Formulation and Evaluation of Dry Powder Inhaler Containing Inhaled Corticosteroids and Long Acting Beta Agonist of Different Fill Weight

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ABSTRACT

The overall objective of this research project was to study the in DPI formulations containing ICH and LABA to achieve efficient drug deposition goals. Hence, this project focused on the formulation development of DPIs and impact of different fill weight or fill volume in performance as well as other physicochemical parameter. The performance mainly APSD of dry powder inhalers containing LABA & ICS was found to be optimum when it is formulated with 30% of fine grade lactose monohydrate. The APSD evaluation was concluded that the deposition of particle of (F8) 12.5 mg is better than (F4) 25 mg. It’s may due to more void space in the 12.5 mg capsule formulation than 25 mg capsule formulation. Due to this good turbulence occurs and separation drug particle form carrier surface is more and give better deposition compared to 25 mg fill weight formulation per capsule. The overall project concluded the 12.5mg formulation (F8) is good. These formulations are advantages over 25 mg formulation such as less carrier residue, cost effective, good therapeutic result.

Keywords: Dry powder inhaler, ICH, LABA, lactose monohydrate, 12.5 mg, 25 mg.

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INTRODUCTION

Pulmonary route serves to be best alternative to the non-invasive administration for systemic delivery of therapeutic agent (mainly proteins and peptides) due to the fact that lungs could provide a large absorptive surface area (up to 100m²) but extremely thin (0.1-0.2mm) absorptive mucosal membrane and good blood supply. The respiratory tract is one of the oldest routes used for the administration of drugs. Over the past decades inhalation therapy has established itself as a valuable tool in the local therapy of pulmonary diseases such as asthma or COPD (Chronic Obstructive Pulmonary Disease). Devices used to deliver drugs by pulmonary route are based on one of three platforms are pressurized metered dose inhaler, nebulizer and dry powder. In the treatment of obstructive respiratory diseases, pulmonary delivery can minimize systemic side effects, and it provides rapid response, and it minimizes the required dose. When developing a pulmonary drug delivery system one of the important parameter to be considered is particle size. Optimum particle size is very important for targeting of drug to lungs. If the particle size is too small they will exhale and if it is too large, they may affect the oropharynx and larynx. Drug can be delivered by using carriers like cyclodextrins, microparticles, liposome, nanoparticles etc.

**PDDS is mainly classified into three classes**

1- Nebulizer
2-Pressurized Metered Dose Inhaler (pMDI)
3-Dry Powder Inhaler [DPI]

Dry powder inhalers have advanced significantly over the past 10–15 years. A Dry powder inhaler (DPI) is a device that delivers medication to the lungs in the form of a dry powder. The dry powder platform comprises devices that generate an aerosol directly from 1-5μm size drug powder, or mixtures with excipients such as Lactose Monohydrate. The development of DPIs has been motivated by the desire for alternatives to pMDIs, to reduce emission of ozone-depleting and greenhouse gases chlorofluorocarbons and hydrofluoroalkanes respectively that are used as propellants. DPIs are commonly used to treat respiratory diseases such as asthma, bronchitis, emphysema and COPD although DPIs have also been used in the treatment of diabetes mellitus.

MATERIALS AND METHOD

METHODS

**Preparation of Dry Powder formulations:**

1. Coarse grade lactose was sifted through 60# and fine grade lactose was sifted through 100#.
2. Both coarse grade lactose and fine grade lactose were mixed geometrically with the help
of a spatula.

Table 1: List of Materials.

| Sr. No | Material                          | Source                |
|--------|----------------------------------|-----------------------|
| 1      | Drug                             |                       |
|        | a) Inhaled Corticosteroid        | Vamsi Pharma          |
|        | b) Long Acting Beta Agonist      | Vamsi Pharma          |
| 2      | Lactose monohydrate              |                       |
|        | Lactohale 200                    | DFE Pharma            |
|        | Lactohale 230                    | DFE Pharma            |
| 3      | Cellulose Capsule size"3"        | Capsugel              |
| 4      | Ammonium Acetate                 | Merck                 |
| 5      | Tetra butyl ammonium hydrogen sulphate | Merck                  |
| 6      | Acetonitrile                     | Merck                 |
| 7      | Methanol                         | Merck                 |
| 8      | Water                            | Milli-Q               |

3. Above lactose blend was then blended in turbula blender for 10 min. For blending, teflon coated stainless steel vessel (SS vessel) was used.

4. Then the vessel was kept for 10 min for conditioning.

5. The lactose blend was unloaded in butter paper & divided into 3 parts.

6. LABA was sifted through 100# & mixed with one part of lactose blend geometrically with the help of a spatula.

7. ICS was sifted through 100# & mixed with another part of lactose blend geometrically with the help of a spatula.

8. Step no. 6 and step no. 7 was mixed geometrically with the help of a spatula & then blended in turbula blender for 10 min at 49 rpm & the vessel was kept for conditioning.

9. The remaining part of the lactose was added in step no. 9 & blended for 30 min in turbula blender at 49 rpm.

10. The drug loaded lactose blend was then filled in to size “3” hard gelatine capsules with partial filling manual capsule filling machine with fill weight of 25 mg and 12.5 mg per capsules. Step involve in preparation of dry powder inhaler as follows is given in figure no5,6,7.
Figure 1: Formulation Chart

FORMULATION

Table 2: Formulation chart of DPI of LABA and ICS using variable % of LH 200 and LH 230. (25 mg)

| Formulation | Active Ingredient | Label claim (mcg) | Lactohale 230 (%) | Lactohale230 (mg) | Lactohale200 (mg) | Unit Formula |
|--------------|-------------------|-------------------|-------------------|------------------|------------------|--------------|
| F1           | LABA+ICS          | 50+250            | 0                 | 0                | 24.700           | 25 mg/capsule |
| F2           | LABA+ICS          | 50+250            | 10                | 2.470            | 22.230           |              |
| F3           | LABA+ICS          | 50+250            | 20                | 4.940            | 19.760           |              |
| F4           | LABA+ICS          | 50+250            | 30                | 7.410            | 17.290           |              |
Table 3: Formulation chart of DPI of LABA and ICS using variable % of LH 200 and LH 230. (12.5 mg)

| Formulation | Active Ingredient | Label claim (mcg) | Lactohale 230 (%) | Lactohale230 (mg) | Lactohale200 (mg) | Unit Formula |
|-------------|------------------|-------------------|-------------------|------------------|------------------|--------------|
| F5          | LABA+ICS         | 50+250            | 0                 | 0                | 12.200           | 12.5 mg/capsule |
| F6          | LABA+ICS         | 50+250            | 10                | 1.220            | 10.980           |              |
| F7          | LABA+ICS         | 50+250            | 20                | 2.440            | 9.760            |              |
| F8          | LABA+ICS         | 50+250            | 30                | 3.660            | 8.540            |              |

*LABA- Salmeterol
*ICA- Fluticasone propionate

PREFORMULATION

Characterization of Drugs

Physical Characteristics
The Physical appearances of the drugs were characterized and recorded in terms of colour & odour.

Solubility studies of APIs
Solubility studies of LABA and ICS were performed by dissolving weight quantity of APIs in different solvent as per IP. And result of solubility study of LABA and ICS are shown in table 5 and table 6 respectively.

Melting point determination
Melting point of LABA and ICS were determined by Capillary tube method.

Evaluation of prepared DPI containing combination drugs

Physical appearance
Accurately 20 capsules of the formulation were placed in a clear and dry Petri dish against white background and inspected for particulate matter, color change of the blend, sticking of blend to the walls of the inner capsule shell and also observed for the softening of the capsules shell.

Average net content
Randomly 20 capsules were weighed, removed the content from each capsule as completely as possible. Weighed together accurately the emptied shell and calculated the average net content. Using following formula for each formulation and the result of this test given in table 14 and 15.

Average net content (mg) = (Wt -We/20)

Where,
Wt = Weight of 20 filled capsules in mg
We = Weight of 20 empty capsules in mg
Locking length of the capsule.
The locking length was determined by using vernier callipers after filling the formulation blend into the size "3" whose specifications are shown in the table 16.

Moisture content by Karl fisher titration method
Transferred about 50 ml of a mixture of methanol to the titration vessel and titrate with Karl Fischer reagent to detect any moisture that may present in the formulation. Quickly add about 100 mg of powder, mix and again titrate with the Karl Fischer reagent (sulphur dioxide, imidazole base and iodine). Calculate the water content of the specimen, in %, taken by the formula and result are shown in table 17.

% Moisture content = BF x 100/W
Where
W = Weight of the Sample, in mg.
B = (Burette reading) Volume of the KF reagent, in ml.
F = the water equivalence factor of KF reagent, in mg.

Assay (By HPLC)

Chemicals & Reagents
Ammonium Acetate : Emplura, AR Grade
Tetra butyl ammonium hydrogen sulphate : AR Grade
Methanol : HPLC Grade
Acetonitrile : HPLC Grade
Water : HPLC Grade

Preparation of buffer solution
Weigh and transfer 15.4 g of Ammonium acetate and 5 g of Tetra butyl ammonium hydrogen sulphate into a beaker containing 1000 ml of water and mix well to dissolve. Filter through 0.45 μm membrane filter.

Preparation of mobile phase (1000 ml)
Prepare a filter mixture of Buffer (0.2M Ammonium Acetate and 5% w/v Tetra butyl ammonium hydrogen sulphate solution), Acetonitrile and methanol in the ratio of 40:30:30 % v/v and degas well by sonication.

Preparation of diluents
Prepare a mixture of methanol & water in the ratio of 70:30 v/v.

Preparation of blank
The diluent was used as blank.

**Chromatographic condition**

- **Column**: Hypersil BDS, C18, 200 x 4.6 mm, 5 μ
- **Flow rate**: 1.5 ml/min
- **Wavelength**: 239 nm
- **Column Oven Temperature**: 40°C
- **Injection Volume**: 15 μl
- **Sampling rate**: 10 points/sec
- **Filter time constant**: Fast
- **Run time**: 20 min
- **Detector**: UV

**Preparation of standard solution**\(^{12,13}\)**

**Preparation of LABA standard stock solution:**
Accurately weighed about 20 mg of LABA working standard was transferred into 100 ml volumetric flask. To this 70 ml of diluent was added & sonicated to dissolve & diluted to volume with diluents & mixed well.

**Preparation of ICS standard stock solution:**
Accurately weighed about 20 mg of ICS working standard was transferred into 100 ml volumetric flask. To this 70 ml of diluent was added & sonicated to dissolve & diluted to volume with diluents & mixed well.

**Preparation of standard solution for strength:**
Transfer 2.9 ml of salmeterol standard stock solution and 10 ml of fluticasone propionate standard stock solution into 100 ml volumetric flask, dilute upto the mark with diluent and mix well. Filter the solution through 0.45 μm nylon syringe filter by discarding first 3 ml of the filtrate.

**Preparation of sample solution**
Accurately weighed and transfer the sample equal to 20 capsule into a 250 ml of volumetric flask, add about 100 ml of diluent and sonicate for not less than 15 minutes with occasional shaking (maintain the sonicator bath temp between 20-25°C). Dilute to volume with diluent and mix well. Filter the solution through 0.45 μm nylon syringe filter by discarding first 3 ml of the filtrate.

**Procedure**
First set the column compartment temperature as per method than gradually increase the flow upto 1.5 ml/min. equilibrate the column for at least 20 min with method condition.

Note: Ensure the column oven temperature before increasing the flow to 1.5 ml/min.
CALCULATION (in mcg for blend sample)

Salmeterol = AT1/AS1 x CS1/CT x mol.wt1/mol.wt2 x P1/100 x 1000

Fluticasone propionate = AT2/AS2 x CS2/CT x mol.wt1/mol.wt2 x P2/100 x1000

Where,

AT1= Area count of Salmeterol peak in the sample preparation
AS1=Average area of Salmeterol peak obtain from five replicate injection of standard solution
CS1=Concentration of Salmeterol Xinofoate standard (w/v)
P1= % purity of Salmeterol Xinofoate standard used (on as is basis)
AT2= Area of Fluticasone propionate peak in the sample solution.
AS2= Average area of Fluticasone propionate peak obtain from five replicate injection of standard solution
CS2= Concentration of Fluticasone propionate standard (w/v)
P2= % purity of Fluticasone propionate standard used (on as is basis)
CT= Concentration of sample.

% of Assay

Salmeterol = Individual content of salmeterol in mcg / label claim of salmeterol in mcg x 100
Fluticasone propionate = Individual content of Fluticasone propionate in mcg / label claim of Fluticasone propionate in mcg x 100

Flow Properties of various grades of lactose monohydrate

Various grades of lactose monohydrate were evaluated for angle of repose (Θ), bulk density (Dv), true density (Dt), compressibility index (CI), Hausner ratio (H).

Uniformity of the delivered dose (DUSA)

Procedure

A dose collection apparatus consists of a filter-support base with an open mesh filter-support, such as a stainless steel screen, a sample collection tube that is clamped or screwed to the filter-support base, and a mouthpiece adapter to ensure an airtight seal between the sample collection tube and the mouthpiece. Use a mouthpiece adapter which ensures that the front face of the inhaler mouthpiece fits with the front face or the 2.5 mm indented shoulder of the sample collection tube, as appropriate. The filter-support base is designed to accommodate 25 mm diameter filter disks. The filter disk and other materials used in the construction of the apparatus must be compatible with the active substance and solvents that are used to extract the active substance from the filter. One end of the collection tube is designed to hold the filter disk tightly against the filter-support
base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection tube passes through the inhaler\textsuperscript{12}.

**DUSA analysis details**

Apparatus: DUSA  
Flow: 60 LPM  
Run time: 4 seconds  
Delay time: 2 seconds  
No. of run: 2 capsule  
No. of capsule analysis: 10

**Preparation of standard solution- Same as use in Assay by HPLC**

**Sample collection from DUSA**

**Sample 1**: added about 6-7 ml of diluent to the DUSA, Closed the end cap and gently shaken by keeping the filter, within the DUSA. Then transpired the sample into a 10 ml of volumetric flask, made up the volume with diluent and mix well. Further diluted 1ml above solution to 10ml with diluent. Then filtered the solution through 0.45 m nylon syringe filter by discarding first 3 ml of filtrate\textsuperscript{12}.

**Sample 2**: Transpired the filter (glass fiber) after capsule activation into a beaker, added 4ml of diluent into DUSA, closed end caps and gently shaken and transpired the solution, to the containing glass fiber filter, added additional 3 ml of diluent, gently shaken and transpired the solution to a 10 ml of volumetric flask, made up the volume with diluent and mix well. Further diluted 1ml above solution to 10ml with diluent. Then filtered the solution through 0.45 m nylon syringe filter by discarding first 3 ml of filtrate\textsuperscript{12}.

**CALCULATION (in mcg for blend sample)**

% of Assay (DUSA)  
Salmeterol= Individual content of salmeterol in mcg / lable claim of salmeterol in mcg x 100  
Fluticasone propionate= Individual content of Fluticasone propionate in mcg / lable claim of Fluticasone propionate in mcg x 100

**APSD by NGI (HPLC method)**

**Chemicals & Reagents**

| Chemical                         | Source                      |
|---------------------------------|-----------------------------|
| Ammonium Acetate                | Emplura, AR Grade           |
| Tetra butyl ammonium hydrogen sulphate | AR Grade                   |
| Methanol                        | HPLC Grade                  |
Acetonitrile : HPLC Grade
Water : HPLC Grade

Chromatographic condition- Same as Assay by HPLC

Preparation of standard solution: Same as Assay by HPLC

APSD analysis details

Device name: Monodose RS 01
Apparatus: Next Generation Impactor (NGI)
Leak: 0.0 kpa (should not more than kpa)
P3 / P2: 0.26 kpa
Flow: 60 LPM
Run time: 4 seconds
Delay time: 2 seconds
No.of round: 2 capsule
No. of capsule analysis: 10

Sample collection from NGI13

Mouth piece: Rinsed the mouth piece with about 6 ml of diluent with the help of syringe needle, transpired solution into 10ml of volumetric flask, make up the volume with diluent and mix well.

Induction Port: Rinsed the induction port with diluent of about 70 ml of diluent with the help of syring needle, transpired the solution into a 100 ml of volumetric flask made up the volume with diluent and mix well.

Pre-separator: Prepared same as like induction port

Stage 1: added about 5 ml of diluent by rinsing the orifice with the help of syringe needle then kept the stage on the gentel rocker for shaking for 5 min transferred the solution into 10 ml volumetric flask and made up the volume with diluent and mix well. Same procedure has been applied for the sample collection from stage 2, stage 3, stage 4, stage 6, stage 7 & MOC.

Note: Well the sample have been filtered through 0.45μm syringe filter by discarding 3ml of each filtrate.

CALCULATION (in mcg for blend sample)

% of Assay

Salmeterol= Individual content of salmeterol in mcg / lable claim of salmeterol in mcg x 100

Fluticasone propionate= Individual content of Fluticasone propionate in mcg / lable claim of Fluticasone propionate in mcg x 100
RESULTS AND DISCUSSION

PREFORMULATION

Physical Characteristics

| Physical Characteristics | LABA                          | ICS                          |
|--------------------------|-------------------------------|------------------------------|
| Colour                   | Off white powder             | A white powder               |
| Odour                    | Characteristic               | Characteristic               |
| Structure                | Crystalline                  | Crystalline                  |

Physical characteristics of both APIs were found as acceptable colour and odour.

| Sr. No. | Solvents | Solubility of LABA |
|---------|----------|--------------------|
| 1       | Water    | Sparingly soluble  |
| 2       | Methanol | Freely soluble     |
| 3       | Ethanol  | Slightly soluble   |

From the results of solubility study, it can be concluded that LABA is freely soluble in organic solvents while it is sparingly soluble in water.

| Sr. No. | Solvents          | Solubility of LABA       |
|---------|-------------------|--------------------------|
| 1       | Water             | Practically insoluble    |
| 2       | Methanol          | Slightly soluble         |
| 3       | Ethanol           | Slightly soluble         |
| 4       | Dimethyl sulfoxide| Freely soluble           |

From the results of solubility study of ICS, it was found that ICS was practically insoluble in water, slightly soluble in methanol & ethanol, freely soluble in dimethyl sulfoxide.

Melting point determination

Melting point of pure LABA and ICS was found as 760°C and 272.60°C respectively

From melting point determination it can be concluded that both API’s were in its pure & pharmacologically active form.

Flow Properties of various grades of lactose monohydrate

Moisture content by Karl fisher titration

| Sr. No. | LABA  | ICS  |
|---------|-------|------|
| 1       | 0.03% | 0.13%|
| 2       | 0.02% | 0.16%|
| 3       | 0.03% | 0.15%|
Moisture content of LABA & ICS was found to be 0.026% & 0.147% respectively.

Assay of LABA and ICS

Table 8: Result of assay of LABA and ICS

| Drug  | Result | Limit as per IP |
|-------|--------|-----------------|
| LABA  | 100.7  | 97-102 %        |
| ICS   | 98.9   | 96-102 %        |

Purity of LABA & ICS was found as 100.7 % and 98.9 %. From the melting point and assay of both APIs, it can be concluded that both APIs were in its pure and pharmacologically active form.

Flow Properties of various grades of lactose monohydrate

Table 9: Result of flow properties of various grades of lactose monohydrate

| Material       | Bulk density (g/ml) | Tapped density g/ml | Hausners ratio | Carr’s index (%) |
|----------------|---------------------|--------------------|----------------|------------------|
| Lactohale200   | 0.346               | 0.578              | 1.67           | 40.1             |
| Lactohale230   | 0.489               | 0.934              | 1.91           | 47.6             |

FORMULATION STUDIES

Physical appearance

All the prepared formulations were observed for particulate matter, colour change, sticking of blend inside the capsule shell, softening of the capsules and the observations are given in the table.

Table 10: Result of physical observation (25mg)

| Formulation | Particulate matter | Colour change of blend | Sticking of blend to capsule shell | Softening of capsule |
|-------------|--------------------|------------------------|-----------------------------------|----------------------|
| F1          | Not observed       | Not observed           | Not observed                      | Not observed         |
| F2          | Not observed       | Not observed           | Not observed                      | Not observed         |
| F3          | Not observed       | Not observed           | Not observed                      | Not observed         |
| F4          | Not observed       | Not observed           | Not observed                      | Not observed         |

Table 11: Result of physical observation (12.5mg)

| Formulation | Particulate matter | Colour change of blend | Sticking of blend to capsule shell | Softening of capsule |
|-------------|--------------------|------------------------|-----------------------------------|----------------------|
| F5          | Not observed       | Not observed           | Not observed                      | Not observed         |
| F6          | Not observed       | Not observed           | Not observed                      | Not observed         |
| F7          | Not observed       | Not observed           | Not observed                      | Not observed         |
| F8          | Not observed       | Not observed           | Not observed                      | Not observed         |

All the formulations were investigated for visual changes. As shown in table 8.6 & 8.7 there was no change observed in following parameters: particulate matter and colour change. There was no
sticking of blend or lump formation inside the capsule shell.  

**Flow property.**

The prepared formulations were evaluated for bulk density, tapped density Haunsers ratio and car’s index. The results obtained are shown in the table.

**Table 12: Result of flow property of each formulation (25mg)**

| Material | Bulk density (gm/ml) | Tapped density (gm/ml) | Haunsers ratio | Carr’s index (%) |
|----------|----------------------|------------------------|----------------|------------------|
| F1       | 0.595                | 1.02                   | 1.72           | 42.10            |
| F2       | 0.609                | 1.04                   | 1.66           | 40.00            |
| F3       | 0.645                | 1.02                   | 1.581          | 36.76            |
| F4       | 0.632                | 1.02                   | 1.61           | 38.33            |

**Table 13: Result of flow property of each formulation (12.5mg)**

| Material | Bulk density (gm/ml) | Tapped density (gm/ml) | Haunsers ratio | Carr’s index (%) |
|----------|----------------------|------------------------|----------------|------------------|
| F5       | 0.598                | 1.00                   | 1.67           | 40.20            |
| F6       | 0.612                | 1.02                   | 1.66           | 40.00            |
| F7       | 0.640                | 1.03                   | 1.60           | 37.86            |
| F8       | 0.628                | 1.00                   | 1.59           | 37.20            |

The bulk densities of the different formulations were within the range 0.523 to 0.672 g/ml and tapped density were within the range of 0.893 to 1.05 g/ml suitable for filling the 25 mg of blend into partial filling dosing plate of the capsule filling machine. The flow property of the formulation was studied by calculating % compressibility index (CI). The CI values were found to be in the range from 31.42 to 45.45%.

**Average net content.**

The average net content of all the formulations (F1-F4) were determined and the results are given in the table.

**Table 14: Result of average net content of capsules (25 mg)**

| Sr. No. | Formulation | Average net content(mg) |
|---------|-------------|-------------------------|
| 1       | F1          | 24.75                   |
| 2       | F2          | 24.81                   |
| 3       | F3          | 25.07                   |
| 4       | F4          | 22.62                   |

**Table 15: Result of average net content of capsules (12.5 mg)**

| Sr.no. | Formulation | Average net content(mg) |
|--------|-------------|-------------------------|
| 1      | F5          | 12.45                   |
| 2      | F6          | 12.62                   |
| 3      | F7          | 12.87                   |
| 4      | F8          | 12.43                   |
All the formulation tested for average net content and results obtained were within the range prescribed in I.P. which is 22.5-27.5 mg for 25 mg and 11.25-13.75 mg for 12.5 mg.

**Locking length of the capsule.**

Table 16: Specifications of the empty size 3 capsule

| Sr. No | Closed Joined Length (mm) |
|--------|---------------------------|
|        | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
| 1      | 15.45 | 15.4 | 15.6 | 15.44 | 15.43 | 15.31 | 15.63 | 15.68 |
| 2      | 15.48 | 15.31 | 15.33 | 15.49 | 15.31 | 15.4 | 15.43 | 15.63 |
| 3      | 15.44 | 15.43 | 15.54 | 15.38 | 15.4 | 15.68 | 15.4 | 15.56 |
| 4      | 15.56 | 15.63 | 15.32 | 15.51 | 15.68 | 15.63 | 15.68 | 15.69 |
| 5      | 15.69 | 15.68 | 15.44 | 15.45 | 15.63 | 15.56 | 15.31 | 15.52 |
| 6      | 15.43 | 15.56 | 15.49 | 15.48 | 15.56 | 15.6 | 15.56 | 15.43 |
| 7      | 15.45 | 15.69 | 15.38 | 15.44 | 15.69 | 15.33 | 15.69 | 15.44 |
| 8      | 15.56 | 15.43 | 15.51 | 15.56 | 15.52 | 15.54 | 15.52 | 15.69 |
| 9      | 15.69 | 15.52 | 15.45 | 15.69 | 15.43 | 15.32 | 15.43 | 15.52 |
| 10     | 15.43 | 15.45 | 15.48 | 15.43 | 15.45 | 15.43 | 15.45 | 15.43 |
| AVG    | 15.50 | 15.51 | 15.45 | 15.48 | 15.51 | 15.47 | 15.51 | 15.55 |
| LIMIT  | 15.4 - 16.2 |

The formulation capsule tested for locking length and results obtained were within the limit.

**Moisture content by Karl fisher titration**

Table 17: Result of moisture content of formulation (25mg)

| Sr. No | Formulation | Moisture content % |
|--------|--------------|-------------------|
| 1.     | F1           | 4.99              |
| 2.     | F2           | 4.97              |
| 3.     | F3           | 4.96              |
| 4.     | F4           | 4.97              |

Moisture content of F1-F4 formulation was found to be within limit.

Table 18: Result of moisture content of formulation (12.5mg)

| Sr. No | Formulation | Moisture content % |
|--------|--------------|-------------------|
| 1.     | F5           | 4.99              |
| 2.     | F6           | 4.97              |
| 3.     | F7           | 4.96              |
| 4.     | F8           | 4.97              |

Moisture content of F5-F8 formulation was found to be within limit.

**Assay of Formulation by HPLC**

The assay was performed for all the formulation by randomly collecting 20 capsules in each formulation and the results are given in the table.
Table 19: Results of assay values of 25 mg formulation

| Formulation | Assay (%) | % LABA | % ICS |
|-------------|-----------|--------|-------|
| F1          | 92.4      | 100.8  |       |
| F2          | 101.7     | 105.9  |       |
| F3          | 102.6     | 105.6  |       |
| F4          | 103.0     | 106.1  |       |

Table 20: Results of assay values of 12.5 mg formulation

| Formulation | Assay (%) | % LABA | % ICS |
|-------------|-----------|--------|-------|
| F5          | 102.9     | 105.5  |       |
| F6          | 103.9     | 105.5  |       |
| F7          | 102.9     | 103.1  |       |
| F8          | 99.0      | 100.3  |       |

From the results obtained, the assay value of LABA and ICS were found in the range of 92.4-103.0% and 100.8-106.1%, as shown in the table 8.12 for 25 mg. From the results obtained, the assay value of LABA and ICS were found in the range of 99.0-103.9% and 100.3-105.5%, as shown in the table 8.13 for 12.5 mg. The assay results of all the formulations were within the limit of NLT 90% and NMT 125%.

Uniformity of the delivered dose by DUSA

Table 21: Percentage of drug delivered dose per capsule of F1, F2, F3 & F4 (25mg)

| Sr. No. | % LABA | F1 | F2 | F3 | F4 | % ICS | F1 | F2 | F3 | F4 |
|---------|--------|----|----|----|----|-------|----|----|----|----|
| 1       | 96.3   | 91.8 | 90.4 | 95.2 | 99.8 | 95   | 99.5 | 99.56 |
| 2       | 97.5   | 99.5 | 91.2 | 97.3 | 99.4 | 94.6 | 91.2 | 101.25 |
| 3       | 102.3  | 97.2 | 93.4 | 95.1 | 97.3 | 100.2 | 94.7 | 98.35 |
| 4       | 101.2  | 93.1 | 94.8 | 99.5 | 100.1 | 96.2 | 101.6 | 94.25 |
| 5       | 98.9   | 99.8 | 96.3 | 97.2 | 97.2 | 94.9 | 92.8 | 101.25 |
| 6       | 98.6   | 103.2 | 95.1 | 103.4 | 102.3 | 101.3 | 96.25 | 99.2 |
| 7       | 92.3   | 91   | 100.1 | 97.1 | 95.3 | 98.3 | 99.8 | 95.26 |
| 8       | 96.2   | 94.3 | 94.7 | 97.4 | 97.1 | 94.38 | 102.3 | 99.7 |
| 9       | 99.9   | 97.3 | 96.3 | 95.3 | 100 | 93.25 | 99.2 | 100 |
| 10      | 92.3   | 100.9 | 99.9 | 99.5 | 99.9 | 98.9 | 93.56 | 96.8 |
| AVG     | 97.55  | 96.81 | 95.22 | 97.7 | 98.84 | 96.703 | 97.091 | 98.562 |
| SD      | 3.4    | 4.1 | 3.2 | 2.6 | 2.1 | 2.8 | 3.9 | 2.4 |

Table 22: Percentage of drug delivered dose per capsule of F5, F6, F7 & F8 (12.5mg)

| Sr. No. | % LABA | F5 | F6 | F7 | F8 | % ICS | F5 | F6 | F7 | F8 |
|---------|--------|----|----|----|----|-------|----|----|----|----|
| 1       | 91.8   | 99.56 | 90.4 | 90.4 | 107.2 | 95.3 | 99.3 | 98.5 |
| 2       | 99.5   | 101.25 | 91.2 | 91.2 | 99.3 | 98 | 95.6 | 102 |
| 3       | 97.2   | 98.35 | 93.4 | 99.4 | 99.3 | 94.3 | 105.3 | 99.3 |
From the results the delivered dose for the 25mg & 12.5mg formulations, comply within the limits as all the results lie between 75% and 125% of the assay value.

**APSD by NGI (HPLC method)**

| NGI Stages | Fluticasone Propionate (Deposition in MCG) | Salmeterol (Deposition in MCG) |
|------------|------------------------------------------|--------------------------------|
|            | F1 | F2 | F3 | F4 | F1 | F2 | F3 | F4 |
| MP         | 1.12 | 1.50 | 6.5 | 9.2 | 0.43 | 0.43 | 1.1 | 2.0 |
| IP         | 18.92 | 17.9 | 23.6 | 28.2 | 3.81 | 3.63 | 3.46 | 4.54 |
| PS         | 157.80 | 153.6 | 120 | 104.4 | 33.01 | 30.61 | 28.1 | 26.6 |
| Stage1     | 4.2 | 5.3 | 6.7 | 12.7 | 0.91 | 1.10 | 1.31 | 2.66 |
| Stage2     | 4.8 | 6.7 | 9.2 | 18.9 | 1.12 | 1.38 | 1.51 | 3.0 |
| Stage3     | 4.3 | 7.0 | 10.8 | 16.3 | 1.21 | 1.32 | 1.40 | 2.52 |
| Stage4     | 4.8 | 7.0 | 11.0 | 15.10 | 1.19 | 1.31 | 1.61 | 3.43 |
| Stage5     | 5.2 | 5.5 | 6.4 | 7.3 | 0.76 | 0.86 | 1.58 | 2.21 |
| Stage6     | 1.34 | 1.7 | 2.01 | 2.9 | 0.55 | 0.59 | 0.70 | 0.85 |
| Stage7     | 1.06 | 1.21 | 1.38 | 1.6 | 0.42 | 0.44 | 0.47 | 0.50 |
| MOC        | 0.42 | 0.63 | 0.94 | 1.1 | 0.20 | 0.25 | 0.29 | 0.33 |
| ISM        | 21.92 | 29.74 | 41.73 | 63.2 | 5.42 | 6.15 | 7.56 | 12.84 |
| Group1     | 182.04 | 178.3 | 156.8 | 154 | 38.16 | 35.77 | 33.97 | 35.82 |
| Group2     | 20.44 | 27.9 | 39.41 | 60.5 | 4.83 | 5.46 | 8.11 | 12.01 |
| Group3     | 12.5 | 19.5 | 28.2 | 38.7 | 3.16 | 3.49 | 4.59 | 8.16 |
| Group4     | 2.82 | 3.54 | 4.33 | 5.6 | 1.17 | 1.28 | 1.46 | 1.68 |
| Citdias data | | | | | | | | |
| FPF ≤ 5µ   | 18.192 | 24.554 | 34.639 | 48.528 | 4.584 | 5.081 | 6.390 | 10.504 |
| FPF% ≤ 5µ  | 8.968 | 11.888 | 18.038 | 21.827 | 10.617 | 12.247 | 15.806 | 22.291 |
| MMAD       | 2.891 | 3.101 | 3.126 | 3.735 | 2.883 | 2.993 | 2.632 | 3.046 |
| GSD        | 2.559 | 2.524 | 2.378 | 2.372 | 2.617 | 2.620 | 2.920 | 2.556 |
| TDD in MCG | 203.9 | 208.40 | 198.53 | 217.6 | 43.610 | 41.920 | 41.530 | 48.1 |
| % TDD      | 87.51 | 89.44 | 85.20 | 93.4 | 95.6 | 91.11 | 92.28 | 106.9 |

**Table 24: Result of APSD of Formulation (12.5mg)**

| NGI Stages | Fluticasone Propionate (Deposition in MCG) | Salmeterol (Deposition in MCG) |
|------------|------------------------------------------|--------------------------------|
|            | F5 | F6 | F7 | F8 | F5 | F6 | F7 | F8 |
| MP         | 2.1 | 4.3 | 8.7 | 13.5 | 0.54 | 0.71 | 1.12 | 2.93 |

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The APSD evaluation of all formulation (F1-F8) is obtained and 30 % (F4 and F8) formulation is good particle size deposition than other Formulation.

**Table 25: Result of APSD of Formulation F4 & F8**

| NGI Stages | Fluticasone Propionate (Deposition in MCG) | Salmeterol (Deposition in MCG) |
|------------|-------------------------------------------|---------------------------------|
|            | F4 (25mg) | F8 (12.5mg) | F4 (25mg) | F8 (12.5mg) |
| MP         | 9.2       | 13.5       | 2.0       | 2.93        |
| IP         | 28.2      | 27.1       | 4.54      | 4.1         |
| PS         | 104.4     | 129        | 26.6      | 26.92       |
| Stage1     | 12.7      | 8.2        | 2.66      | 1.45        |
| Stage2     | 18.9      | 12.1       | 3.0       | 1.93        |
| Stage3     | 16.3      | 17.7       | 2.52      | 2.88        |
| Stage4     | 15.10     | 24.0       | 3.43      | 5.19        |
| Stage5     | 7.3       | 13.5       | 2.21      | 3.98        |
| Stage6     | 2.9       | 2.9        | 0.85      | 0.85        |
| Stage7     | 1.6       | 0.9        | 0.50      | 0.23        |
| MOC        | 1.1       | 0.6        | 0.33      | 0.12        |
| ISM        | 63.2      | 71.7       | 12.84     | 15.18       |
| G1         | 154       | 177.8      | 35.82     | 35.4        |
| G2         | 60.5      | 70.2       | 12.01     | 14.83       |
| G3         | 38.7      | 55.2       | 8.16      | 12.05       |
| G4         | 5.6       | 4.4        | 1.68      | 1.2         |
| Citdas Data|           |            |           |             |
| FPF ≤ 5μ   | 48.528    | 62.473     | 10.504    | 13.7        |
### Graphical Representation of F4 and F8 Formulation

#### Graph 1: Graphical representation of Fluticasone Propionate deposition in NGI stages for (F4&F8)

The deposition of Fluticasone propionate (F4-F8) in NGI stages is obtained and graphical data shows that comparable deposition in F8 & F4 formulation. But some stages of NGI significant difference has been observed in both formulation. The optimum result observe in F8 formulation.

#### Graph 2: Graphical representation of Salmeterol Xinafoate deposition in NGI stages for (F4&F8)

The deposition of Salmeterol Xinafoat (F4-F8) in NGI stages is obtained and graphical data shows that comparable deposition in F8 & F4 formulation. But some stages of NGI significant difference has been observed in both formulation. The optimum result observe in F8 formulation.

**Graph 3: Group wise representation for fluticasone propionate (F4&F8)**

The group wise representation of Fluticasone Propionate is observed. The group 1, 2 and 3 result is significant result for F8 than F4 formulation. The group 4 is comparable result observed.

**Graph 4: Group wise representation for Salmeterol Xinafoat (F4&F8)**

The group wise representation of Salmeterol Xinafoat is observed. Group 1 & 4 is also comparable result observe. Group 2 & 3 is significant result observed.
Graph 5: Graphical representation of CITDAS for Fluticasone Propionate. (F4&F8)
According to CITDAS Fluticasone Propionate is the fine partical fraction (FPF) is significant result of F8 formulation than F4 formulation. MMAD and GSD is similar result found F4 and F8 formulation.

Graph 6: Graphical representation of CITDAS data for Salmeterol Xinafoat (F4&F8)
According to CITDAS of Salmeterol Xinafoate is the fine partical fraction (FPF) is significant result observe of F8 formulation than F4 formulation. MMAD normally less F4 and F8 formulation. GSD is comparable result observe in F4 and F8 formulation.
CONCLUSION

The overall project concluded the 12.5mg formulation (F8) is good. These formulations are advantages over 25 mg formulation such as less carrier residue, cost effective, good therapeutic result.

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