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Formulation and Evaluation of Dry Powder Inhaler Containing Inhaled Carticosteroids 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)[1]. 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[2].

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[3]. 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[4].

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 Carticosteroid	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	Methenol	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 no[5,6,7].

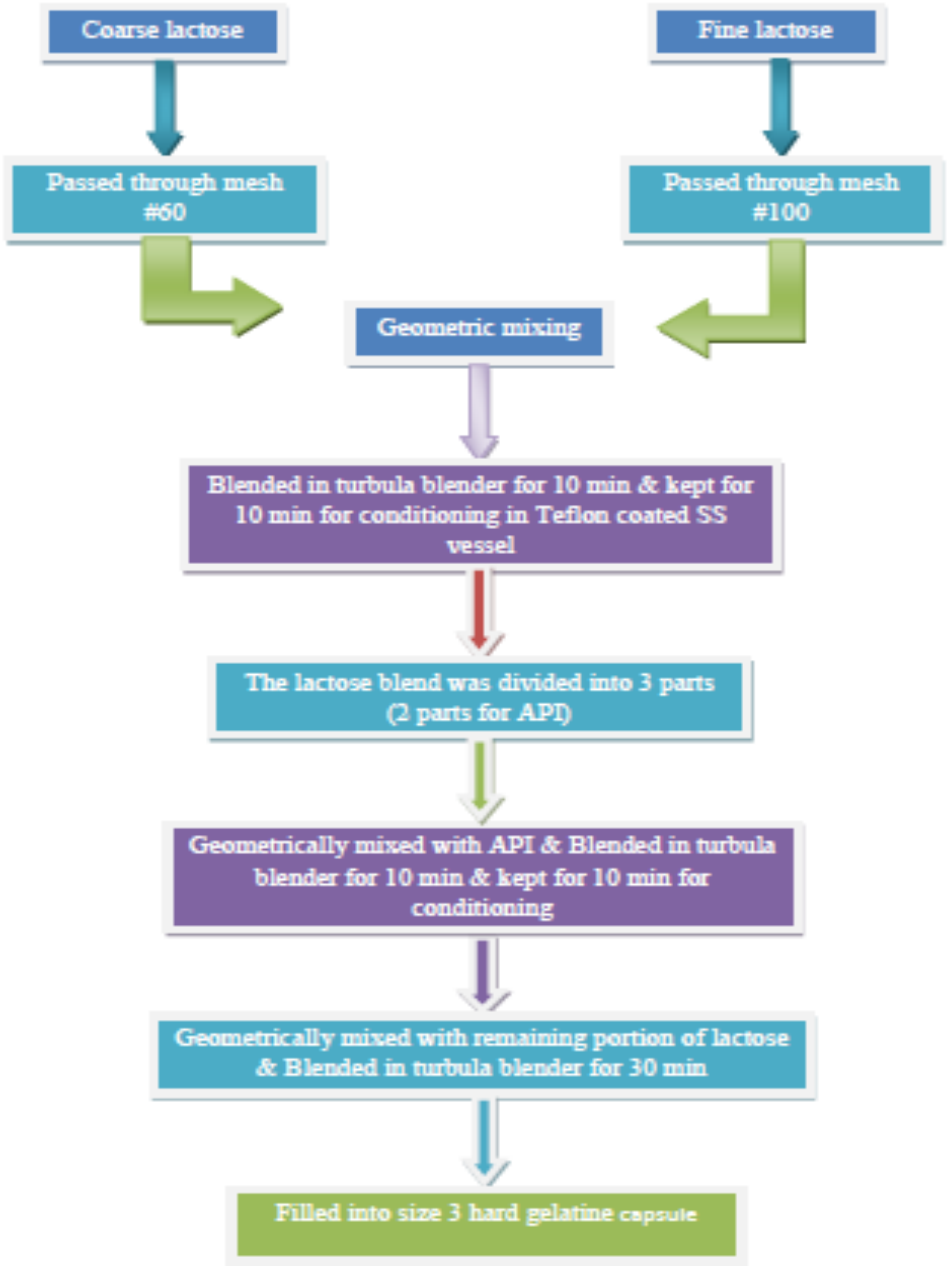


Figure 1: Formulation Chart

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
F2	LABA+ICS	50+250	10	2.470	22.230	mg/capsule
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
F6	LABA+ICS	50+250	10	1.220	10.980	mg/capsule
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 [8,9,10]

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[5].

Melting point determination

Melting point of LABA and ICS were determined by Capillary tube method[5]

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[5].

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[5].

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[6].

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[6].

% 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) [9,10,11]

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 into100 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 20-25^0 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[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[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[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

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)

P_3 / P_2: 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 NGI[13]

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

Table 4: Result of Physical Characteristics of LABA and ICS

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.

Table 5: Solubility studies of APIs

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.

Table 6: Result of solubility study of ICS

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 **760C** and **272.60C** 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

Table 7: Result of moisture content of APIs

Sr. No.	LABA	ICS
1	0.03%	0.13%
2	0.02%	0.16%
3	0.03%	0.15%

Avg	0.026%	0.147%
Limit	0.5 %	0.5 %

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	Color 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				% ICS			
	F1	F2	F3	F4	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				% ICS			
	F5	F6	F7	F8	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

4	93.1	94.25	94.8	99.3	108.3	99.3	99.2	102.3
5	99.8	98.5	96.3	97.3	107.3	97.3	102.3	95.8
6	103.2	99.2	95.1	96.3	103.2	102.3	103.2	99.3
7	91	95.26	93.2	100.1	98.3	101.2	102.3	96.2
8	94.3	91.7	94.7	104.2	109.2	109.2	105.2	105.2
9	97.3	100	96.3	106.3	98.4	105.3	103.6	102.3
10	100.9	96.8	88.4	99.9	106.4	105.3	108.2	103.3
AVG	96.81	97.487	93.38	98.44	103.69	100.75	102.42	100.42
SD	4.12	2.96	2.63	4.99	4.47	4.81	3.62	3.09

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)

Table 23: Result of APSD of Formulation (25mg)

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
Citdas 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

IP	4.9	9.03	18.3	27.1	3.82	3.71	3.57	4.1
PS	155.2	140.2	132.2	129	35.02	31.61	29.1	26.92
Stage1	1.01	2.7	5.1	8.2	0.47	0.59	1.06	1.45
Stage2	2.30	3.9	7.4	12.1	1.12	1.25	1.49	1.93
Stage3	3.41	6.3	11.9	17.7	1.18	1.38	1.56	2.88
Stage4	4.90	11.4	18.3	24.0	2.05	3.01	3.43	5.19
Stage5	5.9	8.12	10.9	13.5	1.94	2.87	3.16	3.98
Stage6	0.9	1.1	1.8	2.9	0.58	0.64	0.71	0.85
Stage7	0.3	0.4	0.7	0.9	0.11	0.17	0.20	0.23
MOC	0.01	0.04	0.4	0.6	0.07	0.09	0.09	0.12
ISM	17.22	31.26	51.04	71.7	7.05	9.41	10.64	15.18
Group1	163.21	156.23	164.3	177.8	39.85	36.62	34.85	35.4
Group2	17.41	30.82	50.3	70.2	6.87	9.15	10.35	14.83
Group3	10.61	25.82	41.1	55.2	5.17	7.26	8.15	12.05
Group4	1.12	1.54	2.90	4.4	0.76	0.90	1.0	1.2
Citdas data								
FPF ≤ 5µ	16.019	28.301	45.771	62.473	6.220	8.481	9.506	13.7
FPF% ≤ 5µ	8.958	15.449	22.112	25.039	13.417	18.481	21.423	27.1
MMAD	2.117	2.343	2.538	2.711	2.184	2.056	2.155	2.3
GSD	2.243	2.210	2.234	2.291	2.397	2.371	2.616	2.4
TDD in MCG	180.930	187.49	215.70	249.50	46.90	46.03	45.49	50.60
% TDD	77.25	80.46	92.27	107.1	104.2	102.2	101.1	112.4

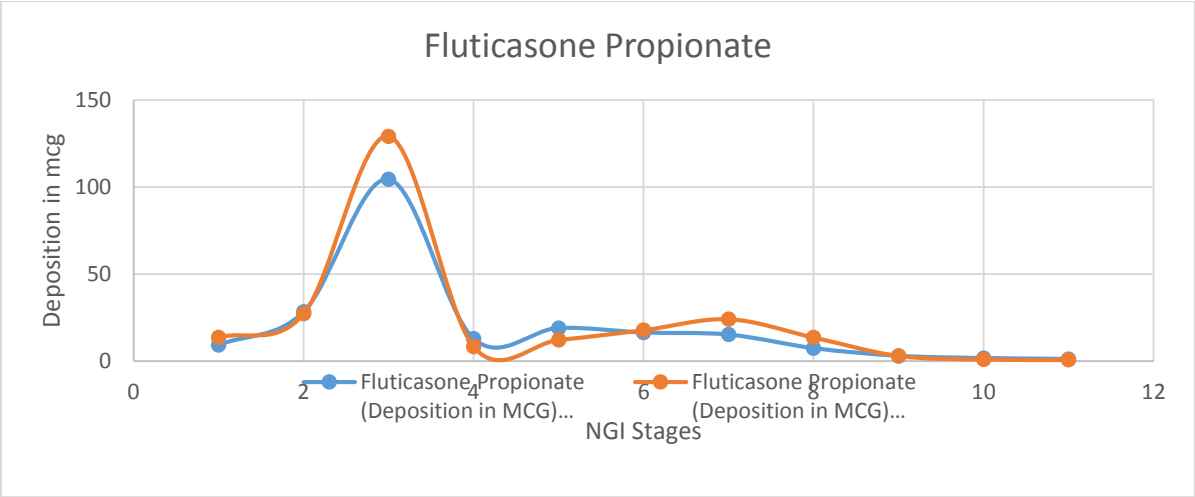
The APSD evaluation of all formulation (F1-F8) is obtained and 30 % (F4 and F8) formulation is good particle size deposition than other Formulation.

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

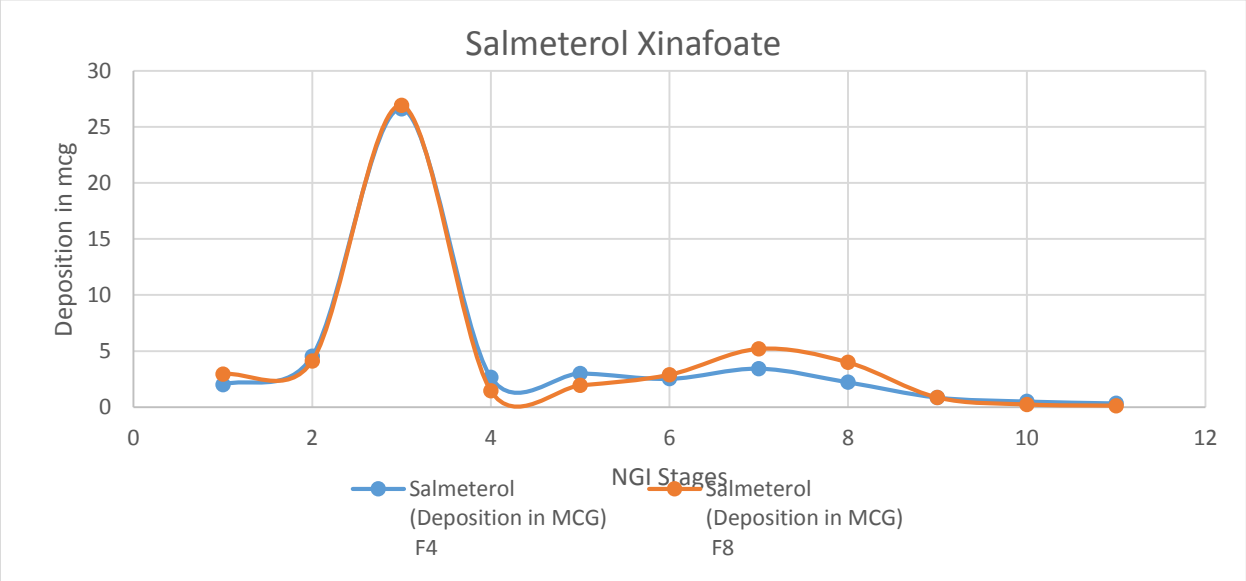
FPF% ≤ 5µ	21.827	25.039	22.291	27.1
MMAD	3.046	2.711	3.735	2.3
GSD	2.556	2.291	2.372	2.4
TDD in MCG	217.6	249.5	48.1	50.6
% TDD	93.4	107.1	106.9	112.4

Graphical Representation of F4 and F8 Formulation



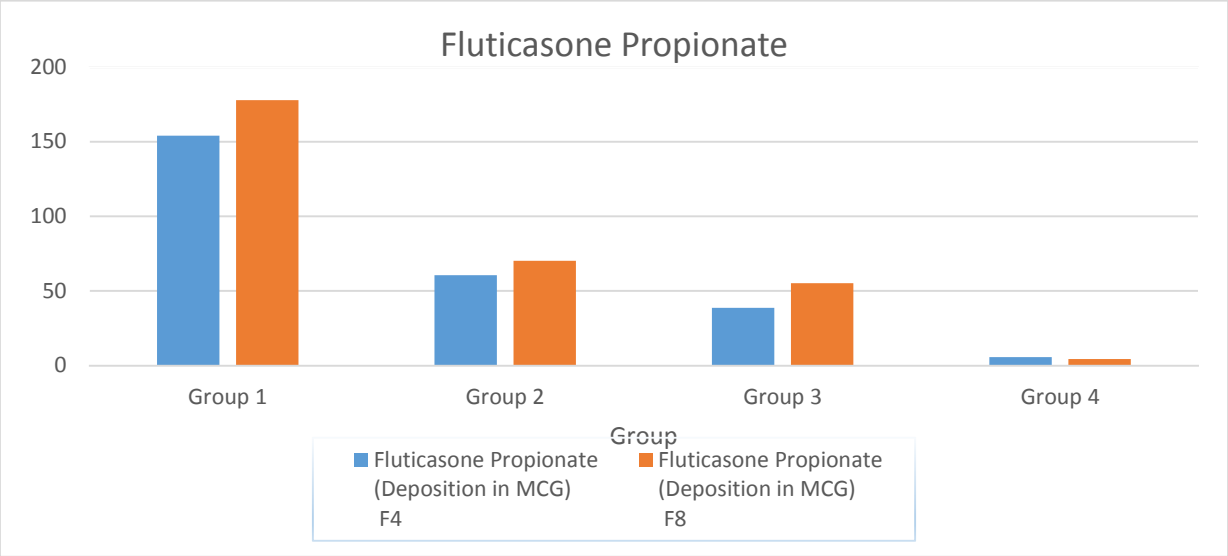
Graph 1: Graphical representation of Fluticasone Propionate deposition in NGI stages for (F4&F8)

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.



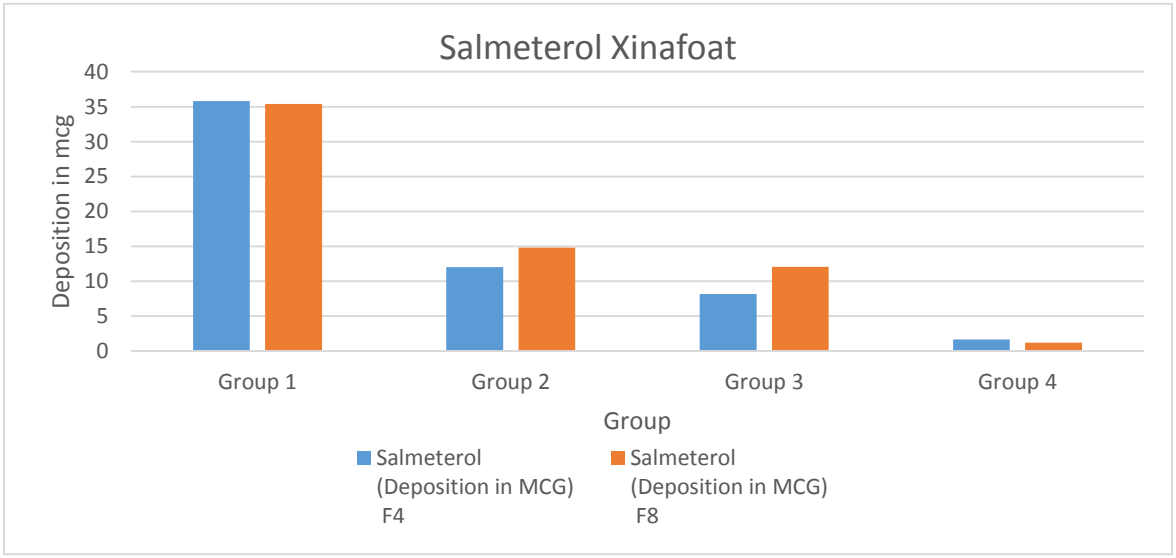
Graph 2: Graphical representation of Salmaterol Xinafoate deposition in NGI stages for (F4&F8)

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.



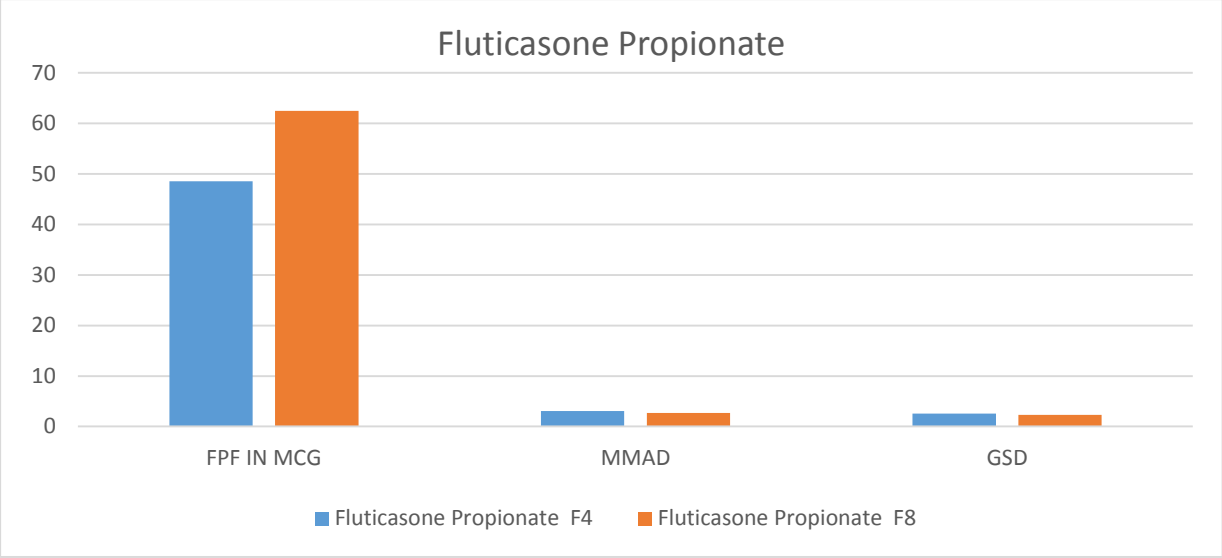
Graph 3: Group wise representation for fluticasone propionate (F4&F8)

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



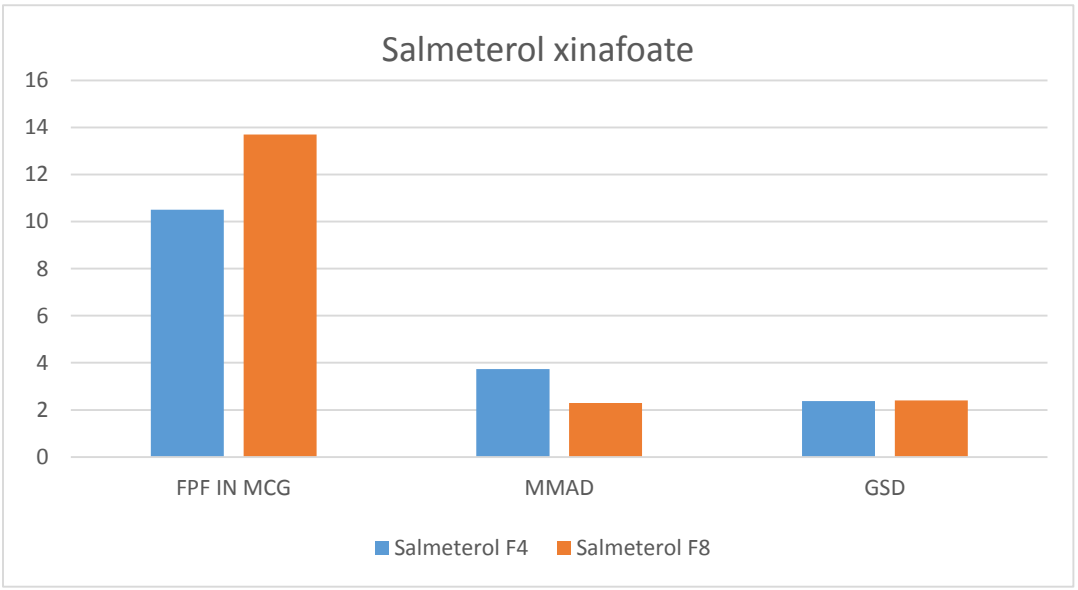
Graph 4: Group wise representation for Salmaterol Xinafoat (F4&F8)

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 similer result found F4 and F8 formulation.



Graph 6: Graphical representation of CITDAS data for Salmaterol 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 comparible result obsrve 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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