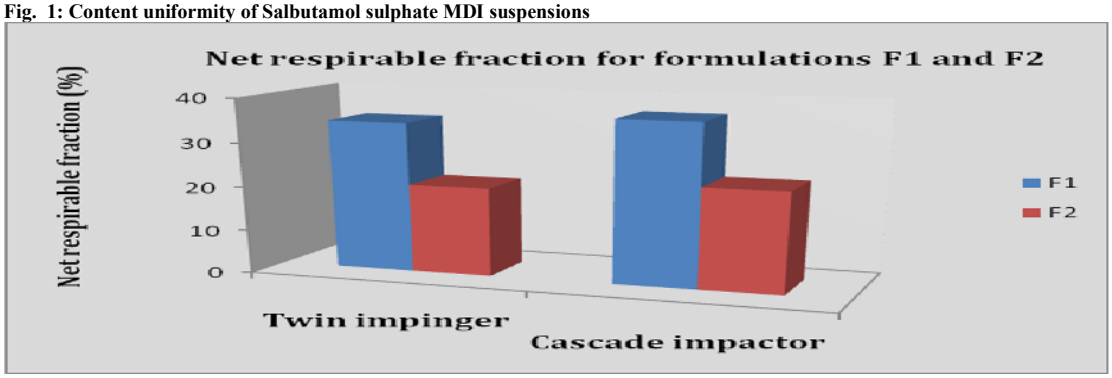

Fig. 2: *In-vitro* drug deposition studies for developed formulations

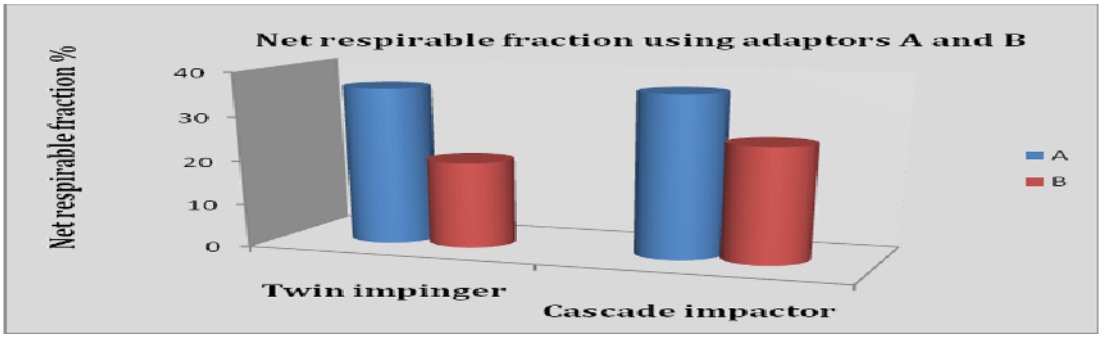

Fig. 3: *In-vitro* drug deposition studies for different adaptors

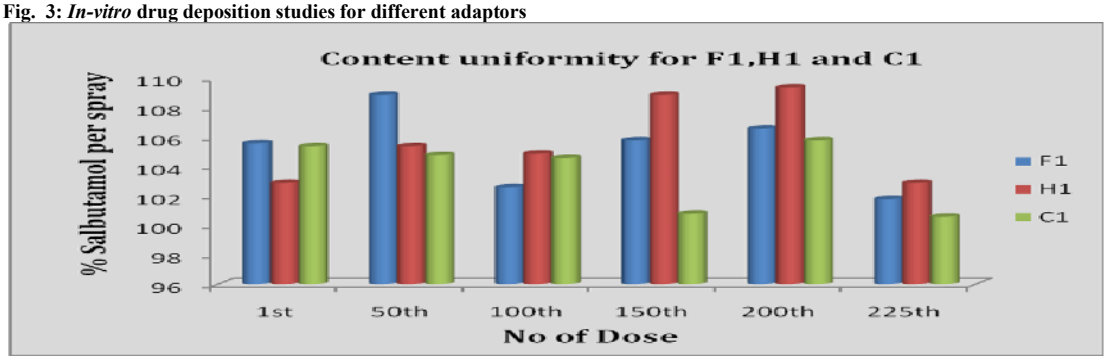

Fig. 4: Content uniformity of optimized and marketed formulations

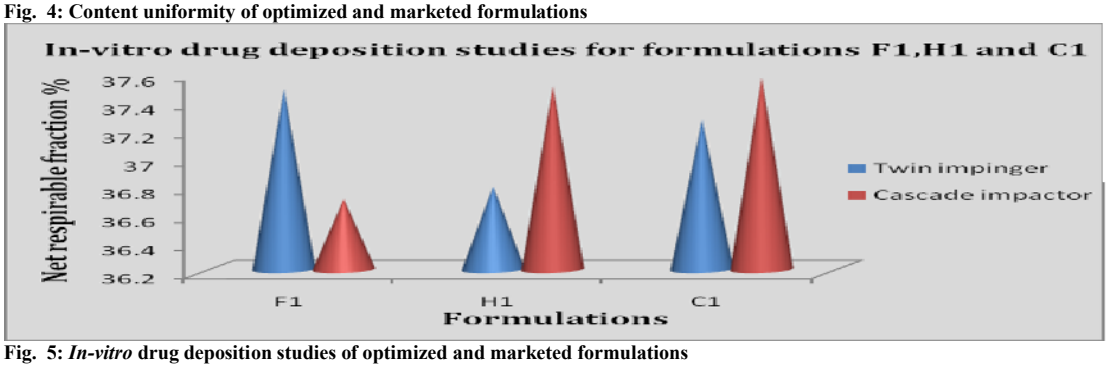

Fig. 5: *In-vitro* drug deposition studies of optimized and marketed formulations

Also, the uniformity of content was maintained for all the formulations till the last dose. Lesser diameter of droplets delivered by C1 in case of spray pattern test was due to the low vapour pressure of propellant P-12 used in that formulation. Net respirable fractions for all the formulations were around 36.9% indicating equivalent performance.

Metered dose inhalation formulations using non-CFC propellant Tetrafluoroethane were developed and optimized. Metered dose inhalation formulations containing Salbutamol Sulphate with Tetrafluoroethane were found to possess the desired properties as indicated by the results of qualitative and quantitative tests and in-vitro drug deposition studies. The orifice diameter of the adaptor was found to have a great impact on net respirable fraction. Tetrafluoroethane based formulation showed equivalent performance characteristics in comparison with marketed formulations and could be used as an environment friendly substitute for CFC propellants in metered dose inhaler formulations.

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