'FORMULATION AND DEVELOPMENT OF RECONSTITUTABLE ORAL SUSPENSION OF ANTI-ASTHMATIC DRUG″

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MASTER OF PHARMACY

IN

PHARMACEUTICS

BY

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Under the guidance of

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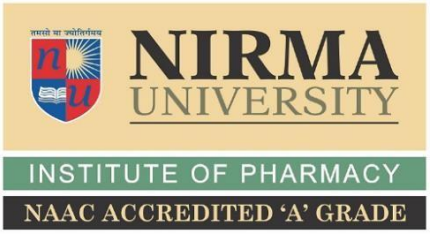

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CERTIFICATE

This is to certify that the dissertation work entitled "Formulation and development of reconstitutable oral suspension of anti-asthmatic drug" submitted by Ms. Mrunal Bhide with Regn. No. (17MPH108) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under my/our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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CERTIFICATE OF ORIGINALITY OF WORK

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DECLARATION

I hereby declare that the dissertation entitled "Formulation and development of Antiasthmatic dry powder for oral suspension", is based on the original work carried out by me under the guidance of Dr. Jigar Shah, Assistant Professor, Department of Pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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MRUNAL BHIDE

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BCS	Biopharmaceutical Classification System
ROS	Reconstituted Oral Suspension
API	Active Pharmaceutical Ingredient
US-FDA	United States- Food and Drugs Administration
IP	Indian Pharmacopoeia
RT	Room Temperature
LOD	Loss on Drying
NMT	Not more than
RS	Related Substances
PhEur	European Pharmacopoeia
IPQC	In Process Quality Control
CU	Content uniformity
BU	Bulk uniformity
FTIR	Fourier Transform Infra-red
HPLC	High Performance Liquid Chromatography
WV	Weight variation
COA	Certificate of Analysis
Aq.	Aqueous
RH	Relative Humidity

FORMULATION AND DEVELOPMENT OF RECONSTITUTABLE ORAL SUSPENSION OF ANTI-ASTHMATIC DRUG

ABSTRACT:

Asthma is a chronic inflammatory disorder characterized by airway obstruction and hyperresponsiveness. Drug X has been used in this case commonly to prevent the wheezing and shortness of breath. Conventional oral tablets and granules are available. However as an alternative dosage form, reconstitutable oral suspension (4mg/5ml) will provide ease of administration and convenience to paediatric patients. It is a multi-dose formulation (6 doses) which is to be reconstituted with water and to be consumed within 7 days. Formulation of reconstitutable oral suspension was carried out by sifting, geometric mixing and direct blending approach. Mannitol, Silicon dioxide (Syloid AL1 FP), Sodium saccharin, xanthan gum, sodium bicarbonate, orange flavor and sunset yellow colour were used for formulation of reconstitutable oral suspension. The drug being highly susceptible to heat, light, pH and moisture, special care was taken while selecting the excipients and during manufacturing of batches. Drug X impurities were a major concern during stability. The drug has a tendency to form impurities during the shelf life. However the excipients were selected accordingly and impurity levels were monitered during the time period. During study, suspension was evaluated for various parameters like viscosity (80-120 cps), pH (6 -8) and % assay of reconstituted suspension (94 -105%). Stability study of dry powder (40ºC/75%RH) and reconstituted suspension (30ºC/65%RH and 2-8ºC) was carried out for reproducible batch for two months and it was within preferred criteria. This ROS formulation shall be advantageous for pediatric asthma patients. Further extensive in-vitro and in-vivo evaluation shall be carried out to confirm its effectiveness.

1. AIM OF INVESTIGATION

DrugX which is a Leukotrine receptor antagonist used in the treatment of Asthma is available in market in various dosage forms such as tablets, granules and conventional suspensions. DrugX is approved by USFDA to be used in pediatric patients from 6 months to 12 years of age. The conventional dosage forms like tablets are inconvenient to consume by the pediatric population. Also the drug is prone to degradation if present is aqueous environment for a longer period of time. Hence aqueous suspensions are not generally preferred. Granules are available as sinlge dosage form. Therefore the aim of this investiagation is to formulate a dry powder which is to be resuspended in water at the time of administration. This formulation will thus provide stability to the drug and also patient complience to pediatric population as it will be a multiple-dosage form. Being a pediatric based formulation, the aim also included taste masking of the drug.

The batches shall be evaluated for characteristic properties of dry powder like flow properties, physico-chemical properties, blend uniformity, content uniformity, assay, related substances and dissolution. The evaluation parameters after recosntitution shall include sedimentation rate, sedimentation volume, viscosity, pH, redispersibility and assay.

Aim of investigation therefore majorly includes protecting the drug against moisture, heat, light and acidic conditions and formulating the product accordingly. The percentage impurity and related substances in the final product should be maintained within the limits specified by Indian Pharmacopoeia .

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2. INTRODUCTION

2.1 Introduction to pharmaceutical suspension

A Suspension is a scrupulous type of dispersed system or in which suspended phase is scattered uniformly into the external phase. The internal phase consists of homogenous solid compound having a particular array of particle size and is maintained evenly in the suspending vehicle with the aid of suspending agent. A suspension containing particles range 1 nm - 0.5 μm size is the colloidal suspension. When the particle size range is between 1 - 100 μm is known as coarse suspension. Most of the pharmaceutical suspensions are coarse suspension.

2.1.1 Pharmaceutical applications of suspensions:

Poorly soluble drugs are mostly preferred in oral liquid dosage forms particular for geriatric, pediatric and patients having difficulty in swallowing solids dosage form.

Rationale for Suspension

1. To reduce the instability of certain drugs in aqueous solution.
2. To mask unacceptable taste of drug, e.g. Paracetamol
3. Suspension can be used topical application: In case of calamine lotion evaporation of dispersing media will leave the active agent by light deposit.
4. It can be used for parenteral administration by intramuscular route. It controls rate of absorption of drug.

2.1.2 Characteristics of Ideal Suspension:

1. The dispersed particles of must not be settle down readily and the settled particle should redisperse easily on shaking. Since one cannot absolutely avoid the sedimentation, it is ideal that the particles should settle slowly.
2. The dispersed particle should not form cake upon settling.
3. The viscosity of the formulated suspension is easily pourable.
4. Formulated suspension should be chemically and physically stable.
5. It should have pleasant taste when administration through oral route.

2.2 Reconstitutable Oral Suspension

Since a long time, the oral drug delivery has gained a higher scope and reputation and has been widely engaged in the systemic release of drugs among all the pharmaceutical products available. The positive aspect regarding the oral dosage form like the ease of administration, patient compliance and stability of formulation created its high level of acceptance.[1]

Although conservative oral suspension can be administered without delay, there exists an additional class of suspension known as dry powders or Reconstituted Oral Suspension (ROS) which is reconstituted at the time of administration. ROS is preferred when drug stability is the foremost concern. After reconstitution, these suspensions have a short but reasonable shelf life when stored at refrigerated temperature and must be used within 7 to 10 days.

Reconstitutable oral suspensions illustrate sufficient chemical stability of the drug throughout the shelf life, avoids the problems of physical stability that are related to solubility, pH, and incompatibilities with other ingredients and it also trims down the weight of the final product since the aqueous vehicle is absent and hence consequently the transportation charge may be abridged.[2]

2.2.1 Disadvantages of liquid oral suspensions :

1. It is a bulk formulation so there is probability of inaccuracy in single dosing.
2. Dose of the drug depends on a range of physical factors of the dosage form for instance storage temperature, rate of sedimentation of the formulation, liquid flow properties like pourability, viscosity, redispersion, flocculation and content uniformity.
3. Stability of the liquid suspension mainly depends on the storagetemperature.
4. Storage can result in caking phenomenon.

2.2.2 Advantages of dry powder for oral suspension:

1. It is advantageous for administration to the pediatric population because of colored, flavored, sweetened formulation.

2. It remains stable on storage and on reconstitution with an ingestible liquid for administration, the corresponding liquid suspension is stable for the extent for which the therapy is required. **(3)**
3. The most common reason of formulation of ROS is insufficient chemical and physical stability of the drug in aqueous vehicles. Conventional suspensions have very short shelf life where as ROS on the other hand have a shelf life of at least 2 years in terms of physical stability.
4. Formulation of ROS reduces transportation expense as an aqueous vehicle is notpresent. It is least susceptible to temperature extremes as compared with conventional suspension.

2.2.3 Required Characteristic of Reconstitutable Oral Suspension :

1. Satisfactory properties of ROS must be maintained before, during, and after reconstitution.
2. There should be no segregation of the dry blend or mixture at the time of development.
3. The powder bring together should disperse effortlessly and should be homogeneous in the aqueous vehicle at the time of reconstitution..
4. The reconstituted suspension have to be easily redispersed and easy to pour which will provide accurate & homogeneous dose.
5. The finished product must have an acceptable oraganoleptic property such as color, odor and taste.

The reconstituted products also require taste-masking. Reconstitution occurs during at the point of dispensing. (4)

The dry blend, or mixture,should be uniform mixture of the proper concentration of every constituent during manufacturing. It must not separate into a non homogeneous mixture as it may cause errors in dosage. The powder blend must disperse rapidly and entirely in the aqueous vehicle during reconstitution. The reconstituted suspension must be effortlesslyredispersed and it should be easy to pour for the patient to provide an precise and uniform dose.(5)

2.2.4 Commonly used excipients :

Since this is a pediatric formulation, the use of excipientsis depends uponpractical requirements and must be reasonable through a risk-based assessment, factors such as the paediatric age group, frequency of dosing and duration of treatmen should be taken into account while selecting excipients for pediatricfomulations. An added challenge is faced by the paediatric medicines as compared to adult medicines. The excipients may show adverse reactions in children that arenot experienced by adults or are not seen to the identicaldegree.(6)

The number of excipients used and their quantity in the development of paediatric medicine formulation should be minimum so as to ensure ansuitable product regarding the performance, stability, palatability, microbial control, dose uniformity and other considerations that are essential to support the quality of the product. Risks for adverse reactions are predominantlyrelated with excipients used for liquid dosage forms.

While choosing the excipients,following aspects should be considered :

- Paediatric safety profile of excipient
- The route of administration
- The single and daily dose of the excipient
- Treatment duration
- Acceptability for the intended paediatric population
- Potential alternatives

Oral suspensions needs a scrupulous combination of ingredients to execute various functions likestabilization,wettingand to impart appropriate co, taste and viscosity. The mixture has to be compatible, non reactive and stable.

The number of excipients was compared with suspensions. The selection criteria for excipients are based on the physical category of powder blend preferred and appropriateness for reconstitution. Excipient number should be kept minimum, because as additional excipients are used, there are probability of problems like –

- The problem of compatibility with API or other excipients is increased.
- Additional processing is required for more number of excipients.
- More excipients will necessitateadditional sampling and testing for quality control

A broad-spectrumtechniqueto reduce the number of excipients is to make use of an excipient that can performmultiple roles, e.g. sucrose, sorbitol acts - as a sweetener as well as a solid diluent.

Table 2.1 Commonly used excipients in ROS

Functional category	**Examples**
Suspending agents	Sodium Alginate , Methylcellulose HydroxyEthyl Cellulose , HydroxyPropyl Cellulose , Xanthan Gum , Acacia , Tragacanth.
Wetting agents	Soybean Lecithin, Polysorbate 80, Sodium Lauryl Sulphate
Sweetner	Sucrose , Aspartame, Sodium sachharin
Preservatives	Sorbic acid, Methyl Paraben , Propyl Paraben
Flavor	Cherry flavor, Vanilla flavor, Banana flavor, Mix Fruit Flavor
Buffer	Trisodium citrate dihydrate, Citric acid anhydrous
Color	FD&C Red No.3, FD&C Red No. 40, D&C Yellow No. 1
Anti-caking agents	Colloidal Silicon dioxide, Amorphous silica gel

2.2.4.1 Suspending agents :

Selection of a suitsble suspending agent is one of the most important factors involved in formulation a pharmaceutical suspension. Suspending agents hinder particle sedimentation by imparting viscosity to the phase. Suspending agents are classified into cellulose derivatives, clays, natural gums, and synthetic gums. In many cases, a combinationof these excipients are used.[8]Suspending agent have to be simply dispersed by vigorous hand shaking at the time of reconstitution.Table 2.2 enlists some suspending agents that are suggested for utilization in formulation of ROS. A commonly used suspending agent in suspension is Xanthan gum because it provides a good batch-to-batch

uniformity, hardly any microbial problems and solution viscosity is nearly independent of pH and temperature.

Table 2.2 List of suspending agent used in ROS[9]

Suspending agents	Stability pH range	Concentrations
Sodium alginate	4-10	1–5 %
Xanthan gum	4-10	1–2 %
Hydroxypropyl cellulose	6-8	1-2%
Hydroxypropyl methylcellulose	3-11	1-2%
Carboxy methyl Cellulose	7-9	1-2%
Colloidal silicon dioxide	0-7.5	2-4%

2.2.4.2 Wetting agents :

The wettbility of the drug depends on it's affinity for water. Many drugs in suspension are hydrophobic; they refuse to accept water, hence are not easily wetted and habitually float on the water surface due to entrapped air. The use of wetting agent aids in the dispersion of hydrophobic drugs. Selection of the wetting agent is based on low concentration and maximum outcome of optimal dispersion. Wetting agent is used as surfactant to slow downthe crystal escalation in range of 0.05%w/w to 0.5 % w/w. Excess wetting may lead to foaming and can give unpleasant taste. Another concern with wetting agents is that itamplifies the risk of caking because coated particles oppose the aggregate development, settle independently and may form dense or caked sediment.

SYLOID® FP silica has a great moisture adsorption capacity because of its highly porous and micronised nature. So when added to a formulation, it is capable of adsorbing a large amount of moisture, keeping the product dry and improving the stability.

Examples: Polysorbate 80, Soybean lecithin, Glycerin , Propylene glycol , Sodium Lauryl Sulphate.

2.2.4.3 Sweetener :

Sweetener has significant role in reconstitute oral suspension. Frequently the drugs have a bitter taste also the suspending agents used in the formulation, particularly clays, might have a bland taste. Sweeteners are useful in masking the unfavourable taste and hence improvethe patient acceptance especially in the pediatric population that uses this product. It is used for taste masking of drug. It can be concluded into 3 main groups-

(A) Bulk sweeteners: Sugars such xylose, glucose, dextroseand sucrose are used at concentration of 15% w/w - 70 % w/w of the total weight of the suspension. Sucrose is used as sweetener, suspending agent and bulking agent in the dry mixture. Sweetening agents can also be used in combination. Taste-masking composition consists of any one sweetening agent and one flavoring agent. The concentration of artificial sweetening agents is between 0 to 0.05 gm/ml.

Sucrose performs functions of both sweetener and suspending agent and can also serve as a diluent in the dry mixture.

(B) Sugar alcohols: Xylitol, Sorbitol, Mannitol and Glycerin

(C) Artificial sweetening agents: Sodium saccharin, Aspartame

Aspartame has reasonable acid stability but its heat stability is poor. The Food and Drug Administrationmight restrict the use of saccharin because of its carcinogenic potential.

2.2.4.4 Preservatives:

Microbial growth affects the chemical stability of excipient, acceptability and safety of the product , so addition of preservatives is obligatory in most of the suspensions. Also the suspending agents and sweeteners added in formulation are subject to microbial contamination. Microbial activity may lead to stability problems if thesuspension is not stored properly or insufficient concentration of preservatives are added.

Table 2.3 List of preservatives used in ROS

Preservative	Concentration
Benzalkonium chloride	0.01-0.02 %

Sodium benzoate	0.02-0.5 %
Sorbic acid	0.05-0.2 %
Methyl paraben	0.015-0.2 %

2.2.4.5 Flavoring agent:

They are used to provide organoleptic preparation to the patient.Flavors improve the patient acceptability of product. They play significant role especially in thepediatric formulations. Natural as well as artificial flavors are used such as orange, raspberry, pineapple, cherry, Banana & Mix Fruit flavor.

2.2.4.6 Buffering Agent:

pH of an oral liquid formulation is an important point in several aspects. Controling the pH of the formulation, can avoid significant changes during storage. Hence, the majority formulations make use of a buffer to manage the probable changes in the solution pH.The selection of a appropriate buffer must be based on –

i. Whether the acid-base forms are mentioned for use in oral liquids
ii. Buffer stability of drug and excipient.
iii. The buffer and container compatibility.

To gain a wider choice of pH as compared to the individual buffer alone, aamalgamation of buffers can also be used. However, all buffers are not appropriate to be used in oral liquids. Buffers are use to maintain pH and provide stability of the suspension. Buffers are used to keep the drug in insoluble form by the maintaining pH. Application of buffer is mention below.

- By change in pH, it prevent decomposition of active pharmaceutical excipients.
- Maintained the Physiological stability

Examples: Sodium Citrate, Citric acid, Sodium bicarbonate.

2.2.4.7 Coloring Agent:

The selection of color should be allied with theflavor that is used to enhance the attractiveness of the product e.g. green color is used for mint-flavour, red for strawberry-flavour and orange/yellow for orange flavoured formulations. Color aids in identification of the product.Colors are obtained either from natural or from synthetic sources. Minerals, plant and animal are the sources for natural colors. Mineral colors, which are also called as pigments, are used to impartcolorto lotions, cosmetics, and other external preparations. Plant colors are extensively used for oral suspension. The synthetic or artificial dyes should be used within a limit of 0.0005 % to 0.001 % . It depends upon the intensity of color required and thewidth of wall of the container to be viewed in it.[10]

Table 2.4 List of coloring agents

White	• Titanium dioxide
Blue	• Brilliant blue • Indigo carmine • Indigo
Red	• Amaranth • Carmine
Yellow	• Tartarazine • Sunset yellow • Carrots • Saffron • Annatto seeds(yellow to orange) • Madder plant(reddish yellow)
Green	• Chlorophyll
Brown	• Caramel (brown)

2.2.5 Preparation of Dry Mixture

2.2.5.1 Powder Blend :

Powder blend known as the powder mixture, is prepared by combining all the excipientsin powder form. When excipients are in small quantities then blending of powder may bedone in two or more stages. This helpds the excipient to be mixed

uniformly with part of major excipient to aid their dispersion. Later stage involves mixing of other excipients.

Advantages :

- Less Cost
- Easy to clean
- These is less chance of physical and chemical stability problem because no use
- of heat and solvent for preparation.
- Low moisture content can be achieved.

Disadvantages:

- Chances of homogeneity problem.
- Poor flow can be the reason for demixing.
- The material lost throughout powder blending will have even greater importance if API is potent drug.

2.2.5.2 Granulated Product:

Most of all excipients are processed by granulation in granulated products. Wet granulation is the frequently used procedure for formulating ROS. Granulating fluid can be aqueous or non aqueous binder solution. There are two approach of incorporating the drug

(1) Excipients and drug can be mix with other.

(2) It should dissolved in granulating fluid.

Wet granulation generally depends on the following steps.

Granulating fluid is used to mix the solid excipients and mass them together. The wet mass is then transformed into dried granules. These granules are then milled with the aid of vibratory sieve or oscillating granulator. For drugs that are susceptible to hydrolysis, non aqueous granulating fluids should be used.

Disadvantages:

- More capital requirement and energy.
- The trace fluid may be produce instability.
- All other exicipient should be stable in granulating fluid.

Suggestion for processing the dry mixture:

- Use efficient mixer.
- Evaluate processing performance of batches on pilot scale up equipment.
- Determine the duration of time required for blending.
- Keep the mixture away from heat and moisture while mixing and the finished batch should also be protected from moisture.
- For the sake of blend uniformity, the sample should be taken from different place of the blender.

Poor flow ability or caking often occurs when individual particles fuse together. There are several reported causes, which include: Poor high temperature stability, Surface charges, Variation of relative humidity and crystallization.

2.2.6 Stability:

Chemical stability is a major issuein reconstituted suspension as compared to that in conventional suspension because drug usually has reduced stability in the presence of water. Separation may be observed after reconstitution of the dry powder. Reconstitution and physical stability of the suspension is still a concern.

2.2.6.1 Chemical Stability:

The main chemical reactions that affect the stability of a drug are oxidation and hydrolysis.[11] Evaluated of chemical stability should be for both dry mixture as well as the reconstituted suspension at controlled temperature, room temperature and storage temperature. While performing the stability evaluation of reconstituted oral suspension, it should be carried out in a container of the identical material and size in which the product is to be marketed.

Degradation of the preservative is tolerableas long asacceptable preservative is present to maintainthe effectiveness. Evaluation at higher temperatures can cause major changes in physical properties like viscosity. Higher Temperature can also considerably transform the solubility of the drug that is suspended.

2.2.6.2 Physical stability:

Physical stability of a suspension includes evaluation of pH, viscosity, sedimentation volume and effortlessness of redispersion. Measure the height of settled drug particles in undisturbed bottles at intervals of time in order to evaluate the sedimentation volume. Sedimentation volume is the sign of the good suspendibility. Exposuring the formulation tofreeze-thaw cyclecan be done to evaluate its physicalstability. After some of cycle, parameters like the particle size distribution, crystal changes, viscosity and sedimentation volume can be measured. Crystallization can also be enlisted as problem.

Table 2.5 Marketed Preparation of ROS

Marketed Product	Drug	Strength	Company
Penbritin Syrup contains 125 mg/5 ml and Penbritin Forte Syrupcontains 250 mg/5 ml	Ampicillin	125, 250mg/ 5 mL	Chemidex Pharma
Co-amoxiclav 400/57mg/5ml	Amoxicillin trihydrate , amoxicillin. Potassium clavulanate	400/57 mg/5 ml	Sandoz
Suprax	Cefixim	100mg/5ml	Sanofi
Zithromax	Azithromycin	300/600/900/1200 mg/ml	Pfizer

2.3 Overview of Asthma

Asthma is a chronic inflammatory disorder characterized by airway obstruction and hyperresponsiveness."The medical term "asthma," which derives from the Greek for "panting," was named by Hippocrates around 400 BC.Sir William Osler described asthma in his Principles and Practice of Medicine in the early 20th century as "swelling of the nasal or respiratory mucous membrane, increased secretion, and.spasm of the bronchial muscles with dyspnea, chiefly expiratory.

Asthma is a multifaceted diseaseof airway swelling characterized by airway inflammation, remodeling, and hyperresponsiveness. Asthma is characterized by gradually worsening shortness of breath, cough, tightness of chest, and/or wheezing.[12]

Asthma and COPD are largely prevalent unceasing respiratory conditions affecting more than 500 million people allacross the world and resulting in considerable morbidity and rise in health-care expenditure.[13] It is estimated that athma affects over 4–11% of the general population, whereas allergic rhinitis is approximated to affect between 10% and 30% of the common population.[14],[15]Although asthma and COPD are usually easy to differentiatein their classic presentations, however many patients demonstrate the features of both.Patients with asthma have also been observed to suffer from allergic rhinitis. It is reported that about 80% of patients who have asthma, also have rhinitis,whereas a considerable number of patients suffering from rhinitis also have asthma. [16]

Table 2.6 Key indicators in asthma[12]

Symptoms	Physical Examination Findings	Modifying Factors
• Wheezing (recurrent) • Cough worse at night Difficulty breathing (recurrent) • Chest tightness	• Thoracic hyperexpansion • Wheezing during normal breathing • Prolonged phase of forced	• Exercise • Viral infection • Animals with fur or hair • Dust

(recurrent)	exhalation • Rhinorrhea • Nasal polyps • Atopic dermatitis	• Mites • Mold • Smoke • Pollen • Changes in weather • Airborne chemicals or dusts • Menstrual cycles

2.3.1 Pathophysiology :

2.3.1.1 Inflammation and Airway Remodeling :

Numerous cell types such as eosinophils, T lymphocytes, mast cells, macrophages andneutrophils are known to mediate the inflammation in asthma.[17] Symptoms of mild asthma are reversible and episodic.,however withsequence of the disease, it can cause long-term and permanentchanges in airway . Longterm changes such as smooth muscle hypertrophy and hyperplasia; amplified production of mucus (and associated risk of mucus plugs); and edemaare foreseen. Permanent changes are thickening of subbasement covering, subepithelial inflammation and fibrosis, hypertrophy and hyperplasia of airway muscle, proliferation and dilation of blood vessel, and mucus gland hyperplasia and hypersecretion.[18],[19]

2.3.1.2 Bronchoconstriction and Airway Hyperresponsiveness:

Bronchoconstriction can be provoked by numerous pathways. IgE-dependent mast cell degranulation causes allergen-induced bronchoconstriction, as a result of which the release of histamine, tryptase, leukotrienes, and prostaglandins takes place.[20] Bronchoconstriction can also occur by mast cell degranulation, secondary to osmotic stimuli, which most likely causes the bronchoconstriction induced by regular exercise.

2.3.2 Types of Asthma :

Since different factors coalesce reason for the asthma, several diverse types of the disease, split by age and severity.

2.3.2.1 Paediatric asthma :

Children are more prone to have an alternating form of asthma that manifests in severe attacks. Some kids might feelasthma on daily basis, but the frequent characteristic of the children suffering from asthma is aincreased sensitivity to substances that are likely to cause allergy. Mild asthma canbe resolveddevoid of treatment during childhood. However, there is a risk that the condition might worsen later on, especially when symptoms are either moderate or severe.

2.3.2.2Adult-onset asthma:

Asthma in adults is consistent and needs administration on daily basis of flare-ups and preventive symptoms. Asthma can commence at whichever age. At least 30 percent of adult presentations of asthma are a result of different allergic incidences. Fatness is a great risk reason for beginning of adult asthma, and the females are expected to growthis disorder beyond the stage of 20 years. Individuals more than the age of 65 years, make up a large fraction of deceases caused because of asthma.

2.3.2.3 Occupational asthma :

Occupational Asthma is well-defined as asthma caused by work exposure.[21]

Briefly, OA is a ailment that is characterized by uneven airflow restriction and/or airway overresponsiveness caused deu tospecific occupational surroundings and not because of the stimuli metseparate the workplace.[22][23]

This is a category of asthma observeds as a result of aparticluar type of job or profession.Symptoms will become noticeable after attending a particular type of workplace. Industries which have regular associations to occupational asthma consist of baking, laboratory work, or manufacturing. This type of work environment leads to the return of childhood asthma or the commence of adult-onset asthma. Other indications maycomprisered eyesand runny nose.

2.3.2.4 Severe asthma :

Such type involves a consistent, unbearable asthma symptoms and difficulties in breathing.

Table 2.7 Classification of severe asthma according to WHO recommendation (2010)[24]

WHO class	Name	Explanation
I	Untreated severe asthma	Uncontrolled. as yet untreated asthma
II	Difficult-to-treat asthma	Uncontrolled asthma due to adherence problems. persistent triggers. or comorbidities
III	Therapy-resistant asthma	Uncontrolled asthma despite maximum therapy or asthma control that can only be maintained with maximum therapy

2.3.3 Asthma management and treatment:

There are no definite remedies for asthma, but with effective asthma treatment and management, the symptoms can be controlled.

- Controller medications should be taken daily. These consist of inhalation **corticosteroids** (fluticasone, budesonide (Pulmicort Flexhaler), mometasone

(Asmanex), ciclesonide (Alvesco), flunisolide (Aerobid), beclomethasone (Qvar) and others).

- Amalgamation inhalers include an inhaled hormones along with a **beta-agonist.** LABAs act as symptom-controllers that are useful in opening the airways. However,they may carry some risks in certain people.
- **Anticholinergics** are a kind of inhaled medications that aid in opening of the blocked airways. The medication tiotropium (Spiriva Respimat) is used as maintenance therapy in asthma.
- **Leukotriene modifiers** are oral medications that include Drug X (Singulair), zafirlukast (Accolate).

2.3.4 Treatment of asthma with drugs modifying the leukotriene pathway

Leukotrienes and former commodities of the 5-lipoxygenase pathway provoke the pathophysiologic responses analogousto those associated with asthma in addition to their potent bronchoconstrictor properties.[25]Particularly, 5-lipoxygenase products can lead to tissue edema[26]and movement of eosinophils[27] and are observed to stimulate airway secretions. Cell cycle stimulation and proliferation of smooth muscle and different hematopoietic cells is also brought about by the leukotrines[28-30], these observations present further confirmation of a probable function of leukotriene modifiers in changing the biology of the airway barrier in asthma.

- **The 5-lipoxygenase pathway :**

A precursor fatty acid known as Arachidonic acid is transformed into the leukotrienes through the 5-lipoxygenase pathway. (Fig. 1)[31,32]

This is available in the intracellular microenvironment when one of the various forms of phospholipase A2 cleaves it from cell-membrane phospholipids.

Leukotriene A4 is unbalanced and gets quickly transformed to leukotriene C4 orelse leukotriene B4.[33] A specific transmembrane transporter then exports the Leukotriene C4 to the extracellular space.

- **Leukotriene receptors:**

It exercise their biologic behaviour by binding and activate the specific receptors. Two subtypes of the receptorhave been identified pharmacologically for cysteinyl leukotrienes, , however their molecular structures are unknown. Most of the activities of the cysteinyl leukotrienes that are mediated by the CysLT1 receptor include human airway smooth muscle contraction, chemotaxis, and improved vascular permeability.[34]

Table 2.8 Drugs that act on the 5-lipoxygenase pathway

Drug class and name	Trade name	Recommended oral dose	Other information
Leukotriene-receptor antagonist			
Drug X	Singulair	10 mg each night in adults; 5 mg each night in children (6–12 yr)	Pediatric dose is a chewable tablet.
Pranlukast	Onon, Ultair	225 mg twice daily	
Zafirlukast	Accolate	20 mg twice daily	Take 1 hr before or 2 hr after eating.
5-Lipoxygenase inhibitor			
Zileuton	Zyflo	600 mg 4 times daily	Measure serum alanine aminotransferase before treatment, every month for 3 months, and periodically thereafter.

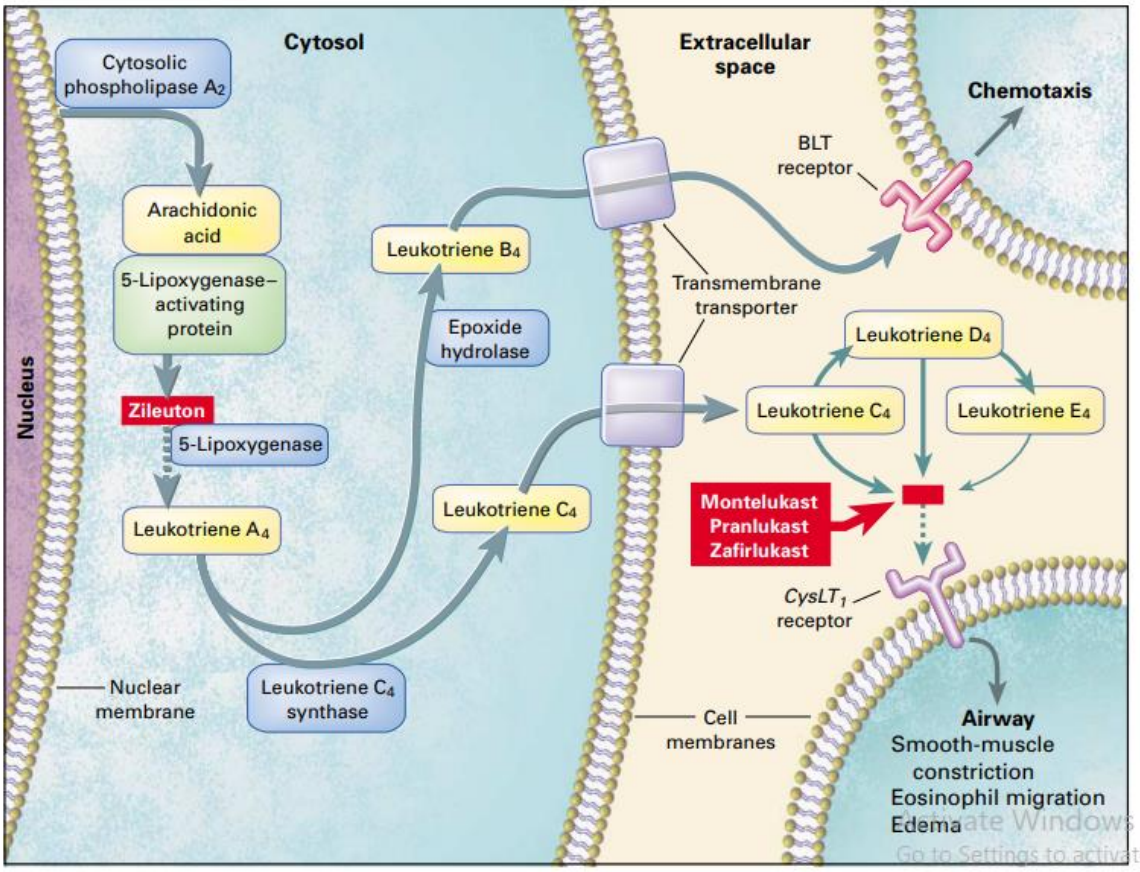


Figure 2.1. Biochemical Pathways of the Formation and Action of the Leukotrienes and Sites of Action of Leukotriene-Modifying Drugs. Enzymes are shown in blue, products in yellow, essential cofactor in green, and drugs in red. Although the synthesis of leukotrienes B4 and C4 probably takes place in close proximity to the nuclear membrane, for clarity they are shown throughout the cytosol. BLT denotes the B leukotriene receptor. An individual cell may produce the cysteinyl leukotrienes, leukotriene B4, or in rare cases both.[35]

2.4 DRUG PROFILE: DRUG X

Table 2.9 Pharmacopoeia status of Drug X

Monograph	IP	BP	USP	EP
Drug X	Yes	Yes	Yes	Yes

(A) Description: A White powder.

(B) Category: Leukotriene receptor antagonists

(C) pKa: pKa1 = 4.4 ; pKa2 = 3.12

(D) Log p: 8.98

(E) Water Solubility: 0.2 mg/ml

(F) BCS class: Class II (Low Solubility, High permeability)

(G) Indications: For the treatment of asthmand to relieve symptoms of seasonal allergy.

(H) Mechanism of action: In the human airwayDrug X selectively antagonizes leukotriene D_4 (LTD_4) at the cysteinyl leukotriene receptor, $CysLT_1$.

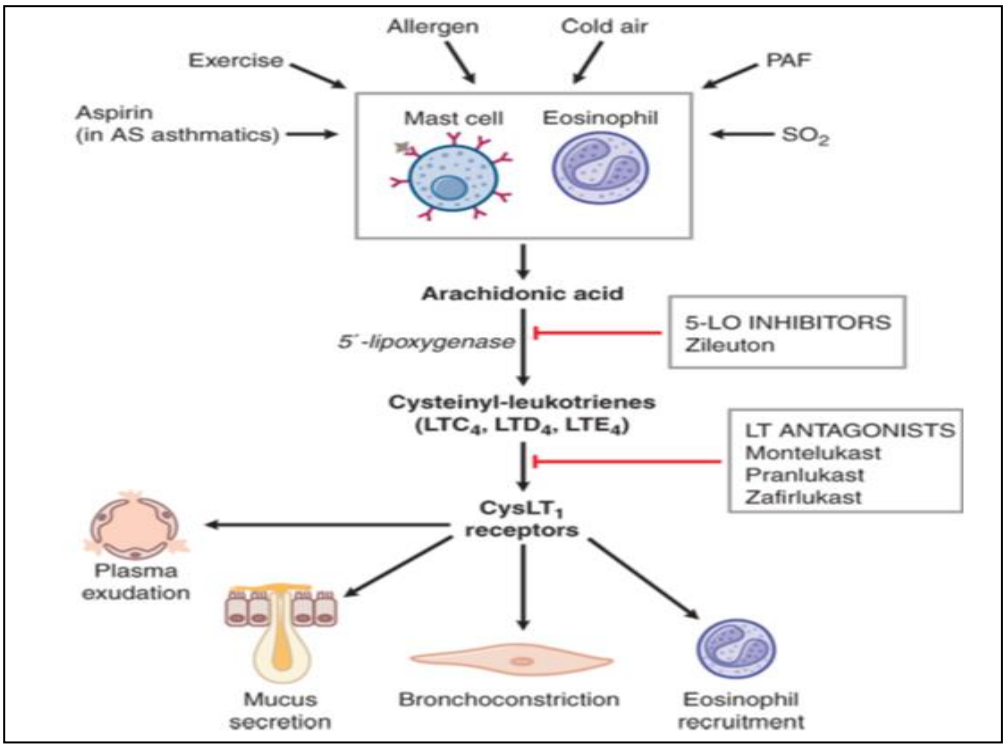


Figure 2.2 : Mechanism of action of Drug X

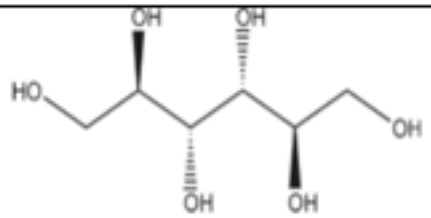
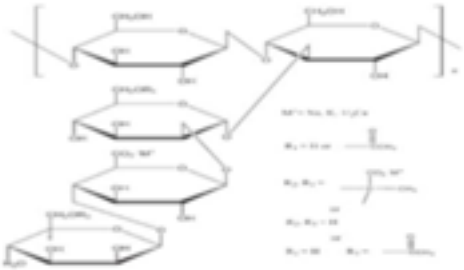
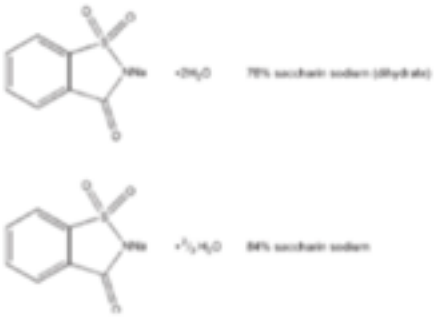
Table 2.10 Pharmacokinetic parameter of Drug X

Pharmacokinetic parameter	Reported values
Tmax	2-3 hrs
Absolute bioavailability	64%
Protein binding	99% (to plasma proteins)
Clearance	45 mL/min [healthy adults]
Route of elimination	exclusively via the bile
Elimination half life of active metabolite	2.7-5.5 hours

(I) Toxicity : Side effects of this drug includes headache, dizziness,abdominal or stomach pain, fever, cough, dental pain, heartburn, stuffy nose, skin rash, weakness or unusual tiredness.

2.5 EXCIPIENT PROFILE

Table 2.10 Excipient profile

Name of excipient	Structure	Functional category	Application
Mannitol		Diluent; plasticizer ; sweetening agent	As an Diluent in tablet formulation, manufacturing of chewable tablets.
Silicon dioxide	$(SIO2)n$	Anticaking agent, viscosity control agent	In range of food products. Tableting agent.
Xanthan gum		Stabilizing agent, suspending agent	Manufacturing of chewable tablet formulations
Saccharin sodium		Sweetening agent	Beverages, Food products
Sodium bicarbonate	$NaHCO3$	Therapeutic agent, alkalizing agent	In effervecent tablets and granules, maintain an alkaline pH

3. REVIEW OF LITERATURE

3.1 Review of literature for reconstitutable oral suspension

Kotliar, E. M. et al [40]hasillustrated a composition of powder for oral suspension containing Azithromycin. He used direct blending methodin order to formulate a non cackingoral suspension of Azithromycin. Excipient chosen were sucrose which functioned as diluent (50% to 98% w/w) based on the bulk weight of the powder for oral suspension, sweeteners used in the range of (50% to 98% w/w), binders utilized between (0.1% to 10% w/w), suspending agents (0.1% to 10% w/w), buffers, gliding agents, flavoring agents, dispersing agent (0.1 % to 4%,) coloring agents in the range of (0.005% w/w to 0.15%w/w) and wetting agents(0.1 %w/w to 4%w/w). The powder was subjected to stability study at room temperature of 25°C/60% RH and at accelerated conditions, i.e. about 40°C/75% RH for 6 month.

Yuqian Du et al [41]performed this work with the inntension to camouflage the bitter taste of Cefuroxime axetil and increase its oral bioavailability. Preparation of dry suspensions wasdone by the way of wet granulation as well as by the method of solid dispersion.It was indicated by DSC analysis that CA was in amorphous form in the solid dispersion when stearic acid acted as the carrier, contributing to an enhancement in the rate of dissolution.On performance of tasteevaluation by three volunteers it was observed that the taste masking was effectively achieved. The observed values of C_{max} and AUC_{0-12} for the experimental suspension were found to be 1.78-times and 2.17-times advanced than that ofthe reference suspension used. The results obtained confirmed that solid dispersion can effectively cover the bitter taste of CA and drastically improves its oral bioavailability.

Scheler, S. et al[44] formulated powder mixtures for antibiotic dry syrup. In this formulation dry syrup of powder mixture of beta-lactam antibiotics as API and excipients were using direct blending approach of dry powder. It contained 80.0 to 95.0 %w/w powdered sugar, up to 1.0 %w/w preservative, up to 2.0 %w/w colloidal silicon dioxide.

Jain, D. et al[45] formulated and evaluated ROS of Ambroxol HCl and Azithromycin using direct blending approach. Xanthan gum and acacia were used as suspending agent. ROS showed sufficient chemical stability of the drug throughout shelf life. The prepared

suspensions were evaluated by following parameter: flow properties, rheological and sedimentation behavior. The ROS of Azithromycin and Ambroxol HCl were provide to be stable over its proposed shelf life of 15 days after reconstitution. The reconstituted suspensions were stored at 4°C, 25°C and 45°C for 15 days. The reconstituted suspension stored at different temperature was evaluated after reconstitution and after 7th and 15th day of reconstitution.

Wang, L. et al[46] prepared and evaluated taste masked oral suspension of Arbidol hydrochloride. Taste masking of bitter taste of Arbidol hydrochloride (ARB) with the combination of solid dispersion and flavors. Taste masking was effectively done by solid dispersion with octadecanol as the carrier by fusion method. Suspending agents, carriers and other excipients were selected.

3.2 Review of literature for Drug X

Tiwari, S. K Et al[47]performed a study was to understand the degradation actions of the drug in diverse oxidative media that contained of hydrogen peroxide, Fenton's reagent , AIBN, Fe^{3+}, and O_2environment underneathstandard laboratory light conditions. It was observed that a total of nine degradation products (X 1 to X 9) were formed from the drug substance as well as the marketed formulation of tablet on storage when placed under controlled oxygen environment in standard laboratory conditions for light and temperature. The information regarding the structure of DPs helped in postulating the degradation pathway of the drug. Also, mechanism for the formation of each DP was estimated. Finally, physicochemical as well as absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of the DPs were predicted by ADMET Predictor™ software.

Al Omari, M. Et al [48]in his study performed the chemical stability ofdrugX in solution and in its solid state. The drug which was incorporated in the solution form, displayed instability on exposure to light leading to the formation of its major photoproduct in the form of *cis*-isomer. In solid state, there was more than 20% decline in its potency after exposure to daylight for 1 week. The main degradation product in the concluding solvent was DrugX *S*-oxide which was also detected as a chief degradation product in the tablet dosage form for the duration of incubation at 40°C/75% RH for 6 months. Findings of this study have helped to comprehend the stability pattern of the drug and to create the critical parameters in a way that might affect the analysis and manufacturing activities.

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4. EXPERIMENTAL WORK

4.1 List of materials and equipments

Various materials and equipments used to carry out the experimental work are listed below**.**

List of Materials

Table 4.1 List of Materials

Sr. no.	Name	Category	Suppliers of Material
1	Drug X	Anti-asthmatic	Morepen Lab
2	Silicon dioxide NF (Syloid AL1-FP)	Moisture protectant	Grace GMBH & CO KG
3	Mannitol	Diluent	Simbhaoli sugars
4	Saccharine sodium	Sweetner	Blue circle specialities chemicals
5	Xanthural 75 (Xanthural Gum)	Suspending agent	C P Kelco
6	Sodium bicarbonate	Alkalizing agent	Sujata chemicals
7	Disodium Edetate	Complexing agent	PolypharmPvt. Ltd
8	Orange flavour 501071 AP0551	Flavour	Ferminch
9	Sunset yellow FCF	Coloring agent	Koel colours Pvt. Ltd

List of Equipments:

Table 4.2 List of Equipments

Sr. no.	Equipment	Manufacturer	Model
1	Digital weighing balance	Mettler Toledo	PB602 – S
2	Sieve 20/40/60/80/100#		
3	pH meter	Labindia	Pico pH meter
4	Viscometer	BrookField	Brookfield LV
5	Dissolution apparatus	Electro lab	TDT-08L
6	HPLC apparatus	Perkin Elmer	LC2010
7	Conical Blender	Kalweka	VDM4

4.2 Pre-formulation studies :

The principal steps in the process formulation growth is the preformulation testing. The purpose of preformulation testing is to study both the physical as well as the chemical properties to produce information that can be of use informulation and development of a dosage form that is therapeutically efficient and pharmaceutically stable.

4.2.1 Characteristics of Drug X

4.2.1.1 Organoleptic Properties

The color of **Drug X**was observed by visual analysis in which sufficient quantity of sample was spread on a glass. Physical nature and other observations were recorded**.**

4.2.1.2 Solubility

Drug X was found to be freely soluble in 95% CH3CH2OH, in methanol and in water; practically insoluble in ACN.

4.2.1.3 Identification of Drug X

(A) By Fourier Transforms Infrared Spectrophotometer (FTIR) Spectra of Drug X:

The infrared spectrum of the sample in potassium bromide dispersion is concordant with the spectrum obtained from the similar preparation of Drug X RS or with the reference spectrum of Drug X.The FTIR spectrum of the substance being examined should be match with the FTIR spectrum of Drug X working standard.

(B) By High Performance Liquid Chromatography (HPLC) : Assay

- **Solvent mixture :-** A mixture of 1: 3 water and methanol
- **Test solution :-** To a quantity of powder containing 60mg of Drug X, add sufficient solvent mixture to obtain a solution containing the equivalent of 0.024% w/v of DrugX.
- **Reference solution :-** A solution containing 0.033% w/v of dicyclohexylamine RS form of the drug in solvent mixture.
- **Chromatographic System:**
  - **Mode:** LC
  - **Detector:** UV 255 nm
  - **Column:** 10 cm X 4.6 mm ; 5µm packing of phenylhexysilane
  - **Column Temperature:** 50 °C
  - **Mobile phase :**Mixture of 2 volumes of acetonitrile and 3 volumes of methanol
  - **Flow Rate :**1.5ml/min
  - **Injection Volume:** 15 µL
- **System Suitability:**
  - **Sample :** STD Solution
  - **appropriateness Requirement:** Tailing Factor : NMT 2.0
  - **Relative STD Deviation:** NMT 1.0%

Analysis: $\frac{ru}{rs} X \frac{Cu}{Cs} X 100$

$\mathbf{r_u}$: Peak Response from sample solution

$\mathbf{r_s}$**:** Peak Response from Standard solution

Cs: Conc. of DrugX RS in STD solution (mg/ml)

Cu: Conc. of DrugX RS in Sample solution (mg/ml)

4.2.1.4 Flow Properties:

20 g powder was taken and placed in 100 ml of measuring container . Volume engaged by the powder noted down as V_0, without disturbing the cylinder. Then cylinder was fitted in instrument and 10 taps were preformed. After 10 taps, volume was noted down as Va. Again after 500 taps volume was noted down as Vb. The difference between Va and Vb was less than 2.0% so tapped volume was noted down without further processing. Bulk density and tapped density, Carr's index, Hausner ratio was calculated.

4.2.1.5 Particle size analysis :

Particle size of the drug was affected on physical and chemical properties. Particle size analysis was done with the help of microscope by the inhouse method.

4.2.1.6 Related Substances :

HPLC system: HPLC with auto sampler, UV detector, pump and inbuilt column

compartment (Make: Agilent 1100 series or equivalent)

- **Chromatographic parameters:**
  - **Mode:** LC

- **Detector:** UV 255 nm
- **Column:** 10 cm X 4.6 mm ; 5µm packing of phenylhexysilane
- **Column Temperature:** 50 °C
- **Mobile phase :**Mixture of 2 volumes of ACN and 3 volumes of methanol
- **Flow Rate :**1.5ml/min
- **Injection Volume:** 15 µL

Solvent mixture :- A mixture of 1 volume of H2O and 3 volumes of Methanol

Test solution :- To a quantity of powder containing 60mg of Drug X, add sufficient solvent mixture to obtain a solution containing the equivalent of 0.024% w/v of DrugX.

Reference solution :- A solution containing 0.033% w/v of dicyclohexylamine RS form of the drug in solvent mixture.

System suitability:

- **Sample:** System Suitability Solution
- **Suitability Requirement:** Resolution: not less than 1.5 between DrugXrelated Compound A and DrugXRelated Compound B

Analysis: Sample Solution

Calculate other percentage of each impurity in the portion of DrugX

$$\frac{Ru}{Rs} X100$$

Ru: Response of peak after each impurity

Rt: Sum of all peak response

4.2.1.7 Water content

Moisture content of the drug sample was determined using Karl Fischer instrument. Drug was directly placed in an open Petri dish to high humidity levels (40°C /75 % RH and 25°C / 60 % RH) and the moisture gain was monitored till equilibrium % loss on drying (LOD) was achieved. Loss on drying was measured by moisture analyzer.

4.2.1.8 pH – Solubility Profile

The solubility of drug in different pH range of the gastrointestinal tract was determined to aid in selecting suitable dissolution media for development purpose and to ensure that 'sink conditions' are maintained therein.

10mg of drug was dispersed in 250ml of aqueous pH solutions.

4.2.2 Drug-excipient compatibility study:

Drug- Excipient compatibility study was carried out by placing drug alone or drug along with excipients in specific ratio in stopper vials at 2–8˚C, 30˚C/60%RH and 40°C/ 75% RH for 1 month and compatibility study was also carried out in open vial 40˚C/75%RH for 1 month for assay and related substances of the mixture were carried out at initial, 15 days and after 1 month.

4.2.2.1 Condition for Drug - Excipient compatibility study

Drug excipients compatibility study was carried out by placing drug alone and drug with excipients in different ratios. One month compatibility study was carried out at 30°C/ 60% RH and 30°C/ 60% RH. The results were evaluated on the basis on Assay value.

4.2.2.2 Drug - Excipient compatibility study: Related Substance

Drug excipients compatibility study was carried out by placing drug alone and drug with excipients in different ratios. One month compatibility study was carried out at 30°C/ 60% RH and 40°C/ 75 % RH. The chemical analysis results of the samples are shown in Table 4.14 and Table 4.15.

4.3 REFERENCE PRODUCT CHARACTERIZATION

4.3.1 Physical Characterization of Reference Product

The reference product Delpomont® oral suspension (4mg/5ml) was manufactured by Akums drugs and Pharmaceuticals Ltd, India. Delpomont® Oral Suspension is available as 4mg/5ml in Indianmarket. The physical characteristics of Delpomont® Oral Suspension (4mg/5ml) are given in Table 4.16. The product details of Delpomont® Oral Suspension are given inTable 4.17.

4.3.2 Chemical Characterization of Reference Product

The reference product **Delpomont® Oral Suspension 4mg/5ml** was evaluated for chemical characteristics i.e., related substances and assay. The results are described in Table 4.18

4.4 EVALUATION PARAMETER FOR RECONSTITUTABLE ORAL SUSPENSION

Procedure for Reconstitution

Labeled Claim: Each bottle contains 24mg DrugX in 30gof Powder Blend. 5ml of the reconstituted suspension contains 4mg of drug.

Procedure:

- The bottle should be tapped multiple times to slacken the powder. Measure 30 ml water in cylinder.
- Add around half quantity of water that is used for constitution in the bottle, closed the bottle and shake it well for around 1 minute.
- Add remaining half quantity of water then shake bottle for approximately 1 minute.

4.4.1 Evaluation Parameter

4.4.1.1 For Powder before Reconstitution:

(A) Flow Properties:

Bulk density : It is used to portray a stuffing of particles. The formula for bulk density determination is:

$$\text{Bulk Density} = \frac{\text{Weight of powder}}{\text{Bulk volume}}$$

Tapped density: For measurement of tapped density, powder should be packed in a cylinder. Then tap on Taped density apparatus (Electrolab – ETD1020). After 10 taps, volume is measured and not more than 2% variation. If variation is more than 2%, it should be observed after 500 times tapping. If still variation is more than 2%, powder is tapped for 1250 times.

$$\text{Tapped Density} = \frac{\text{Weight of powder}}{\text{Tapped volume}}$$

Compressibility Index (CI): Compressibility is associated with the flow

rate, cohesive property and particle size distribution.

The flow characters of blend are given in table 4.20

$$\text{Carr's Index} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \text{ X } 100$$

Hausner's Ratio: It is the relation between tapped density and bulk density.

$$\text{Hausner's Ratio} = \frac{Tapped\ density}{Bulk\ density}$$

Table 4.3 Effect of Hausner's Ratio and Carr's Index on flow property

Carr's index (5)	Flow character	Hausner's ration
<10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
>38	Very, very poor	>1.60

(B) Particle size determination:

100 gm of moisture free dried powders was transferred to digital sieve shaker. Sieves (Electrolab) of different mesh size were used for analysis and amount of powder retained on sieves was calculated.

(C) Loss on Drying:

Loss on Drying (LOD) indicates % content of water present in sample. Samples weighing 1-2 gm were kept in Halogen moisture analyzer (Mettler Toledo) at 75ºC.

(D) Blend Uniformity: (Assay)

Standard Preparation: Transfer an precise quantity of weighed powder i.e about 100 mg of DrugX working standard in 100 ml of flask. Dissolve and adulterate to make up the volume with methanol and then mix. Final concentration of solution was 2µg/ml.

Assay Preparation: Transfer an precise quantity of powdered sample weighed which is equivalent to 100 mg of DrugX in 100 ml flask. Put in 70 ml of Methanol and sonicate for

15 minute taking care to maintain temperature of ultrasonic bath below 10ºC, dilute to required volume using methanol and then mix. Filter it using 0.45µ Nylon filter discarding first 5 ml of filtrate. Final concentration of solution was 2µg/ml.

System Suitability: Measure the absorbance of standard preparation six times at 250 nm using UV/VIS Spectrophotometer (Shimadzu UV-1800). Methanol was used as a blank and the results were recorded. Relative standard deviation of six absorbance observation should not be more than 2.00%.

Procedure:

Measure the absorbance of assay preparation at 255 nm using appropriate UV/VIS Spectrophotometer. Use methanol as a blank. Estimate the content of DrugX in % of label claim using the values of absorbance obtained from the standard preparation, Assay preparation and percentage potency of working standard used.

Calculation:

$$Assay = \frac{Au}{As} X \frac{W1}{100} X \frac{5}{50} X \frac{10}{50} X \frac{100}{W2} X \frac{50}{5} X \frac{50}{10} X \frac{100}{L.c} XP$$

Au: Absorbance of Assay preparation.

As: Mean absorbance of standard sample.

W1: Weight of DrugXworking sample in mg.

W2: Weight of sample taken in mg.

L.C.: Label Claim in %w/w.

P: Potency of DrugX working standard in percentage on as is basis.

Table 4.4 Inprocess Sampling Protocol : Blend Uniformity

Sampling Positions	Top	Bottom
Left Front	S1	S2
Left Back	S3	S4

Right Front	S5	S6
Right Back	S7	S8
Middle Centre	S9	S10

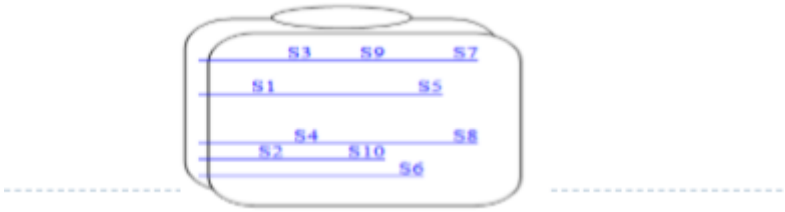


Figure 4.1: Sampling Point for the Blend Uniformity

4.4.2.2 Evaluation of Reconstituted oral suspension:-

(A) %Assay (Content Uniformity)

Detrmined by liquid cgromatography

Test solution :- To the filling of one bottle of powder, add 100ml of methanol, mix using ultrasound. Add adequate methanol to obtain a solution containingthe corresponding of 0.002 % w/v of the drug.

Reference solution :- A 0.00264% w/v solution containing of DrugXdicyclohexylamine RS in methanol.

Table 4.5 Chromatographic system for assay determination

Column	stainless steel column 10 cm *3.0mm, packed with phenylsilane bonded to porous silica (5 µm)
Column temperature	50°C
Mobile phase	equal volumes of 0.2 % v/v solution of trifluoroacetic acid in water and a 0.2% v/v solutin of trifluoroacetics acid in acetonitrile
Flow rate	0.9 ml per minutes
Specrtrophotometer set at	389 nm
Injection volume	5 µl

Inject the reference solution and the test solution.

Analysis:

Sample: Standard Solution , Sample Solution

Calculate % of DrugXin portion of DrugX for oral suspension taken

$$Assay = \frac{Au}{As} X \frac{W1}{10} X \frac{2}{20} X \frac{200}{W2} X \frac{50}{5} X \frac{W3}{L.C} X \frac{p}{100} X 10$$

Where,

Au: Mean peak area of sample solution

As: Mean peak area of the standard solution

W1: Weight of standard mg

W2: Weight of sample taken mg

W3: Weight / ml of suspension in mg/ml

L.C.: Label Claim

P: Potency

(B) Dissolution:

Medium: A 900 ml of 0.5 % w/v solution of sodium dodecyl sulphate in water.

Apparatus: IP Apparatus No. 1 (Paddle)

RPM: 50

Time: 20 min

Pull out a appropriate volume of medium, filter this solution.

Test solution : Make use of the filtrate

Standard Solution: A 0.033% w/v solution of Drug X dicyclohexylamine RS in the methanol. Dilute 1.0 ml of this solution upto 50.0 ml using a 0.5 % w/v solution consisting of sodium dodecyl sulphate.

Table 4.6 Chromatographic system for dissolution :-

Column	stainless steel column 10 cm * 4.6mm, packed with phenylsilane bonded to porous silica (5 µm)
Column temperature	50°C
Mobile phase	equal volumes of 0.2 % v/v solution of trifluoroacetic acid in water and a 0.2% v/v solutin of trifluoroacetics acid in acetonitrile
Flow rate	0.9 ml per minutes
Specrtrophotometer set at	389 nm
Injection volume	5 µl

Add the reference solution and the test solution.

$$\%\ Drug\ release = \frac{Au}{As} X \frac{W1}{100} X \frac{900}{W2} X \frac{W3}{L.C} X \frac{p}{100} X 10$$

Au: Absorbance value of sample

As: Mean Absorbance value of Standard.

W1: Weight of DrugX working standard in mg

W2: Weight of sample taken mg/ml (After weight – Before Weight)

L.C.: Suspension Label Claim of DrugX (mg/ml)

P: Strength of working standard solution is in % on as such basis.

Tolerance: NLT 80% (Q) of the mentioned amount of DrugX is Dissolved.

(C) Related Substance

Determined by liquid chromatography

Table 4.7 Solution preparation for Related substances :

Solvent mixture :	A mixture of 1 volume of water and 3 volumes of methanol
Test solution :	To a quantity of powder containing 24mg of DrugX add 250ml of the solvent mixture with the aid of ultrasound.
Reference solution (a) :	Dilute 1.0ml of the test solution to 100.0ml with the solvent mixture. Further dilute 1.0ml of this solution to 5.0 ml with solvent mixture.
Reference solution (b) :	To 10 ml of test solution add 4µl of hydrogen peroxide solution (100vol) and mix. Expose the solution to ambient light for 1 hour.

Table 4.8 Chromatographic system for Related substances :-

Column	A stainless steel column 10 cm * 4.6mm, packed with phenylhexylsilane bonded to porous silica (5 µm)
Column temperature	50°C
Mobile phase	a solution containing 0.2 % v/v of trifluoroacetic acid in water and a mixture of 2 volumes of acetonitrile and 3 volumes of methanol
Flow rate	1.5 ml per minutes
Specrtrophotometer set at	255 nm
Injection volume	15 µl

Calculate % of each impurity in portion of the drug for oral suspension taken.

Time (in min)	Mobile Phase A (% v/v)	Mobile Phase B (% v/v)
0	48	52
5	48	52
10	45	55
17	45	55
27	25	75
28	25	75
30	48	52
40	48	52

$$Relative\ Substance = \frac{Ru}{Rs} X \frac{Cs}{Cu} X \frac{1}{F} X100$$

Where;

Ru: Peak response of each individual impurity from the sample solution.

Rs: Peak response of each individual impurity from standard solution.

Cs: Concentration of DrugXin standard solution.

Cu: Concentration of DrugXin sample solution.

F: Relative response factor

Table 4.9 Relative Substance profile for DrugX ROS

Name	Relative retention time	Acceptance criteria NMT%
DrugX Impurity C	0.45	1.7
DrugX Impurity G	0.92	0.15
DrugX	1.0	--

DrugX Impurity F	1.04	0.15
DrugX Impurity D	1.16	0.15
DrugX Impurity E	1.18	0.15
DrugX Impurity B	1.55	0.3
Individual unspecified impurity	--	0.2
Total impurity	--	**2.1**

(D) Deliverable Volume:

The following test is give guarantee that when oral liquids are transferred from the container that they were originally placed in, will carry the quantity of dosage form that is confirmed on its label. Such tests are relevant for less than 250 ml products, which are given as liquid preparations or reconstituted suspension. When content of the container was transfer to measuring cylinder , avoid the air bubble. Every container is allowed to drain for not more than 30 min for container for multiple unit dose and 5 sec for single unit containers. Measure the volume of each mixture when it is free from air bubble.

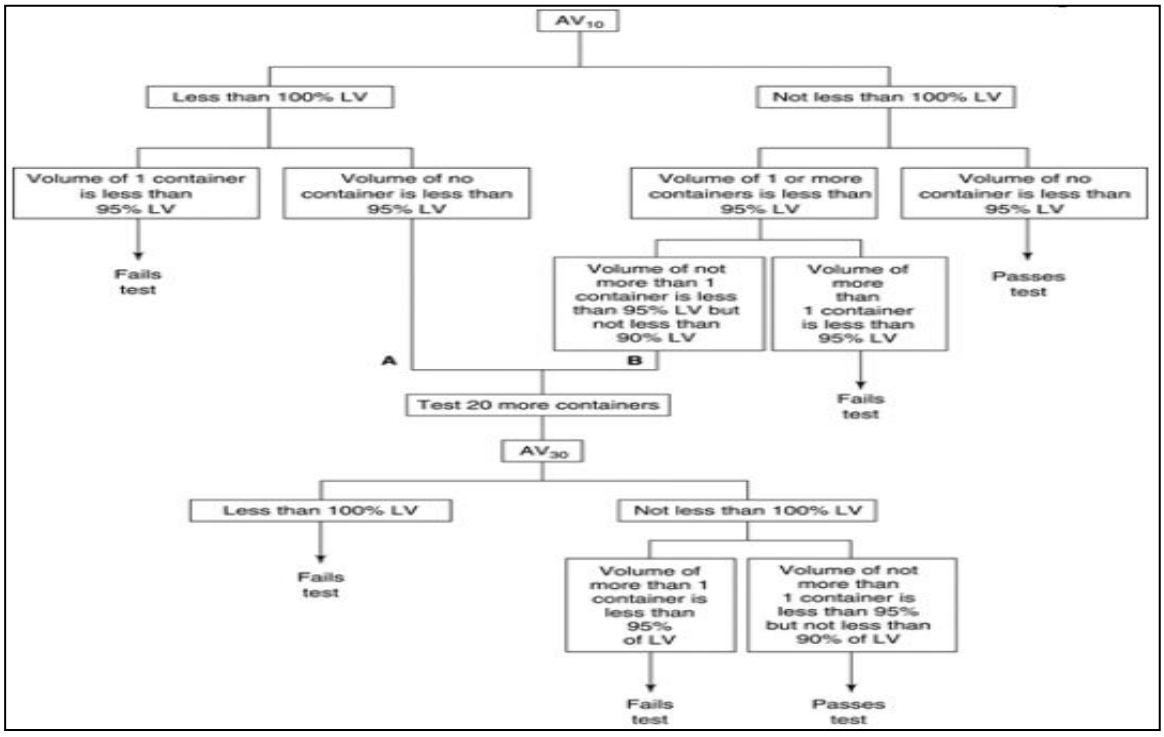


Figure 4.2 Procedure for measuring deliverable volume for multiple unit containers

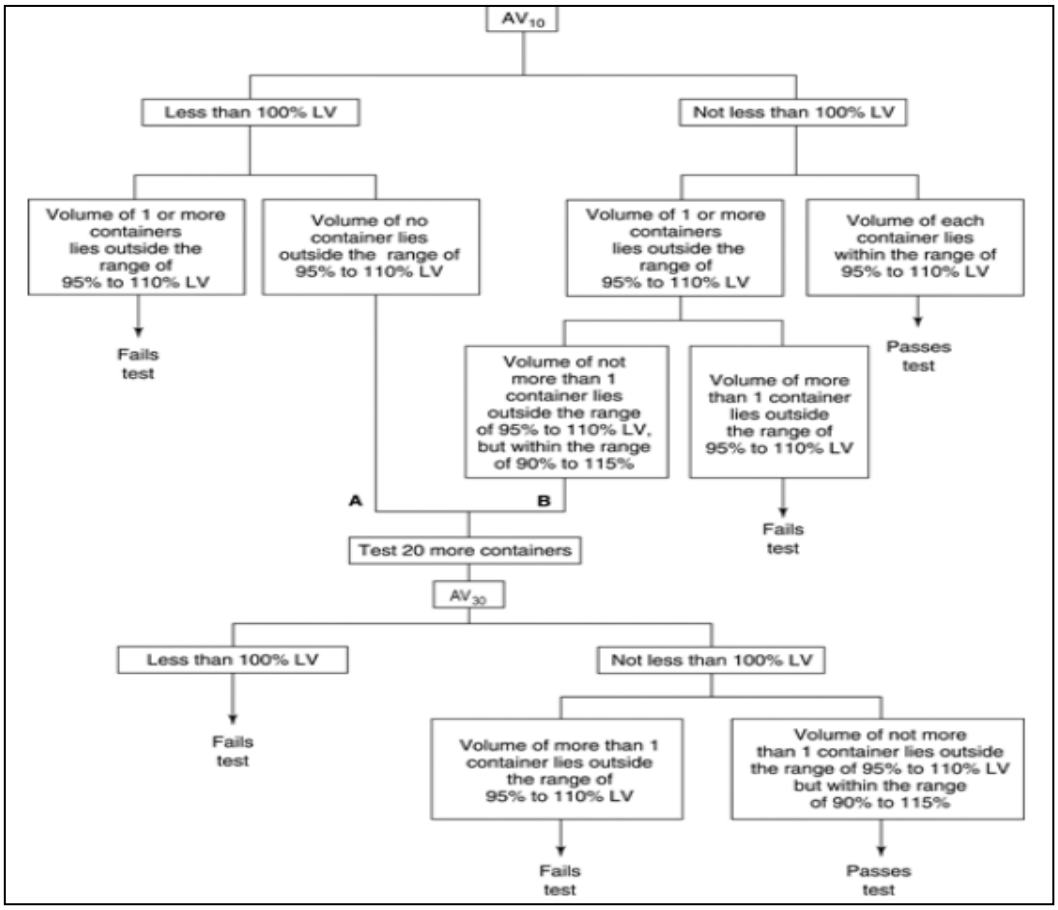


Figure 4.3 Procedure for measuring deliverable volume for single unit container

(E) pH measurement

(F)Viscosity

(G) Sedimentation Volume

$$Sedimentation\ Volume = \frac{Hu}{Ho}$$

(H) Homogeneity

After minimum shaking of the package containing suspension, the amount of drug substance present at top, bottom, middle of the packed suspension, will be equivalent within 10%.

(I) Percentage easy of redispersibility:

It is a significant parameter that gives an idea about the quality of suspensions. It is evaluated after a period of 1 week. The suspension which was stored in a measuring cylinder was upturned by 180° and number of times inversions are required to re-establish a homogeneous suspension was determined.

4.5 FORMULATION AND DEVELOPMENT USING DIRECT BLENDING APPROACH

4.5.1 Composition of preliminary batches :

Table 4.10 Composition of formulation trials using direct blending approach F1-F6

Components	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)
Drug X	4.150	4.150	4.150	4.150	4.150	4.150
Syloid	8.394	8.394	8.394	8.394	8.394	8.394
Orange flavor	1.300	13.00	1.300	1.300	1.300	1.300
Disodium EDTA	4.00	4.00	4.00	4.00	4.00	4.00
Sunset Yellow	0.200	0.200	0.200	0.200	0.200	0.200
Methyl paraben	7.206	7.260	7.260	7.260	7.260	7.260
Propyl	0.800	0.800	0.800	0.800	0.800	0.800

paraben						
Xanthan gum	55	52	50	11	11	11
Sodium saccharine	25	25	25	25	10	5
Sodium bicarbonate	61	61	61	61	61	61
Mannitol 200DC	332.896	-	-	-	391.896	-
Sorbitol	-	335.896	-	-	-	396.896
Sucrose	-	-	337.896	376.896	-	-
Qty(mg/5 ml)	500	500	500	500	500	500

Table 4.11 Composition of formulation trials using direct blending approach F7-F11

Components	**F7(mg)**	**F8(mg)**	**F9(mg)**	**F10(mg)**	**F11(mg)**
Drug X	4.150	4.150	4.150	4.150	4.150
Syloid	8.394	8.394	8.394	8.394	8.394
Orange	1.300	1.300	1.300	1.300	1.300

flavor					
Disodium EDTA	4.00	4.00	4.00	4.00	4.00
Sunset Yellow	0.200	0.200	0.200	0.200	0.200
Methyl paraben sodium	7.260	7.260	-	-	-
Propyl paraben sodium	0.800	0.800	-	-	-
Xanthan gum	11	11	11	11	11
Sodium saccharine	6	6	6	6	6
Sodium bicarbonate	61	61	10	3	7
Mannitol 200DC	-	395.896	-	-	-
Sorbitol	-	-	454.956	-	-
Sucrose	395.896	-	-	461.956	457.956
Qty(mg/5 ml)	**500**	**500**	**500**	**500**	**500**

- Concentration of Xanthan gum, sodium saccharine , sodium bicarbonate and different diluents were varied in order to achieve desired results.\
- **Batches F1-F4:** Mannitol 200DC, sorbitol, sucrose as diluents with xanthan gum as viscosity modifier (11%, 10.4% and 10%).
- **Batches F5-F7:** Mannitol 200DC, sorbitol, sucrose as diluents with sodium saccharine as sweetner(2%, 1% and 1.2%).
- **Batches F8-F11:** Mannitol 200DC, sorbitol, sucrose as diluents with sodium bicarbonate as alkanizingagent (12.2%, 2% , 0.6% and 1.4%)

Manufacturing procedure :

Table 4.12 Manufacturing method

Sr.no.	Process	Details
1	**Premix 1** Dispensing, Co-sifting, Sifting	Dispense the approprite quantity of drug X and all excipients on calibrated balance. Co-sift the drug X with syloid and ¼ th quantity of sucrose through mesh 40. Sift it again.
2	**Premix 2**	Co-sift the xanthan gum with orange flavour, sodium bicarbonate disodiumedetate and sodium saccharin through mesh no. 40 Sift it again.
3		Sift colour through mesh no. 80 OR 100 and collect in the same pack . Keep it aside
4	**Premix 3**	Sift the premix 1 with equal quantity of sucrose through mesh no. 40
5	**Premix 4**	Sift the premix 3 with remaining quantity of sucrose and colour through mesh 40

6	**Premix 5**	Co-mill Premix 4 and premix 2 through screen 1.2mm greater
7	**Blending**	Blend in suitable blender

4.5.2 Composition of optimized batch:-

Table 4.13 Final formula with Mannitol

Sr. no	Ingredients	Quantity /batch (g)
1	Drug	4.15
2	Silicon dioxide NF (Syloid AL1 FP)	16.788
3	Mannitol (Perlitol 100 SD)	439.762
4	Saccharin sodium IP	6
5	Xanthan Gum IP (Xanthural 75)	2
6	Sodium bicarbonate IP	20
7	Aerosil	10
8	Orange flavour	1.3

Final manufacturing method :-

A) Sifting/ Co-milling:

Ingredients	Mesh
Sifting	
Mannitol	40

1) Sfit mannitol through 40 mesh sieve. Comill the retention if any thtough Quadro Comill 0.6mm screen.

B) Preparation of Premix I :

Ingredients	**Mesh**
Co-sifting	
DrugX + Mannitol + Syloid AL1FP	40

2) Spread apptoximately 0.5 % of total quantity of mannitol through 40 mesh initially to avoid sticking of API.

3) Mix the API and silicon dioxide AL1FP thoroughly.

4) Ad mannitol to above blend and mix well. Sift the blend through 40 mesh sieve.

5) Collect the sifted material and again sift through 40 mesh sieve.

6) Rinse API polybag with mannitol and sift through 40 mesh sieve.

7) Rinse the sieve by sifting remaining quantity if mannitol through above 40 mesh sieve vibratory mixer by keeping the mixer on.

8) Label it as Premix I.

C) Preparation of Premix II :

Ingredients	**Mesh**
Xanthan gum	40
Sacharin Sodium	
Orange flavour 501071	
Mannitol	

9) Co-sift Xanthan gum, saccharin sodium, orange flavour and mannitol through 40 mesh sieve.

10) Collect the sifted materail and again sift through 40 mesh sieve.

11) Label it as Premix II

D) Preparation of Premix III :

12) Co-sift Premix I and Premix II with some quantity of mannitol through 40 mesh sieve.

13) Label it as Premix III.

E) Preparation of Premix IV :

14) Co-sift Premix III with remaining quantity of amnnitol through 40 mesh sieve.

15) Label it as Premix IV.

F) Blending :

16) Load Premix IV into the blender and blend using following parameters :

Capacity of blender	10 L
Total no. of revolutions	30 ± 10
% occupancy	60% ± 5%

G) Co-milling :

17) Co-mill above blend through Quadro comill 0.6mm screen.

H) Blending :

18) Load the final comilled blend into the blender and blend using following parameters:

Capacity of blender	10 L
Total no. of revolutions	30 ± 10
% occupancy	60% ± 5%

19) Collect the blend samples from 10 different positions of the blender and analysing the same for uniform distribution of the drug.

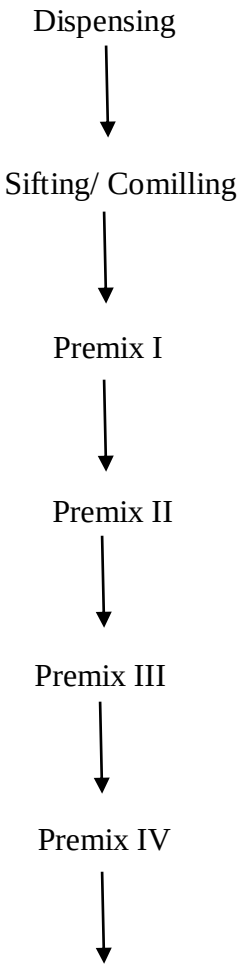
Description : White to off white powder with no agglomerates.

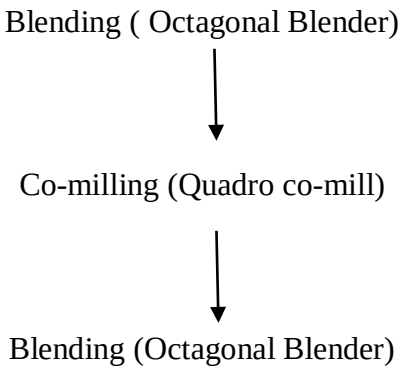
20) Unload the blend in double lined black plybag with silica gel desiccant (400 gm x 2 no's) between two polybags and place these bags in triple laminate high barrier bags tightened with fastner in product containers.

Below mentioned physical parameters of the blend were checked.

- Appearance : White to off white powder with no agglomerates.
- LOD (at 80°C) : NMT 3.0% w/w
- Bulk density : 0.80-1.20 gm/ml
- Tapped density : 0.90-1.30 gm/ml
- Water content : NMT 5% w/w

4.5.3 Process flow chart along with equipments :





Storage condition : Store protected from light and moisture, at a temperature not exceeding 30°C.

5. RESULTS AND DISCUSSION

5.1 Result of Organoleptic properties

Table 5.1 Organoleptic properties specifications of API

IP Specification	Inference
A white to pale yellow powder	Almost white powder

5.2 Result of Drug identification by FTIR

Figure 2.6 : FTIR spectrum of Test sample of DrugX

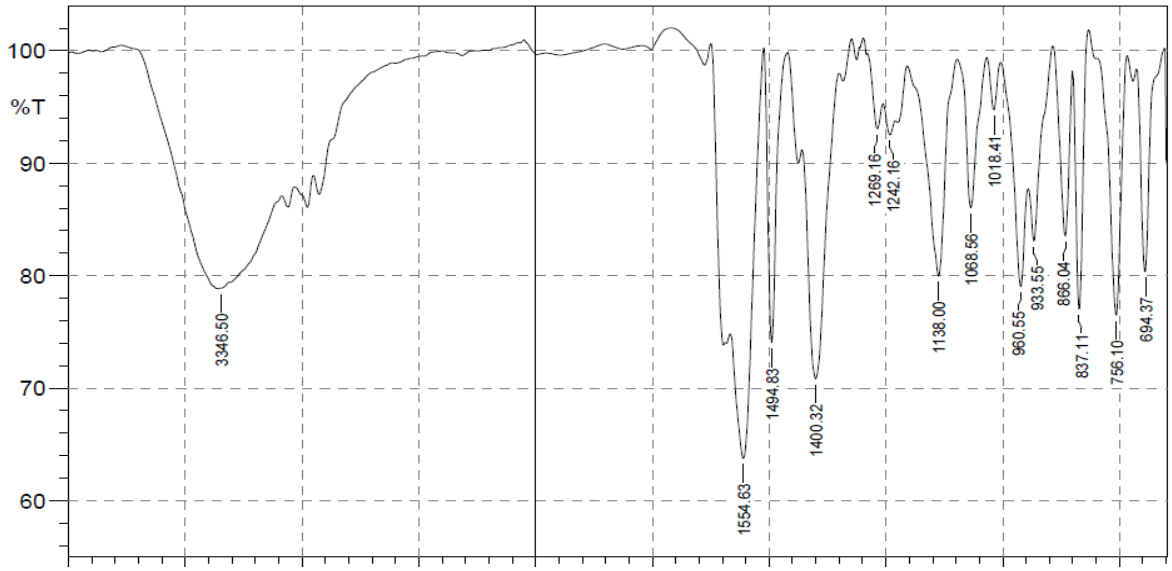


Table 5.2 FTIR Value

Peaks (1/cm)	Functional Group
3346	OH (Hydroxyl)
1400-1554	Alkene (C=C), Aromatic
1242-1269	C-O- C Stretch
1099,1074	C-OH Stretch

Discussion :-

The sample spectrum of Drug X was compared with reference standard and both spectra were found similar in peak values representing wave numbers.

5.3 Result of DrugX assay

Table 5.3 Assay specification of DrugX (As per COA)

IP Specification	98.0- 102.0 % wt/wt
Result	99.9 % wt/wt

Discussion :-

Assay of the drug sample was compared with reference data, it was found that assay of the API was within the limit (~98% – 102% w/w).

5.4 Result of Evaluation of flow properties of DrugX

Table 5.4 Flow Properties of DrugX

A. R. No	Bulk Density	Tapped Density	Carr's Index	Hausner ratio	Flow Properties
DMIT-60240	0.648	0.768	1.18	15.62	Good
	0.670	0.783	1.16	14.43	

Discussion :-

From the above results it can be concluded that DrugX exhibits fair to good flow property.

5.5 Result of Particle size evaluation of API

Table 5.5 Particle size distribution of DrugX(As per COA)

A.R No	Batch / Lot No.	Source	Particle size (µm) (% particles under size)		
			10%	50%	90%
DMIT-	MIT 6237	Morepen	1 µ	5 µ	12µ

60240					

Discussion :- From the results it can be concluded that the DrugXis a micronized powder which d_{90} = 12 µm.

5.6 Result of related substances in API

Table 5.6 Related Substance (As per CoA) of DrugX

Impurities	IP Specification	Result
Impurity C (sulfoxide isomer)	NMT 0.30%	0.04
Impurity D(methyl styrene)	NMT 0.30%	0.20
Single maximum impurity	NMT 0.30%	0.06
Total impurities	NMT 1.0%	0.5

Discussion :-

The Related Substance determined was within the range of standard value, hence, it can be concluded that the drug sample had similar physical property as standard drug.

5.7 Result of water content evaluation

Table 5.7 Water content (As per CoA) of DrugX

IP limit	NMT 3.0% w/w
Result	0.6% w/w

Discussion :-

The water content of the API was observed well within the limits specified by IP

5.8 Result API pH solubility profile

Table 5.8 pH solubility profile of DrugX

Sr. No.	Medium	Quantity	% drug recovery
1	Amt of drug (mg)	10 mg	
2	Vol of aq pH solution (ml)	250 ml	
3	Distilled water (5.9)	250 ml	57.3
4	0.1 M HCl (1.1)	250 ml	11.6
5	Aq. Ph Solution (1.2)	250 ml	15.0
6	Aq. Ph Solution (4.5)	250 ml	11.3
7	Aq. Ph Solution (5.5)	250 ml	38.0
8	Aq. Ph Solution (6.8)	250 ml	45.4
9	Aq. Ph Solution (7.5)	250 ml	48.7

Discussion :-

From the above findings for drug X, the highest orally administered dose (10mg) is not soluble in 250ml of aqueous medium over a pH range of 1-7.5 i.e. **' low solubility drug'**. The solubility is higher in the alkaline media and reduces in the acidic media.

5.9 Result of Drug-Excipient compatibility

Table 5.9 Drug - Excipient compatibility study

Sr. No	Ingredients	Ratio	After 1 month			
			30°C/ 60% RH		40°C/ 75% RH	
			Physical Appearance	Total Impurity(%)	Physical Appearance	Total Impurity (%)

1	**API**		NC	0.20	NC	
2	**API + Syloid**	1:1	NC	0.45	NC	0.50
3	**API + Mannitol 200 DC**	1:10	NC	0.48	NC	0.64
4	**API + Sodium saccharin**	1:10	NC	0.64	NC	0.68
5	**API + Xanthan gum**	1:10	NC	0.35	NC	0.40
6	**API + Sodium bicarbonate**	1:10	NC	0.67	NC	0.77
7	**API + Disodium edetate**	1:1	NC	0.30	NC	0.38
8	**API + Orange flavour**	1:0.25	NC	0.46	NC	0.54
9	**API + Sunset yellow colour**	1:0.25	NC	0.15	NC	0.20

Discussion :-

Assay of DrugXwith other excipients, was within the acceptable range value (~98 – 102 %w/w) at the condition 30°C/ 60% RH & 40°C/ 75% RH. So it can be concluded that in presence of these excipients % impurity of DrugX was not affected. During study it was observed that there was no significant change in assay of blends due to thermal stress. Therefore, it can be concluded that selected excipients were compatible with DrugX.

Drug was found to be compatible with all the excipients at both the conditions. Total impurities were found to be less than 1%.

5.10 Result of RS in Drug - Excipient compatibility study:

Table 5.10 Drug - Excipient compatibility study: Related Substance (open vials)

Ingredients	Ratio	Relative susbtances (30 days 40°C/ 75 % RH (open vials))						
		B	C	D	E	F	Other	Total
API	1:0	0.06	BQL	ND	ND	ND	ND	0.1
API + Syloid AL1 FP	1: 1	0.07	ND	BQL	ND	ND	ND	0.13
API + Mannitol	1:10	0.06	ND	ND	ND	ND	ND	0.06
API + Sodium saccharin	1:10	0.13	BQL	BQL	ND	ND	BQL	0.16
API + Disodium edetate	1:1	0.08	BQL	BQL	ND	ND	BQL	0.12
API + Xanthan gum	1:10	0.10	BQL	BQL	ND	ND	BQL	0.13
API + Sunset yellow	1:0.25	0.07	BQL	BQL	ND	ND	BQL	0.14
API + Orange flavour	1:0.25	0.06	BQL	ND	BQL	ND	BQL	0.12

*ND= Not Detected BQL: Below Quantified Limit

Table 5.11 Drug - Excipient compatibility study: Related Substance (Sealed vials)

Ingredients	Ratio	Relative Substance(50°C (Sealed vials))						
		B	C	D	E	F	Other	Total
API	1:0	0.06	ND	ND	ND	ND	ND	0.15
API + Syloid AL1 FP	1: 1	0.07	ND	BQL	ND	ND	BQL	0.1
API + Mannitol	1:10	0.06	BQL	BQL	ND	ND	BQL	0.04
API + Sodium saccharin	1:10	0.10	BQL	BQL	ND	ND	BQL	0.12
API + Disodium edetate	1:1	0.08	BQL	BQL	ND	ND	BQL	0.06
API + Xanthan gum	1:10	0.10	BQL	BQL	BQL	ND	BQL	0.20
API + Sunset yellow	1:0.25	0.07	ND	ND	BQL	ND	ND	0.23
API + Orange flavour	1:0.25	0.10	BQL	BQL	BQL	ND	BQL	0.20

*ND = Not Detected, BQL= Below Quantified Limit

Discussion:-

Based on the result, it was observed that there was no significant change in physical property of blend due to thermal stress. Besides, data related to relative substance also indicated that there was no significant change in levels of impurities in the above mentioned blends. Therefore, it can be concluded that the selected excipients were compatible with the drug.

5.11 Result of Reference product characterization

Table 5.12 Description of Delpomont® Oral Suspension 4mg/5ml

Description	Delpomont® Oral Suspension 4mg/5ml
Label claim	Each 5ml contains: DrugX IP equivalent to DrugX 4mg Colour- Sunset yellow FCF
Excipients	Aspartame, Anhydrous citric acid, Colloidal silicon dioxide, Methylparaben, Mixed fruit flavor, Sodium citrate dihydrate,

Table 5.13 Product details of Delpomont® Oral Suspension 4mg/5ml

No	Description	Delpomont® Oral Suspension 4mg/5ml
1	Name of product	Delpomont® Oral Suspension 4mg/5ml
2	Pack profile	100ml ambered coloured PET bottle with ROPP cap
3	Manufactured by	Akums drugs and Pharmaceuticals Ltd
4	Marketed by	DelCurelifesciences Ltd
Dosage form details		
5	Batch number	GMAG22
6	Manufacturing date	Jan 2017
7	Expiry date	Dec 2018
8	Shelf life	12 months

9	Dosage form	Suspension
10	Dosage and route of administration	• Route of administration : Oral • Delpomont should be taken orally
11	Storage condition	Store protected from moisture and light and moisture, at a temperature not exceding 35°C. Don't freeze.
12	Instructions	Keep out of the reach of children. Close the bottle tightly after each use. **Shake well before use.**
Physico-chemical parameters		
13	Description	Orange to light orange viscous suspension
14	Ph	4.79
15	**Assay**	101.4%
16	Dissolution media	0.5% (w/v) Sodium dodecyl sulfate in water;900ml
Related Substances		
17	Sulfoxide impurity	0.4%
18	Methylstyrene impurity	0.24%
19	SMI	28.2%
20	Total impurity	31.2%
21	Water activity	-
22	Water content	84.40% w/w
23	Bulk density	NA
24	Tapped density	NA

Table 5.14 Chemical characteristics of Delpomont® Oral Suspension 4mg/5ml

Tests	**Results**
Product Name	**Delpomont® Oral Suspension 4mg/5ml**
Batch no	RLD (GMAG22)
Expiry date	June 2017

Assay	101.4
Related substances	
Sulfoxide impurity	0.4%
Methylstyrene impurity	0.24%
SMI	28.2%
Total impurity	31.2%

5.12 Result of Evaluation of Flow properties of Batches F1-F11

Table 5.15 Result of evaluation of flow properties

Batch No.	Bulk density (mg/ml)	Tapped density (mg/ml)	Hausner's ratio	Carr's index (%)	Angle of repose
F1	0.640	0.640	1.25	20.00	16.37°
F2	0.648	0.648	1.18	15.62	18.00°
F3	0.670	0.670	1.16	14.43	19.55°
F4	0.650	0.650	1.20	17.19	18.12°
F5	0.620	0.620	1.25	15.50	20.00°
F6	0.670	0.670	1.17	14.54	20.64°
F7	0.642	0.642	1.12	11.32	20.21°
F8	0.640	0.750	1.17	14.66	22.53°
F9	0.640	0.780	1.21	17.94	23.98°
F10	0.980	1.148	1.16	14.63	23.52°
F11	0.828	1.035	1.25	15.70	24.11°

Discussion :

Powder for reconstitution must shows good free flowing properties so that it can easily reconstitute.

1.Bulk density: Bulk density of all batches was found to be in the range of 0.620-0.980 mg/ml.

2. Tapped density: Tapped density of all the batches varied from 0.724-1.148 mg/ml.

3. Angle of repose: Based on angle of repose all the above batches showed Good flow.

4. Compressibility index: Based on result obtained batches showed Good flow property.

5. Hausner's ratio: Batches showed good flow.

5.13 Result of Evaluation of physicochemical parameters of batches F1-F11

Table 5.16 Result of evaluation of physicochemical parameters

Batch No.	Viscosity (CP)	Drug content (%)	pH	Content uniformity (%)	Dissolution (% drug release)		
					5 Min	10 Min	15Min
F1	171.0	83.4	8.68	82.50	27	32	32
F2	105.2	81.02	8.48	80.00	70	75	75
F3	89.81	89.56	8.08	89.64	80	82	82
F4	100	94.10	8.29	94.50	90	94	94
F5	10	98.07	8.32	98.02	88	91	92
F6	68	101.0	8.56	100	92	94	96
F7	89.6	102.8	8.44	100.2	86	91	91
F8	18.8	98.03	8.52	99.07	87	92	92
F9	76.5	99.0	7.82	99.0	96	97	100
F10	102	99.99	7.56	100.4	98	99	100
F11	108	99.20	7.60	100.5	97	99	99

Discussion :

Viscosity: Batches F1-F3 shows too high viscosity and after reconstitution it was not easily pourable, hence these batches were not carried out for further studies.

Batches F5,F6 and F8 also not carried out for further studies due too much less viscosity and after reconstitution liquidy suspension was obtained.

Batches F7-F11 shows optimum viscosity and after reconstitution easily pourable and viscous suspension was obtained.

Drug content: IP limit for drug content : NLT 90.0% and NMT108% of the labeled amount of drug X.

Bathces F1-F3 showed drug content beyond the USP limits hence, these batches were not carried out for further studies.

Bathches F4-F11 showed drug content within the USP limits.

Viscosity: Batches F1-F3 shows too high viscosity and after reconstitution it was not easily pourable, hence these batches were not carried out for further studies.

Batches F5,F6 and F8 also not carried out for further studies due too much less viscosity and after reconstitution liquidy suspension was obtained.

Batches F7-F11 shows optimum viscosity and after reconstitution easily pourable and viscous suspension was obtained.

Drug content: IP limit for drug content : NLT 90.0% and NMT108% of the labeled amount of drug X.

Bathces F1-F3 showed drug content beyond the USP limits hence, these batches were not carried out for further studies.

Bathches F4-F11 showed drug content within the USP limits.

Content uniformity: Preceeded as directed in dissolution (as per USP).

Batches F3-F11 passed the content uniformity test.

From the above results Batches F7, F9-F11 were selected for further studies:

5.14 Result of Evaluation of selected batches after reconstitution

Table 5.17 : Evaluation of suspension after reconstitution on 0 day

B.No	Appearance	Taste	Viscosity	Assay (%)	Total impurity (%)	pH	Sedimentation Ratio	Redispersibility(No.of stroke)
F7	Uniform	Palatable	89.6	100.2	0.102	8.44	1	0
F9	Uniform	Palatable	76.5	99.0	0.180	7.82	1	0
F10	Uniform	Palatable	102	100.4	0.165	7.56	1	0
F11	Uniform	Palatable	108	100.5	0.142	7.60	1	0

Table 5.18 : Evaluation of suspension after reconstitution on 15th day

B. No	Appearance	Taste	Viscosity (cp)	Assay (%)	Total impurity (%)	pH	Sedimentation Ratio	Redispersibility (No.of stroke)
F7	Uniform	Palatable	80.00	75	4.62	5.50	0.8	3
F9	Uniform	Palatable	70.42	98.00	0.950	8.9	1	1

F10	Uniform	Palatable	98.50	99.90	0.520	8.20	0.933	1
F11	Uniform	Palatable	100.02	95	0.890	8.50	0.866	1

Discussion :

- Batch F7 showed decrease in pH to the acidic but Drug X is unstable at acidic pH hence this batch is not carried out for further studies.
- Batches F9 –F11 were taken without preservatives. This batches showed increase in pH during 15 days stability studies.
- Batch F10 showed higher stability for 15 days after reconstitution.

5.15 Result of Stability Evaluation of Batch number 10 :-

Table 5.19 Stability data for initial sample

Test	Specification	Result
Assay	NLT 94% and NMT 105%	97.8
Related substances		
Impurity C	1.7	0.262
Impurity G	0.15	ND
Impurity F	0.15	ND
Impurity D	0.15	ND
Impurity E	0.15	ND
Impurity B	0.3	0.169
Total	2.1	0.431

Table 5.20 Stability data for exposed sample

Test	Specification	Result		
Assay	NLT 94% and NMT 105%			
DHS (Open)		**95.9**		
DHS (Closed)		93.9		
MHS (Closed)		97.3		
Related substances		**DHS (Open)**	**DHS (Closed)**	**MHS (Closed)**
Impurity C	**1.7**	**0.962**	2.111	0.888
Impurity G	0.15	ND	ND	0.158
Impurity F	0.15	ND	ND	ND
Impurity D	0.15	ND	ND	ND
Impurity E	0.15	ND	ND	ND
Impurity B	0.3	0.201	0.171	0.198
Total	2.1	1.163	2.671	1.244

Table 5.20 Stability data for 2 months exposed sample

Test	**Limits**	**Result**			
		1 month			**2 months**
		25°C/ 60% RH	**30°C/ 65% RH**	**40°C/ 75% RH**	**40°C/ 75% RH**
Assay	**NLT 94% and NMT 105%**	**101.2**	**97.4**	**95.9**	
Related substances					
Impurity C	1.7	0.513	0.699	1.672	3.454
Impurity G	0.15	ND	ND	ND	BLOQ
Impurity F	0.15	BLOQ	ND	ND	0.319
Impurity D	0.15	ND	ND	BLOQ	ND
Impurity E	0.15	ND	0.197	ND	ND
Impurity B	0.3	0.212	0.173	0.458	BLOQ
Total	2.1	0.844	1.069	**2.209**	**3.773**

Discussion :-

- However after a month of stability the impurity level showed a rise.
- Increase in moisture content was observed.
- Impurity C was out of limit.
- It was observed that the use of sucrose led to increase in moisture content.
- Hence sucrose was replaced with mannitol**.**

5.16 Result of Stability evaluation of Optimized batch with Mannitol :-

Table 5.21 Stability data for initial sample

Test	Specification	Result
Assay	NLT 94% and NMT 105%	98.3
Related substances		
Impurity C	1.7	0.324
Impurity G	0.15	ND
Impurity F	0.15	0.148
Impurity D	0.15	ND
Impurity E	0.15	ND
Impurity B	0.3	0.169
Total	2.1	0.662

Table 5.22 Stability data for exposed sample

Test	**Limits**	**Result**			
		15 days open exposed		**1 month open exposed**	
		25°C/ 60% RH	**40°C/ 75% RH**	**25°C/ 60% RH**	**40°C/ 75% RH**
Assay	NLT 94% and NMT 105%	98.3	95.5	103.3	92
Related substances					
Impurity	1.7	0.513	1.663	0.556	1.487

C					
Impurity G	0.15	ND	ND	ND	ND
Impurity F	0.15	0.22	0.137	0.378	0.216
Impurity D	0.15	ND	ND	ND	ND
Impurity E	0.15	ND	ND	ND	ND
Impurity B	0.3	0.164	0.159	0.173	0.154
Total	2.1	0.887	1.928	**1.106**	**1.903**

Table 5.23 Stability data for 2 months exposed sample

Test	**Limits**	**Result**		
		1 month closed		**2 month**
		25°C/ 60% RH	**40°C/ 75% RH**	**40°C/ 75% RH**
Assay	NLT 94% and NMT 105%	102	100.2	98.8
Related substances				
Impurity C	1.7	0.44	1.212	1.304
Impurity G	0.15	ND	ND	ND
Impurity F	0.15	0.283	0.276	0.489
Impurity D	0.15	ND	ND	ND
Impurity E	0.15	ND	ND	ND
Impurity B	0.3	0.154	0.166	0.175
Total	2.1	0.888	1.653	**2.008**

Discussion :

- As per the 2 month stability study it was found that the formulation was stable and the impurities were observed within limits.
- Hence this batch with mannitol was finalized for further studies and feasibility batches were carried out using this formula.

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6. CONCLUSION :

DrugX, a widely used drug in the treatment of asthma possesses many stability issues as it is prone to degradation by light, heat, water and acidic pH. Hence this study was an effort to formulate the drug is such a way as to curb the degradation and make it more convenient for pediatric population. The dry powder for oral suspension was thus formulated by carefully choosing the excipients. From the preliminary trails taken, a few batches which showed satisfactory powder flow characteristics and physico-chemical properties were shortlisted for further studies. When these batches were subjected to stability study upto 2 months in different temperature and moisture conditions, an increase in total impurity percentage was observed. The total impurity exceeded the limits specified by the Indian Pharmacopoeia.

It was observed that the rise in impurity was due to Sucrose which was used in large quantity as a diluent. Sucrose is hygroscopic in nature and hence demonstrated moisture absorption into the product.

Hence Mannitol, which is a less hygroscopic sugar was used to replace sucrose as a diluent. The trail taken with this diluent displayed satisfactory stability when exposed to various temperature and humidity conditons upto 2 months. The degradation process was curbed and the impurity levels were observed well within the limits set by Indian Pharmacopoeia.

It was therefore concluded that the use of mannitol resulted in a formualation that was stable for 2 months. This formulation was successful and therefore considered as optimized. Stability study for 3 and 6 months shall be carried out using this formulation.

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