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OPTIMISATION OF SALBUTAMOL SUSPENSION AS METERED DOSE INHALATION (MDI) AND COMPARISON WITH A MARKETED MDI SUSPENSION

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ABSTRACT

The direct delivery of therapeutically active drug to the pulmonary tract has proved to be a life saving tool for many Asthmatics. Salbutamol being a drug of choice in severe Asthma attacks, in the present study attempt was made to prepare MDI of Salbutamol in suspension formulation and compare it with the marketed MDI of salbutamol suspension. The proportion of C.F.C. propellant blend (p11 & p12) in 30:70 proportion gave optimum vapor pressure; in the range of 70-75 psig was selected. Oleic acid was selected as a surfactant and its concentration was optimized. A series of physicochemical tests were performed simultaneously on both the preparations. Results were compared. The formulations were further subjected to in-vitro evaluation whereby net respirable fraction was determined. Logic behind this study was to create a better suspension than that available in the market. In all qualitative as well as quantitative tests the formulated suspension was comparable with marketed MDI. However our formulation retained minimum amount of drug (8.57%) on adaptor compared to the marketed preparation (10.40%). This shows the maximum availability of the drug in the pulmonary tract.

INTRODUCTION: Delivering small doses of active ingredient directly to the lungs effectively localizes the drug thereby maximizing therapeutic effect while minimizing unwanted side effects. In the treatment of pulmonary disorders and in some cases, for systemic action, inhalation is the preferred route of administration. Bronchodilators [1], anti-inflammatory agents [2], mucolytics, antiviral agents [3] and phospholipids protein mixtures for surfactant replacement therapy are all routinely given as aerosolized formulations. Recently pentamidine has been delivered via the lung to treat *Pneumocystis carinii* pneumonia associated with AIDS [4.] The development of potent protein drugs by biotechnology has also stimulated a growth of interest in inhalation aerosols because of the possibility of systemic delivery of these drugs via the airways [5.]

Metered Dose Inhalations (MDI) are fine dispersions of solid particles in gas packed in pressurized containers. The drug is expelled by a metered valve in a prefixed volume from a volatile propellant. Currently accepted theory of asthma development considers it multifactorial in origin and progression and centers on the clinical triad of bronchial inflammation, hyper-reactive smooth muscle activity and reversible airflow obstruction. The changes that occur with asthma are primarily the result of inflammatory events with the airway.

These events include cellular infiltration and release of inflammatory mediators such as histamine; prostaglandins D and F; thromboxane; leukotrienes C4, D4, and E4; platelet activating factor; bradykinin, adenosine; substance P; neurokinin A and serotonin. These substances all produce bronchoconstriction. Many of these mediators produce at least part of their effect by directly acting on bronchial smooth muscles; while some produce at least part of their broncho-constriction through stimulation of afferent cholinergic receptors [6]. The etiology of asthma is poorly understood, but resistance to airflow is increased by a number of factors including constriction of the airways smooth muscle, the lumen and thickening of the airway epithelium [7].

The principal symptom of Asthma is dyspnea (breathlessness), wheezing, cough and chest tightness. For immediate action of the drug, it should be distributed uniformly in the lungs. Salbutamol or Albuterol is a short-acting β2-adrenergic receptor agonist. β- Agonists are functional antagonists of bronchoconstriction mediators in that they reverse smooth muscle contraction regardless of the stimulus. Salbutamol is used as bronchodilator in the management of disorders involving reversible airway obstruction such as Asthma and in some patients with chronic obstructive airway diseases. Salbutamol (Albuterol) is (RS) - 1- (4-hydroxy- 3- hydroxymethylphenyl) - 2- (tert-butylamino)ethanol.It is sparingly soluble in water.

In case of suspension formulations, the substances that are insoluble in the propellant are dispersed in suitable propellant vehicle. The propellant is described as “liquefied gas with a vapor pressure greater than atmospheric pressure (14.7 psi) at a temperature of 105^{o}F”. It supplies the necessary pressure to expel material from the container, when the valve is opened. Good solvent power, purity, stability, low toxicity, freedom from irritation, non-flammability, non-explosiveness, non-reactiveness, freedom from odor and color are the fundamental requirements of a propellant [8]. It is important to select an appropriate propellant system, so as to get the optimum vapor pressure. Surfactants in aerosol formulation are extremely important. These agents maintain the disperse nature of the drug in propellant blend and provide lubrication for operation of the metering valve. They also help to alter the lipoidal solubility thereby enhancing the pulmonary absorption rates of the active ingredient and act as permeation

enhancer by reducing the interfacial tension between drug surface and lung membrane. Their concentration has to be optimized.

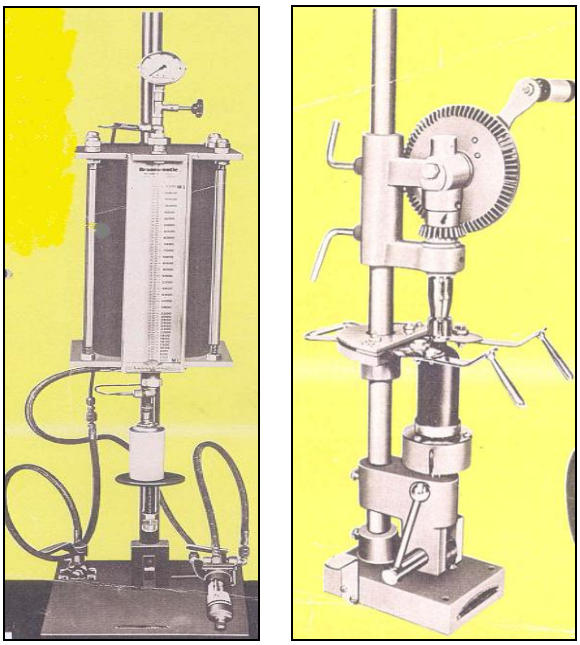
MATERIALS: Salbutamol was a gift sample from Cipla Limited. Marketed MDI was purchased from local market. All other chemicals used were of analytical grade.

METHOD:

Procedure for Filling/Sealing Canisters:

1. In a closed vessel maintained in an ice bath, drug was dissolved in the required quantity of the cosolvent, ethyl alcohol and mixed with the predetermined quantity of the propellant, P-11.
2. To each canister, required quantity of the above product concentrate was added. The canisters were crimped immediately using crimping machine.
3. The sealed cans were then sonicated in the bath sonicator containing cold water at 10ºC for 15 min.
4. The propellant filling unit was used to fill propellant P-12 under pressure with an accuracy of ±1cc. The aerosol filling unit was attached to a propellant storage cylinder along with pressure burette capable of metering small volumes of liquefied gas. The storage cylinder provided with suitable valve, allowed purging of the canister and also permitted required quantity of the liquid propellant to be charged from the storage cylinder into the canister.
5. Thus Propellant P-12 was purged into each canister till the desired weight was achieved.

(The filling of the propellant was carried out with the help of filling unit operated under nitrogen pressure 15 lbs/Kg cm2). Throughout the process, the temperature was maintained at 20-22°C and humidity not more than 40%RH (In order to minimize particulate and microbial contamination).



AEROSOL FILLING AND CRIMPING MACHINE

Development of Formulation:

Formula Optimization:

- **Selection of Propellant Blend:** A series of formulations A1, A2, A3 and A4 was prepared by taking different proportions of propellants P-11 to P-12 such as 30:70, 40: 60, 50: 50 and 60: 40 which are shown in **Table 1**. The formulations were checked for the vapor pressure. The blend giving optimum vapor pressure, in the range of 70-75 psig was selected for further development of salbutamol formulations.

TABLE 1: SELECTION OF A PROPELLANT BLEND

Formulation	P- 11	P - 12	Vapor Pressure (Psig)
A1	30	70	70-75
A2	40	60	55-60
A3	50	50	45-50
A4	60	40	40-45

2. **Selection of Surfactant and Optimization of surfactant concentration:** Uniform dispersion of the micronized drug is extremely important for getting reproducible delivery after each actuation. Smooth dispersion also helps in giving the desired particle size of the spray. Surfactants concentration has to be optimized. Too less concentration affects the dispersibility of the formulation, at the same time excessive concentration of surfactant can affect the spray pattern due to increase in the concentration of non-volatile components. Effect of surfactant was studied by preparing the MDI formulations B1 to B5 using various surfactants such as oleic acid, ethyl oleate, span 20, tween 20, tween 80 respectively in concentration of 10 mg per can. This is shown in **Table 2.** The effect of selected surfactant i.e. oleic acid on the MDI formulation was studied by evaluating content per spray, spray pattern, vapour pressure, evaporation rate and particle size distribution. A series of formulations C1 to C10 was prepared using various concentrations of oleic acid in the range of 2-20mg per canister. The formulations were studied in terms of vapour pressure, evaporation rate, content per spray; spray pattern and particle size distribution. These are the important parameters that are used for the performance evaluation of the MDI formulation and the results are shown in **Table 3.**

TABLE 2: SELECTION OF SURFACTANT FOR MDI SUSPENSION FORMULATION

Formulation	% Particle Size Distribution		Spray Pattern (mm)	Vapor Pressure (psig)	Evaporation Rate	% Content per Spray
	<3µ	3 - 5µ				
B1	97.5	2.5	9.5	70	+++	104.3
B2	94.0	6.0	10.0	70	+++	102.4
B3	93.5	6.5	11.5	70	+++	100.1
B4	92.0	8.0	11.0	70	+++	98.01
B5	91.0	9.0	12.2	70	+++	99.02

TABLE 3: OPTIMIZATION OF CONCENTRATION OF OLEIC ACID IN MDI SUSPENSION

Formulation	Conc. of Oleic Acid mg / can	% Particle Size Distribution		Vapor Pressure (psig)	Evaporation Rate	% Content per Spray
		< 3µ	3 - 5µ			
C1	**2**	**90.0**	**10.0**	**70-75**	**+++**	**99.8**
C2	**4**	**94.0**	**6.0**	**70-75**	**+++**	**101.1**
C3	**6**	**95.5**	**4.5**	**70-75**	**+++**	**102.2**
C4	**8**	**96.0**	**4.0**	**70-75**	**+++**	**103.1**
C5	**10**	**97.5**	**3.5**	**70-75**	**+++**	**104.1**
C6	**12**	**96.0**	**4.0**	**70-75**	**+++**	**100.1**
C7	**14**	**92.0**	**8.0**	**70-75**	**++**	**100.02**
C8	**16**	**90.0**	**10.0**	**70-75**	**++**	**100.01**
C9	**18**	**94.0**	**6.0**	**70-75**	**++**	**99.6**
C10	**20**	**89.0**	**11.0**	**70-75**	**++**	**92.1**

+++ » Excellent; ++ » Good; + » Poor

a. **Qualitative Tests**: These are the physical tests which confirm the satisfactory delivery of the medicine as well as the integrity of the pack.

1. **Dimensional checks**: The components of the MDI pack viz. canister, adaptor (actuator) and the mouthpiece cap were separated and measured individually for length, breadth and height. Uniformity of their dimensions was assessed using vernier calipers. These measurements are indicated in **Table 4**.

TABLE 4: DIMENSIONAL CHECKS ON MDI PACKAGING SYSTEM

Formulation	Average Dimensions (mm)		
	Canister L x B	Adaptor L x B x W	Mouthpiece Cap L x B
C5	75.0 x 16.53	72.84 x 39.4 x 17.03	19.16 x 28.0
M1	75.0 x 16.51	72.84 x 39.4 x 17.03	19.16 x 28.0

C5: MDI Suspension Formulation; **M1:** MDI Marketed Formulation

2. **Spray Pattern:** Spray pattern is useful in determining the performance of a specific formulation-valve combination and ensures therapeutic performance [9]. In addition the test serves as a check on batch to batch uniformity of aerosols. The spray delivered through MDI was impinged onto a glass plate containing activated silica gel-dye mixture. The MDI was held at a distance of 3cm from the plate. The spots formed thereafter were observed under the UV light. The results of the test are reported as an average of six readings in **Table 5A.**

3. **Particle Size Distribution:** This is the most important test that determines the deposition of the aerosol in the lungs. The parameter influences the in vivo performance of the aerosol system as it is one of the governing factors affecting respirable dose of the drug [10]. For optimum therapeutic performance the aerosolized particles should be less than 5µm in size [11]. The particle size distribution of the aerosol suspension was initially determined by the optical microscopy.

The MDI under evaluation was sprayed on a glass slide. The slide was rinsed with CCl_4 to prevent the excipient particles from interfering with the measurement of the drug particles. After sufficient rinsing with CCl_4, the slide was placed under the microscope and particle size was measured by using 100X magnification with oil immersion method. 100 particles in 25 different fields were measured. The results are tabulated in **Table 5B** as the number of particles ≤3µm and between 3-5µm.

TABLE 5A: SPRAY PATTERN

Formulation	Average diameter of the spot (mm)	Description
C 5	11 ± 0.136	Round spot with distinct violet color at the centre and faint pink color at the periphery.
M1	10 ± 0.266	Slightly oval spot with distinct violet color at the centre and faint pink color at the periphery.

TABLE 5B: PARTICLE SIZE DISTRIBUTION

Formulation	Number of Particles of Size		Standard Deviation
	≤ 3µm (%)	3-5µm (%)	
C5	96	4	± 0.69
M1	97	3	± 1.22

4. **Flame Extension:** The test is performed to check if the product is inflammable [12]. The MDI was held at a distance of 18cm from the flame of candle and sprayed by one actuation. Flame extensions greater than 40cm indicate that the product is inflammable. The effect of gradually increasing the distance between the adaptor mouthpiece and the flame on the extension produced was studied and the results are reported in **Table 6A.**

5. **Leak Test:** To ensure integrity of the MDI pack, the test was performed by inserting the cans of the known weight, in a water bath maintained at 50^{o}C. After equilibration, canisters were checked for the presence of any leaks in the form of air bubbles arising from the orifice or the valve crimp. These cans were then wiped clean and their weights were recorded. The cans were placed in up-right position for 3 days and weighed at the end of third day. The leakage rates were then calculated as

explained above. **Table 6B** indicates the average of six readings.

6. **Vapor Pressure:** The vapor pressure serves as a force to expel product out of the canister. The test is applied at the initial stages of development of MDI formulation as a tool to determine the basic formulatory requirement for performance of a MDI product. The test is applied to the finished product, helps to establish the integrity of the pack, as any leakage would be detected by a drop in vapor pressure [13]. It was measured using a specially designed vapor pressure gauge, 'Comes Pressure Gauge'. The pressure gauge had a notch on the top where nozzle of the MDI valve could be fitted. The can was held in this position and pressed against the notch of the gauge. The pressure within the canister was displayed by the gauge. The average of six readings is reported in **Table 6C.**

TABLE 6A: FLAME EXTENSION TEST

Formulation	Distance (cm)		
	18	25	30
C5	Flame Extinguished	Flame Extinguished	Flame Extinguished
M1	Flame Extinguished	Flame Extinguished	Flame Extinguished

TABLE 6B: LEAK TEST

Formulation	Initial Weight (g)	Weight (g) after 3 Days	Leakage
C5	24.362	24.362	No leakage
M1	25.488	25.488	No leakage

TABLE 6C: VAPOR PRESSURE TEST

Formulation	Vapor Pressure (psi) at Room Temperature
C5	75 – 80
M1	75 – 80

Average of six readings;
C5: MDI Suspension Formulation;
M1: MDI Marketed Formulation

Evaporation Rate: With one single actuation of MDI the formulation was sprayed on a dark surface (for visibility) and the time taken by it to disappear was measured. The evaporation performance was rated as (+++) excellent, (++) good and (+) poor based on the time taken for evaporation. When the time taken for the evaporation was less than 30seconds, it was considered as having excellent rate of evaporation and times between 30-60seconds and more than 1 minute were rated as good and poor evaporation rates respectively. The observations of average of six readings are summarized in **Table 7.**

8. **Number of deliveries per container:** These were checked by counting the total number of actuations, till the contents of the canister were exhausted completely. The results as average of six readings are shown in **Table 7.**

TABLE 7: COMPARATIVE STUDY OF SALBUTAMOL MDI PREPARATIONS, BASED ON EVAPORATION RATE AND NUMBER OF DELIVERIES PER CONTAINER

Formulation	Evaporation Rate	Number of deliveries
C5	+++	201
M1	+++	228

b. Quantitative Tests:

1. **Average Weight per Metered Dose:** (Shot Weight Test): It determines the capacity of the metering valve in terms of the weight of the delivered substance. Density of the propellant used mainly affects the shot weight. The canisters under evaluation were first separated from the adaptor and their weights were recorded. The first five sprays were fired in air. ('Test Firing'). After the test fire, the canisters were wiped with a tissue paper and their weights were recorded (W_1). Five successive deliveries were sprayed from the inhaler after placing the canisters back into their actuators. The canisters were subsequently removed from the adaptors and the valve stem and the

orifice were wiped clean. The containers were weighed again and their weights were recorded (W_2). The difference in the initial and the final weights of the canisters divided by number of deliveries fired from the canisters gives the 'Average weight per Metered Dose'.

$$\text{Average weight per Metered Dose} = \frac{W_1 - W_2}{5}$$

The test was performed in triplicate to check the reproducibility of the results. The results are given under **Table 8.**

TABLE 8: DETERMINATION OF AVERAGE WEIGHT PER METERED DOSE OF SALBUTAMOL MDI.

Formulation	W1	W2	(W1 – W 2)/ 5 (mg)
C5	23.624	23.185	87.66 ± 0.045
M1	24.285	23.846	87.80 ± 0.078

Average of six readings; C5: MDI Suspension Formulation; M1: MDI Marketed Formulation

2. **Content per Spray:** The amount of drug delivered per spray was determined by a spectrophotometeric method of analysis and the average of 6 actuations was checked for the compliance with their label claims. In a beaker containing 75ml methanol, ten deliveries were fired below the surface of the methanol from MDI.

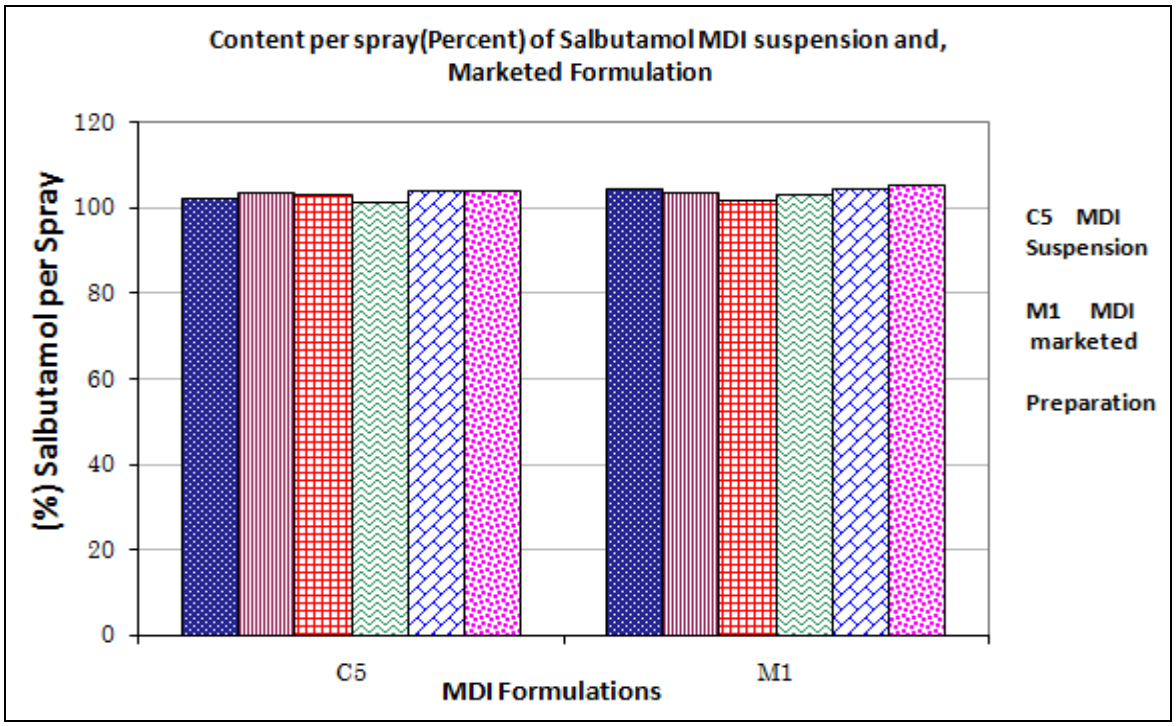
   Each time the canister was shaken thoroughly keeping 5 seconds gap between each spray. Absorbance of the resulting solution was measured at 246nm and the salbutamol content was determined by the method described below. The solution obtained after firing ten deliveries from the MDI was collected in a beaker containing methanol. This was introduced into a 100ml volumetric flask which contained 10ml of 0.1N NaOH solution. The volume was made up to 100 ml mark using methanol and the absorbance of the resulting solution, (conc. 10mcg/ml) was recorded at 246nm (I). To the remaining contents of the volumetric flask 0.5ml of conc. HCL was added and the absorbance was again measured at 246nm (II). The net reading was obtained by subtracting (II) from (I). A standard solution was prepared by weighing approximately 50mg of standard salbutamol (I.P) in a 100ml volumetric flask containing 10ml of 0.1N NaOH. The volume was made up using methanol. From the resulting solution, a 2ml aliquot was taken and diluted to 100ml with methanol.

   The resulting solution (conc.10mcg/ml) was measured for its absorbance at 246nm (I). To the remaining solution 0.5ml of conc. HCL was added and the absorbance was measured again at 246nm (II). The net reading was obtained by subtracting (II) from (I). The content per spray of the MDI was calculated using formula:

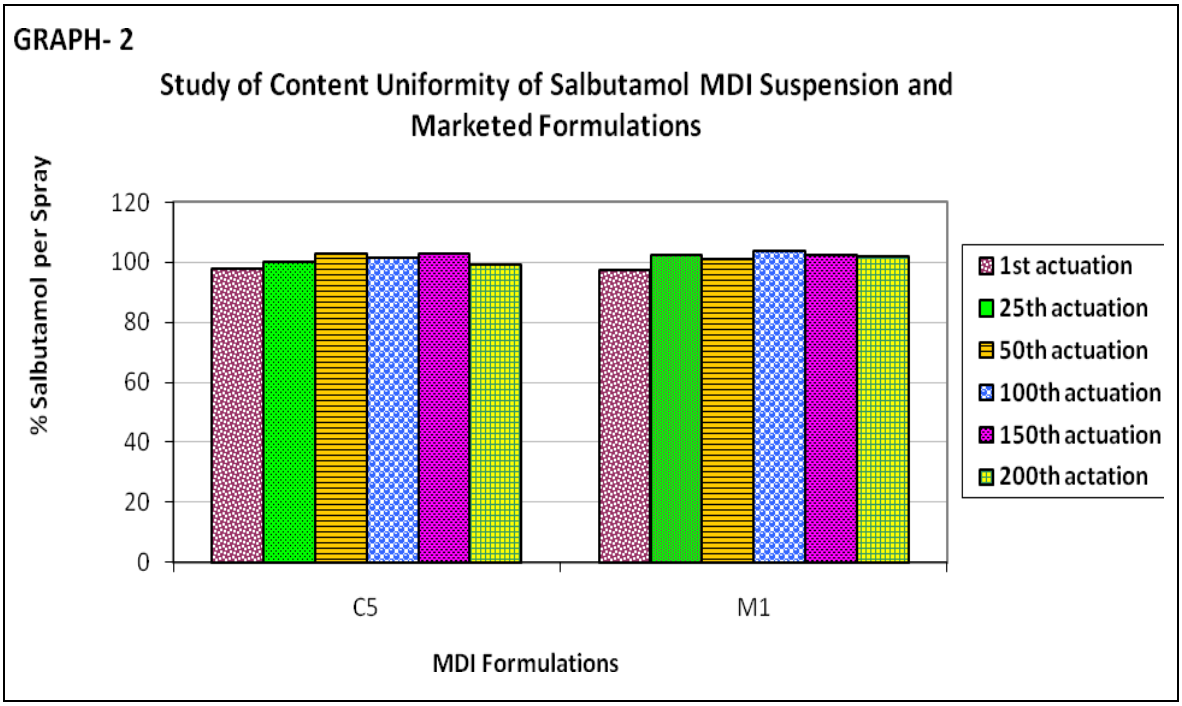
$$\text{Content per spray} = \frac{\text{Sample Abs.}}{\text{Std. Abs.}} \times \frac{\text{Std. Wt.}}{100} \times \frac{2}{100} \times \frac{100}{10} \times 1000$$

The results are recorded under **Graph-1**

3. **Content Uniformity:** The uniformity of content of salbutamol in 10 doses was measured using the method similar to that mentioned for content per spray. In order to check the extent of variation in 1st, 25th, 50th, 100th, 150th, 200th doses sprayed from MDI. These sprays were analyzed for the content of salbutamol per spray. Results are shown in **Graph 2.**



GRAPH 1: CONTENT PER SPRAY(PERCENT) OF SALBUTAMOL MDI SUSPENSION AND, MARKETED FORMULATION



GRAPH 2: STUDY OF CONTENT UNIFORMITY OF SALBUTAMOL MDI SUSPENSION AND MARKETED FORMULATIONS

4. **Retention on Adaptor:** At the time of spraying from MDI, some amount of drug may get retained on the adaptor. This represents the wasted drug which is not available for inhalation and hence to be restricted to minimum. Ten deliveries as indicated in the 'Content per Spray' test were fired through MDI with adaptor attached to it. The components of the MDI were separated and rinsed with methanol. The collected solution was analyzed for the content of salbutamol by the procedure described in the previous test. The results are described under **Table- 9**

TABLE 9: RETENTION OF THE EMITTED DOSE ON THE ADAPTOR OF SALBUTAMOL MDI

Sample No.	Amount of Salbutamol Retained on Adaptor (%)	
	C5	M1
1	8.23	10.12
2	8.02	8.77
3	7.85	11.21
4	9.46	10.71
5	8.68	10.91
6	9.15	10.65
Average	8.57	10.40
Std. Dev. (±)	0.645	0.873

c. **In-vitro evaluation:** The formulations were further subjected to in-vitro evaluation whereby net respirable fraction was determined using Twin Impinger [14, 15]. Twin Impinger Apparatus consists of two stage reservoirs; wherein Stage I represents the extent of drug deposited in the oropharyngeal region and stage II represents the extent of drug deposition in the lungs.

Stage I:

Upper Impinger Chamber: MDI was attached to the equipment by means of a rubber collar of the upper impinger chamber. The throat consists of modified glass tubing with precise diameter. This tubing is attached to a round bottom glass reservoir of 50ml capacity and contained 7ml of the analyzing liquid.

Stage II:

Lower Impinger Chamber: Flask I is connected to the lower impinger by means of a coupling glass tube. The tube has a plastic screw cap and a side arm outlet which can be connected to the vacuum pump. The lower jet assembly is made up of polypropylene filter holder. This is connected to a lower end of a plastic coupling tube. The filter assembly consists of a circular disc comprising of 4 jets arranged on a projected circle of diameter 5.3mm. The stage II terminates with a conical flask of 250ml capacity. This contains 30ml of the analyzing liquid. The amount of active ingredient deposited in this stage represents the "Net Respirable Fraction". The fraction is supposed to mimic the amount of drug bioavailable to the lungs for exerting the required therapeutic effect.

Method: The two collecting chambers were filled with the required amount of distilled water (7ml in stage I and 30ml in stage II). The MDI was attached to the device by means of the rubber collar. 10 sprays were fired into the apparatus.

The side arm tube was connected to the vacuum pump. The flow rate was adjusted to 60ltr/min with the help of Flow-Meter. This mimics the respiratory or Inspiratory flow rate of normal individual. A gap of 5 seconds was maintained between two successive sprays. The reservoir was rinsed with the distilled water. The amount of drug deposited on the adaptor, collar and valve termed as device and stage I represents the fraction of the drug that is not available for inhalation or the 'Non-respirable Fraction'. The amount of drug deposited in stage II, is the amount of drug available to the lungs and represents the 'Net Respirable Fraction'. The results are indicated in **Table 10.**

TABLE 10: NET RESPIRABLE FRACTION OF MDI FORMULATIONS OF SALBUTAMOL BY TWIN IMPINGER TECHNIQUE

Deposition at		% Drug Deposition	
		C5	M1
Device	Average	12.105	12.25
	±S.D	0.728	0.680
Stage I	Average	48.30	45.20
	±S.D	0.892	0.653
Stage II	Average	39.59	42.55
	±S.D	0.862	0.532

RESULTS AND DISCUSSION:

- **Formula Optimization:**

1. **Selection of Propellant Blend:** Different proportions of propellants, P-11 to P-12 viz. 30: 70, 40:60, 50:50 and 60: 40 were tried in formulations A1, A2, A3, A4 respectively, which are shown in **Table 1.** The vapor pressure in the formulation was found to be 75,60,50,45 psig respectively. The formulation A1 showed desired vapor pressure which was 70-75psig. Hence this proportion, P-11: P-12, 30:70 was further used in the MDI formulations.

2. **Selection of Surfactant:** The formulations B1 to B5 were prepared using variety of surfactants viz. oleic acid, ethyl oleate, span 20, tween 20 and tween 80 as given in **Table 2**. These formulations were evaluated on the basis of physicochemical characteristics. All the formulations showed satisfactory evaporation rate and the vapor pressure was found to be 70 psig. All the formulations showed satisfactory content per spray. Spray pattern was as the desired and almost comparable in all the formulations. The formulation B1 showed maximum particles, 97.5% of the size less than 3µ. Thus, considering all the quality control parameters, formulation B1, containing oleic acid was selected for further studies and the amount of oleic acid was optimized.

3. **Optimization of surfactant concentration:** MDI formulations C1 to C10 were prepared with various concentrations of oleic acid ranging from 2-20 mg per can. When studied for physicochemical properties as shown in **Table 3**. It was observed that all the formulations C1 to C10 had same vapor pressure, 70-75psig. However, evaporation rate was found to decrease for formulations C7 to C10 containing 14mg to 20mg of oleic acid. This is because increase in the concentration of oleic acid caused increase in the concentration of non-volatile components, thereby reducing the volatility and hence the evaporation rate. Content per spray was found to be satisfactory with all the concentrations C1 to C9 except the last formulation C10 having 20mg oleic acid. This is because the increase in concentration of the surfactant leads to increase in the solid content resulting into the coarser spray which gives lower content per spray.

Formulation C5 with 10 mg of oleic acid had maximum number of particles in the size range of <3 µ. All the quality control parameters were found to be satisfactory for this formulation. Hence the amount of oleic acid to be added in formulations was optimized for 10mg per canister and further studies were performed.

- **Physicochemical Evaluation of MDI formulations:** The selected MDI suspension formulation C5 were subjected to the physicochemical evaluation tests as per the standard phramacopoeial procedures and the characteristics of the formulations were further compared with a conventional marketed formulation M1.

d. Qualitative Tests: The series of tests performed could successfully assess the test-samples to establish, satisfactory delivery of the MDI products.

1. **Dimensional checks:** This test is essential in order to ensure the proper performance of the MDI during the packaging operation. **Table 4** shows the average dimensions of the individual components of MDI device i.e. the canister, adaptor and mouthpiece cap. The packaging components used for the formulation were from the same batch and were of standard quality, manufactured by reputed suppliers. Both the formulations C5 and M1 satisfactorily passed the test. The dimensions of canister were found to be 75 X 16.5 mm, that of adaptor were 72.85 X 39.5 X 17.0 mm and mouthpiece cap 19.16 X 28 mm respectively.

2. **Spray Pattern:** It is an important test to determine actuator and valve performance. Size and shape of the actuator orifice as well as valve affect the spray characteristics. Smaller the diameter of the spots formed on the silica gel plate lower is the emitted particle droplet size. **Table 5A** indicates average diameters of the spots along with their description. Both the formulations showed good spray pattern with the spot diameter in the range of 10-12mm. The spots were round to oval in shape with distinct violet color at the centre and faint pink color at the periphery.

3. **Particle Size Distribution:** Particle size distribution is the major factor governing the deposition of the emitted dose from the MDI and hence its therapeutic performance. The anatomy and physiology of the respiratory tract makes the therapy difficult as it restricts entry of the particulate matter. Smaller particles in the range of 1-5µm, settle in the lower airways. **Table 5B** shows the particle size distribution of formulations under study. The average of three readings in 25 different fields showed 96% and 97% of the particles below 3µm; 4% and 3% of the particles were found in the range 3-5 µm respectively.

4. **Flame Extension:** Propellants used in the MDI preparations are low boiling point liquids and hence inflammable. The combustibility and flammability of the propellants in the formulations is determined by spraying the MDI on an open flame from a known distance. The observations as reported in **Table 6A** indicated that flame was found to extinguish at the distance of 18, 25 and 30cm by all the formulations under study. Thus it can be concluded that, propellants used in all these formulations were non-flammable.

5. **Leak Test:** The test confirms the effective valve seal and integrity of the MDI product during storage. **Table 6B** indicates the leakage rate of the formulations under study, stored for three days. There was no change in the initial weights of the cans. No leakage was observed in samples of formulations C5 and M1.

6. **Vapor Pressure:** Vapor pressure is a very important parameter to be determined at the developmental stage as well as at the finished product stage. Sufficient development of pressure within the container is absolutely essential so that the desired quantities of the contents propel into the metering valve and then expelled outside as a fine spray. Vapor pressures of the test formulations were measured and the results are tabulated under **Table 6C**. MDI formulations C5 and M1 had vapor pressure in the range of 70-80 psi., which is optimum for an aerosol product with good spray performance.

7. **Evaporation Rate:** It is an important physical characteristic that indirectly determines the spray pattern, and drug deposition in the respiratory tract. The performance was rated as excellent, good and poor, based on the time required for evaporation. The observations are tabulated in **Table 7.** Both the formulations

under study had excellent evaporation rate which was less than 30 second. This indirectly determines the spray characteristics.

8. **Number of deliveries per container:** The test is performed to confirm the proper filling and sealing of the containers. Any leakage from the container would not give complete actuations. The canisters contain 200 metered doses. The number of deliveries possible after actuating container valves till the contents were exhausted, were recorded in **Table 7** for formulations C5 and M1. The numbers of deliveries were found to be 201 and 228 respectively. The excess number of deliveries, in case of marketed preparation shows about 15% filling overages. The formulations pass the test.

e. Quantitative Tests:

1. **Average Weight per Metered Dose:** For therapeutic efficacy, proper dosing is essential. In case of MDIs the metering valve determines the dose. For correct dosing one has to ensure proper design and functioning of the valve. **Table 8** shows the average of the six doses delivered from the MDIs. The average weight per metered dose or shot weight was found to be for C5 and M1 as 87.6 and 87.8mg respectively with standard deviation less than 0.1.

2. **Content per Spray:** The test confirms delivery of the drug as per the labeled claim. Average of six readings is given in **Graph 1** indicated that the three test formulations pass the phramacopoeial limits, not less than 80% of the labeled claim and not more than 120% of the labeled claim. The formulation C5 and M1 showed 103.05% and 103.84% of the drug per spray with standard deviation ±1.086 and 1.320 respectively.

3. **Content Uniformity:** The drug content in the spray was determined at various intervals of actuation to confirm uniform delivery of the drug at each time till the entire content was used up. Content uniformity was assessed at 1^{st}, 25^{th}, 50^{th}, 100^{th}, 150^{th} and 200^{th} actuation. The results recorded under **Graph 2** indicated that the average content of the specified actuations for all the three formulations was found satisfactory, in the range 100-102% with almost same standard deviation, 2-2.3%. The formulations confirm the uniformity of drug content till the last dose is actuated out of the container.

4. **Retention on Adaptor:** The amount of drug retained on the adaptor has to be restricted to minimum for the maximum availability of the drug in the pulmonary tract. **Table 9** show the amount of drug retained on adaptor for the test formulations. The suspension formulations retained minimum amount of drug, 8.57% . The marketed preparation showed 10.40% of the retention on adaptor.

f. *In-vitro* evaluation:

1. **Net Respirable Fraction:** Therapeutic efficacy of MDI is determined by the amount of drug deposited in the lungs. In vitro study is performed using Twin Impinger, an apparatus that mimics the respiratory tract in terms of deposition of the particulate matter. The apparatus has two stages. The stage I indicates the fraction of the sprayed drug deposited in the oropharyngeal region and not available for lung deposition and stage II indicates the fraction that gets deposited in the lungs, called as Net Respirable Fraction. **Table 10** indicate the results of this test. It was observed that the average Net Respirable Fraction was 39.59% and 42.55% for the formulations C5 and M1 respectively.

CONCLUSION: In all the six samples amount of salbutamol retained on adaptor was less than marketed MDI. This represents the wasted drug which is not available for inhalation is restricted to minimum. The study shows that the suspension prepared was better than marketed M.D.I. with respect to retention on adaptor.

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