# FORMULATION DEVELOPMENT OF ANTIHYPERTENSIVE DRUG LABETALOL HCl INJECTION

A Dissertation Submitted to

**THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY**

**CHENNAI – 600032**

In partial fulfilment of the requirements for the award of the Degree of

**MASTER OF PHARMACY**

**IN**

**BRANCH-I -> PHARMACEUTICS**

**Submitted By**

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## THE CERTIFICATE

This is to certify that the dissertation work entitled "**FORMULATION DEVELOPMENT OF ANTIHYPERTENSIVE DRUG LABETALOL HCl INJECTION"** submitted to **THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI-32** in partial fulfilment for the award of the degree of **Master of Pharmacy in pharmaceutics** is a bonafide research work done by **AL.GURU PRASAD (261710006)** under my guidance and supervision was carried out at **Department of Pharmaceutics,** C.L. Baid Metha College of Pharmacy, Chennai-600097, during the academic year of 2019.

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## DECLARATION

I GURU PRASAD A.L **(261710006)** hereby declare that this thesis entitled, "**FORMULATION DEVELOPMENT OF ANTIHYPERTENSIVE DRUG LABETALOL HCl INJECTION"** has been originally carried out by me under the guidance and supervision of **Prof. Dr. R.KUMARAVELRAJAN, M.Pharm., Ph.D.,** Professor, Department of pharmaceutics, C.L.Baid Metha College of Pharmacy, Chennai-97 and **Dr. PAVAN KUMAR POTTURI, M.Pharm., Ph.D.,** Manager (FR&D), Caplin Steriles Ltd, Perungudi, Chennai-600096 during the academic year 2019. This work has not been submitted in any other degree at any other university and that all the sources we have used or quoted have been indicated and acknowledged by complete reference.

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**AL. Guru Prasad**

# CONTENTS

## List of Abbreviations

| Abbreviation | Expansion |
|---|---|
| **%** | Percentage |
| **$^0$C** | Degree Centigrade |
| **µg** | Microgram |
| **µm** | Micrometer |
| **Mg** | Milligram |
| **Min** | Minutes |
| **Sec** | Seconds |
| **mL** | Millilitres |
| **Nm** | Nano Meter |
| **Mm** | Millimetre |
| **Cm** | Centimetre |
| **Hr** | Hour |
| **w/v** | Weight/Volume |
| **w/w** | Weight/Weight |
| **IV** | Intravenous |
| **IM** | Intramuscular |
| **SC** | Subcutaneous |
| **WFI** | Water for injection |
| **SVP** | Small volume parenteral |
| **LVP** | Large volume parenteral |
| **HTN or HT** | Hypertension |
| **HBP** | High blood pressure |
| **mmHg** | Millimetres Mercury |
| **CVD** | Cardiovascular diseases |
| **CKD** | Chronic kidney disease |

| | |
|---|---|
| **BP** | Blood Pressure |
| **pH** | Potential of Hydrogen |
| **FTIR** | Fourier Transform Infrared Spectroscopy |
| **LOD** | Loss on drying |
| **IP** | Pharmacopoeia of India |
| **USP** | United States Pharmacopoeia |
| **API** | Active Pharmaceutical Ingredient |
| **EDTA** | Disodium edetate |
| **RLD** | Reference listed drug |
| **RH** | Relative humidity |
| **HPLC** | High performance liquid Chromatography |
| **NMT** | Not more than |
| **NLT** | Not less than |
| **BQL** | Below quantification limit |
| **I** | Inverted |
| **U** | Upright |
| **ND** | Not detected |
| **NP** | Not performed |
| **PES** | Poly ether sulfone |
| **PVDF** | polyvinylidene difluoride |
| **SS** | Stainless steel |
| **RPM** | Revolution Per Minute |
| **RT** | Room Temperature |
| **FDA** | Food and Drug Administration |
| **Kgf** | Kilogram – force |

# 1. Introduction

## 1.1 Parenteral Formulation

### *1.1.1 Definition*

Parenteral products are intended for administration by injection or implantation through the skin, or other external layers such as stratum corneum, and directly into body fluids, tissues, or organs. From the site of administration, the medicament is readily transported to its site of action. (1)

Parenteral is a solution; emulsion or suspension in water for injection does not preclude the inclusion of suitable excipients used to make preparation isotonic with blood, to adjust pH, increase solubility, to prevent deterioration of active substances or provide antimicrobial properties. (2)

### *1.1.2 Significant of parenteral dosage form*

Parenteral drug delivery has unique advantages. Parenteral drugs can improve adherence, act immediately and allow the administrator to control drug delivery. One advantage of parenteral drugs is their ability to improve medication adherence. Some patients struggle with taking oral drugs as directed. According to a 2014 survey, “Swallowing problems affect 1 in 25 adults, annually.” Other patients cannot take or keep down oral drugs due to nausea. Parenteral drugs allow such patients to adhere to their medication regimens. They also allow physicians and caregivers to administer drugs to patients who are unconscious or otherwise unable to receive medication on their own.

Another advantage of parenteral drugs is that they can act quickly compared to oral medications. “The IV route provides immediate onset of action.” If a patient is unconscious, unresponsive or in any type of emergency situation, a rapid response can be lifesaving. Sometimes, physicians need to administer medication to a specific location in a controlled manner. Parenteral drugs can fulfill this need. For example, dentists need to administer local anesthesia to a small area. Parenteral administration of the localized anesthesia allows this to happen. (3)

## 1.2 Hypertension

Hypertension (HTN or HT), also known as high blood pressure (HBP), is a long-term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure typically does not cause symptoms. Long-term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, atrial fibrillation, peripheral vascular disease, vision loss, chronic kidney disease, and dementia. [4]

High blood pressure is classified as either primary (essential) high blood pressure or secondary high blood pressure. About 90-95% of cases are primary, defined as high blood pressure due to nonspecific lifestyle and genetic factors. Lifestyle factors that increase the risk include excess salt in the diet, excess body weight, smoking, and alcohol use. The remaining 5-10% of cases are categorized as secondary high blood pressure, defined as high blood pressure due to an identifiable cause, such as chronic kidney disease, narrowing of the kidney arteries, an endocrine disorder, or the use of birth control pills. [5]

Blood pressure is expressed by two measurements, the systolic and diastolic pressures, which are the maximum and minimum pressures, respectively. For most adults, normal blood pressure at rest is within the range of 100 – 130 millimetres mercury (mmHg) systolic and 60 – 80 (mmHg) diastolic. For most adults, high blood pressure is present if the resting blood pressure is persistently at or above 130/80 or 140/90 (mmHg). Different number applies to children. Ambulatory blood pressure monitoring over a 24 hours period appears more accurate than office-based blood pressure measurement. [6]

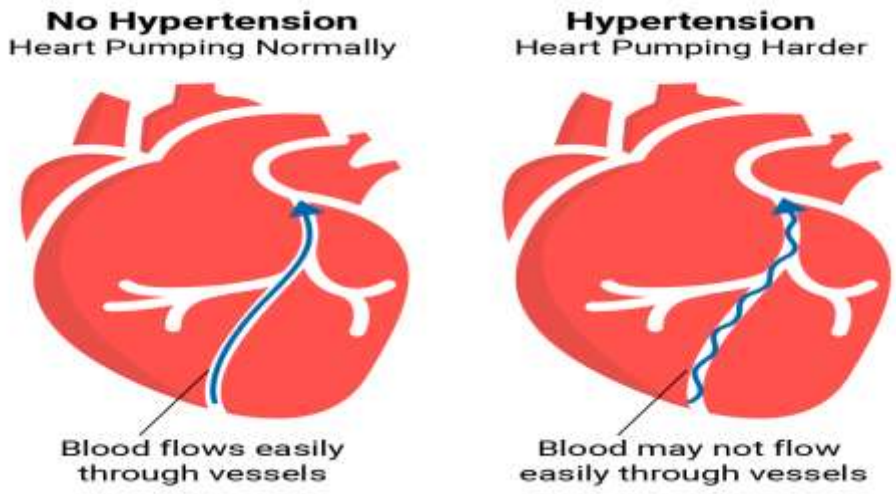



**Fig 1: Module of Hypertension**

Hypertension is the most common modified risk factor for cardiovascular diseases (CVD), stoke and renal failure. It is the second leading cause of chronic kidney disease (CKD). It is estimated that more than one billion adults are hypertensive worldwide and this figure is projected to increase to 1.56 billion by the year 2025, which is an increase of 60 % from 2000. Cardiovascular disease and hypertension are accounting for loss of 4 % gross domestic product for low- and middle-income countries annually which is amounting 500billion US Dollar. Clinical evidence suggests that lowering blood pressure (BP) with antihypertensive drugs reduce the risk of myocardial infraction, stoke, heart failure, revascularization produces and end-stage renal diseases in hypertensive patients. [7]

Lifestyle changes such as weight loss, decreased salt intake, physical exercise, and a healthy diet helps to control blood pressure. If lifestyle changes are not sufficient to control blood pressure then the medications are used. First line medication for the treatment of hypertension are thiazide-diuretics, calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers. These medications may be used alone or in combination. [8]

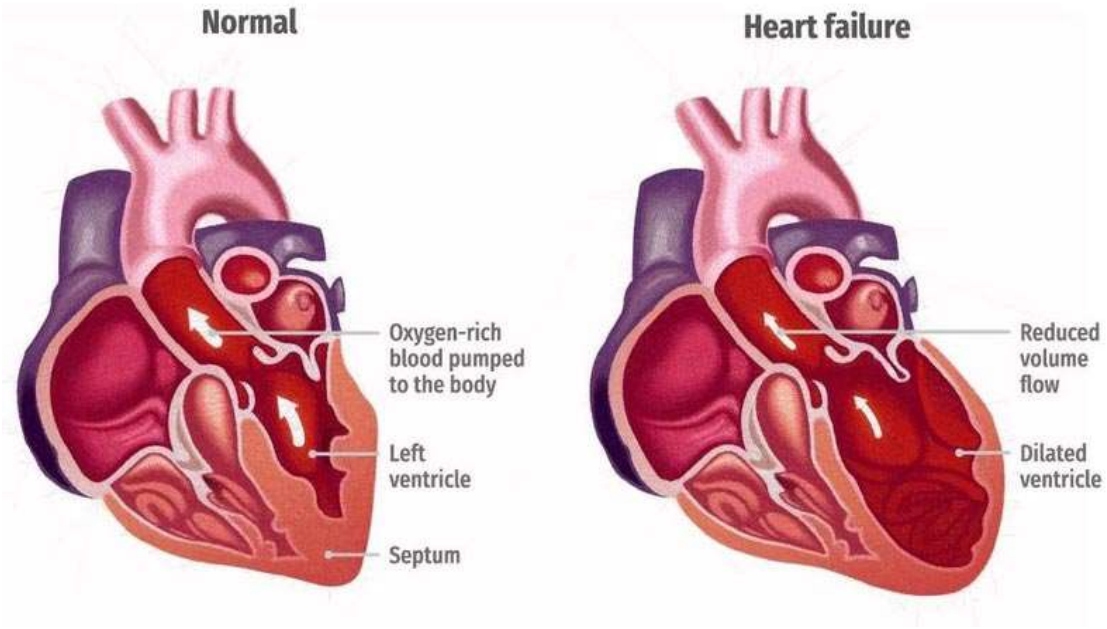



**Fig 2: The Causes of Heart Failure**

### *1.2.1 Causes of high blood pressure:*

✓ ***Primary hypertension***

Primary hypertension is also called essential hypertension. This kind of hypertension develops over time with no identifiable cause. Most people have this type of high blood pressure. Researchers are still unclear what mechanisms cause blood pressure to slowly increase. A combination of factors may play a role. These factors include:

- **Genes:** Some people are genetically predisposed to hypertension. This may be from gene mutations or genetic abnormalities inherited from your parents.
- **Physical changes:** If something in your body changes, you may begin experiencing issues throughout your body. High blood pressure may be one of those issues. For example, it's thought that changes in your kidney function due to aging may upset the body's natural balance of salts and fluid. This change may cause your body's blood pressure to increase.
- **Environment:** Over time, unhealthy lifestyle choices like lack of physical activity and poor diet can take their toll on your body. Lifestyle choices can lead to weight problems. Being overweight or obese can increase your risk for hypertension.

✓ ***Secondary hypertension:***

Secondary hypertension often occurs quickly and can become more severe than primary hypertension. Several conditions that may cause secondary hypertension include:

- kidney disease
- obstructive sleep apnoea
- congenital heart defects
- problems with your thyroid
- side effects of medications
- use of illegal drugs
- alcohol abuse or chronic use
- adrenal gland problems
- certain endocrine tumours

## 1.3 Anti Hypertensives

Antihypertensives are a class of drugs that are used to treat hypertension (high blood pressure). Antihypertensive therapy seeks to prevent the complication of high blood pressure, such as stroke and myocardial infarction. Evidence suggests that reduction of the blood pressure by 5 mmHg can decrease the risk of stroke by 34%, of ischaemic heart disease by 21%, and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease. There are many classes of antihypertensives, which lower blood pressure by different means. Among the most important and most widely used drugs are thiazide diuretics, calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists (ARBs), and beta blockers. [9]

The fundamental goal of treatment should be the prevention of the important endpoint of hypertension, such as heart attack, stroke and heart failure. Patient age, associated clinical conditions and end-organ damage also play a part in determining dosage and type of medication administered. The several classes of antihypertensives differ in side effect profiles, ability to prevent endpoints, and cost. The choice of more expensive agents, where cheaper ones would be equally effective, may have negative impacts on national healthcare budgets. [10]

### *1.3.1 Adrenergic receptor antagonists*

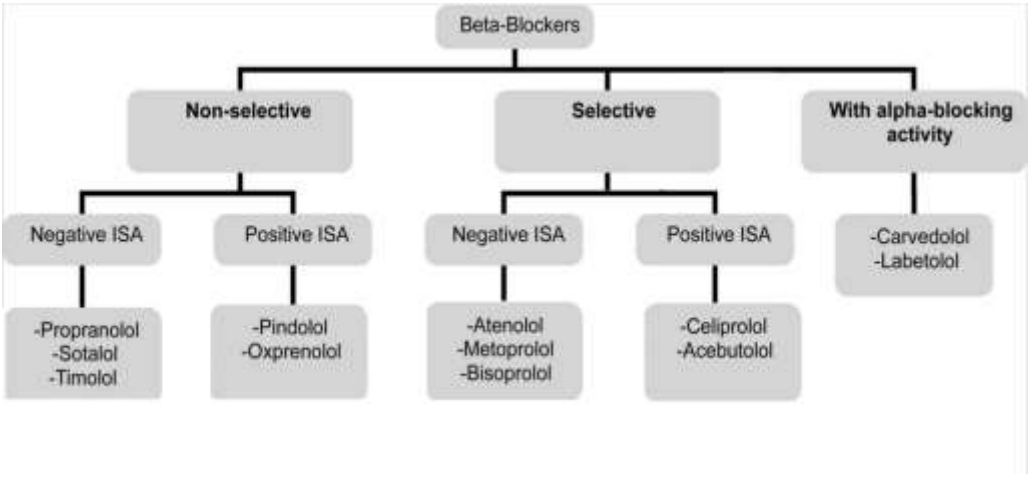



***Fig 3: Classification of beta-blockers***

### *1.3.2 Mechanism of α - β blockers*

Alpha-beta blockers belong to a larger class of medicines called adrenergic inhibitors. They combine the effects of two types of medicines. They behave like alpha blocker medicines when they affect special receptor cells in the smooth muscles of your blood vessels. This action stops your cells from receiving chemicals called catecholamines. These chemicals narrow your arteries. This makes your blood pressure go up. When these chemicals are blocked, your blood vessels can relax. This in turn allows your blood to flow more easily, resulting in lower blood pressure.

These medicines act like beta-blockers when they block these same catecholamines in your brain, heart, and blood vessels. The result is that your heart beats more slowly and with less force. Plus, your blood vessels relax and widen so that blood flows through them more easily. Both of these actions make your blood pressure go down. [11, 12]

## 1.4 Classification of Parenteral

Several categories of parenteral preparations are described as follows

A. Injections
B. Infusions
C. Concentrates for injections or infusions
D. Powders for injections or infusions
E. Gels for injections
F. Implant

### *1.4.1 Injections:*

- These are sterile solutions, emulsions or suspensions.
- Injections are prepared by dissolving, emulsifying or suspending active substances & excipients in water, in non-aqueous vehicle or mixture of both.
- Parenteral solutions are clear, free from particles & emulsions do not show any phase separation.
- Suspensions may show sediment, readily dispersed on shaking gives a stable suspension.
- An Injection is a preparation intended for parenteral administration or for constituting or diluting a parenteral article prior to administration.

- Parenteral articles are preparations used for injection through the skin or other external boundary tissue.
- The active substances are administered, using gravity or force, directly into a blood vessel, organ or tissue.
- Parenteral articles should meet pharmacopoeial requirements for sterility, pyrogens, particulate matter, and other contaminants. (13)

### *1.4.2 Classification of injections*

The following nomenclature pertains to five types of parenteral preparations according to U.S.P.
**[DRUG] Injection—**Liquid preparations that are drug substances or solutions thereof.
**[DRUG] for Injection**—Dry solids that, upon the addition of suitable vehicles, yield solutions conforming in all respects to the requirements for Injections.
**[DRUG] Injectable Emulsion**—Liquid preparations of drug substances dissolved or dispersed in a suitable emulsion medium.
**[DRUG] for Injectable Suspension—**Dry solids that, upon the addition of suitable vehicles, yield preparations conforming in all respects to the requirements for Injectable Suspensions.
**[DRUG] Injectable Suspension**—Liquid preparations of solids suspended in a suitable liquid medium. (14)

### *1.4.3 Infusions:*

- An alternative to oral treatment is infusion therapy: administering medication through the use of a sterile catheter that is inserted into a vein and secured.
- In medicine, infusion therapy deals with all aspects of fluid and medication infusion, via intravenous or subcutaneous application. A special infusion pump can be used for this purpose, A fenestrated catheter is most frequently inserted into the localized area to be treated.
- There are a range of delivery methods for infusion of drugs via catheter:
    - Electronic Pump: Drugs are often pre-mixed from vials and stored in infusion bags to be delivered by electronic pump.
    - Elastomeric pump
    - Pre-Filled Infusion Therapy: with this latest technology, a unit dose can be metered to the location from a pre-filled container.

- Infusion therapy is usually employed to treat serious or chronic infections that do not respond to oral antibiotics.
- Some examples of infusion therapies include **:** Antibiotic/Antiviral, Anti-Coagulation Therapy, Anti-Emetics, Anti-Hemophilic Factors, Blood Component Stimulating Factor, Chemotherapy, Enteral Nutrition, Hydration, Inotropic Therapy, Pain Management, Total Parenteral Nutrition. [15]

### 1.5 Routes of Administration of injection

Injections are often classified according to their routes of administration. These are as follows.

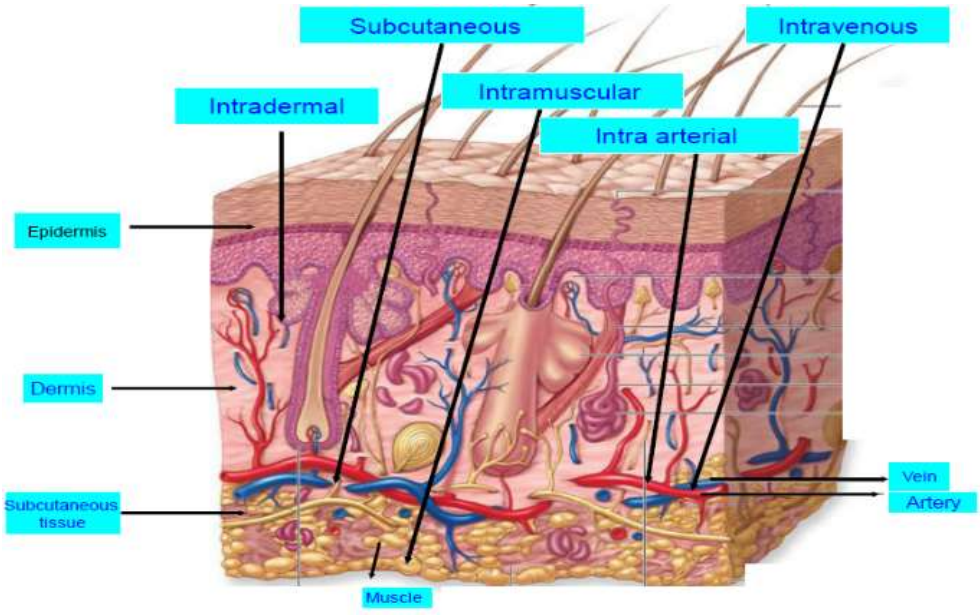



**Fig 4: Routes of Administration**

- Intravenous injections
- Intramuscular injections
- Subcutaneous injections
- Intradermal injections
- Intra-arterial injections
- Intracardiac injections
- Intraspinal injections
- Intra-articular injections
- Intrathecal (IT)

### *1.5.1 Intravenous (IV)*

IV administration introduces drug directly into the venous circulation. IV bolus is used for immediate therapeutic effect, typically for general anaesthesia and for treatment of cardiac arrhythmia. IV dosing is popular for preclinical testing of compounds during drug development and also as a standard to determine absolute bioavailability from other dosage routes.

### *1.5.2 Intramuscular (IM)*

Following intramuscular (IM) administration, drugs must cross one or more biological membrane in order to enter the systemic circulation. Intramuscular injection is used mainly for the drugs and vaccines that are not absorbed orally. The IM route is often used for sustained medication and specialized vehicles, such as aqueous suspensions, oily vehicles, complexes and microencapsulation.

### *1.5.3 Subcutaneous (SC)*

Injection used to deliver drugs between the epidermis and the dermis. The volume of such injections usually varies between 0.1- 0.2 mL. Used mainly for diagnostic purpose in investigations of immunity and allergy.

### *1.5.4 Intra-arterial*

Intra-arterial injection is used to deliver drugs directly to organs, for example, in cancer chemotherapy, and in the use of vasopressin for GI bleeding. Intra-arterial carmustine is effective to treat brain tumours and pelvic intraarterial actinomycin D is used for malignant trophoblastic disease. Intra-arterial drug administration has potential safety implications. Embolization, arterial occlusion, and localized drug toxicity have been reported. [16]

### *1.5.5 Intradermal injections:*

These are given in between dermis and epidermis. Skin of the left for arms usually selected for given injection. Generally, 0.1 to 0.2 ml of parenteral solution is injected by this route. The route is used for diagnostic purposes and for testing the sensitivity of the injectables.

| Advantages | Disadvantages |
|---|---|
| Useful for patients who cannot take drugs orally | More expensive and costly to produce |
| Useful for drugs that require a rapid onset of action (primarily intravenous administration) | Potential for infection at site of injection |
| Useful for emergency conditions | Potential for sepsis |
| Useful for providing sustained drug delivery (implants, intramuscular depot injections) | Potential for thrombophlebitis |
| Can be used for self-delivery of drug (subcutaneous) | Potential for fluid overload |
| Useful for drug that are inactivated in the gastrointestinal tract or susceptible to first-pass metabolism by the liver | Potential for air embolism |
| Useful for injection of drugs directly into a tissue (targeted drug delivery) | Potential for tissue damage upon injection and extravasation |
| Useful for delivering fluids, electrolytes, or nutrients (total parenteral nutrition to patients) | Psychological distress by the patient |
| Useful for providing precise drug delivery by intravenous injection or infusion utilizing pharmacokinetic techniques | Require specialized equipment, personnel, devices, and techniques to prepare and administer drugs |
| Can be done in hospitals, ambulatory infusion centres and in-home health care | Potential for pain upon injection |

**Table 1: Advantage and Disadvantage of parenteral dosage form**

### 1.6 Preformulation research of parenteral medications

Preformulation research relates to pharmaceutical and analytical investigations that both proceed and support formulation development efforts for all dosage forms

### *1.6.1 Drug substance physiochemical properties*

#### *1.6.1.1 Molecular structure and weight*

These are the most basic characteristics of a drug substance and are among the first items to be known. From the molecular structure, the investigator can make initial judgements regarding potential properties and functional group reactivities.

#### *1.6.1.2 Colour*

Colour is generally a function of drug's inherent chemical structure relating to a certain level of unsaturation. Colour intensity relates to the extent of conjugated unsaturation as well as the presence of chromophores such as $–NH_2$, $-NO_2$ and -CO-(ketone), which intensify colour. A significant colour change can become a limiting factor to the shelf life of the parenteral product even before a significant change in chemical stability is noted.

#### *1.6.1.3 Particle size, shape and crystallinity*

The particle size of a water-soluble drug is not of concern unless it exists in large aggregates and an increase in rate of solution is desired to reduce manufacturing time. Under such circumstances milling through appropriate size sieve will be sufficient. Particle size and shape characteristics can be determined by microscopic evaluation using either an optical microscope, preferably with polarizing attachments or by scanning electron microscope.

#### *1.6.1.4 Melting Point*

The melting point of the substance is thermodynamically defined as the temperature at which the solid and liquid phases are at equilibrium. The melting point determination is a good first indication of purity since the presence of relatively small amounts of impurity can be detected by lowering as well as widening in the melting point range.

#### *1.6.1.5 Thermal Analytical profile*

During synthesis and isolation, a sample may have been exposed to changes in the temperature environment that may be exhibited as a thermal profile when the sample is heated between ambient temperature and its melting point. When no thermal history exists, the sample will neither absorb nor give off heat prior to its melting point. The basic technique used to study this phenomenon is called differential thermal analysis. (DTA)

#### *1.6.1.6 Hygroscopicity*

A compound is hygroscopic if it picks up significant amount of moisture under a specific condition of temperature and humidity. A high degree of hygroscopicity may adversely affect the physical and chemical properties of a drug substance, making it either pharmaceutically difficult or unsatisfactory to work with.

Hygroscopicity studies are usually carried out over a range of humidity conditions relevant to the general laboratory and manufacturing areas as well as un-controlled storage environment. A low humidity condition can be used to determine whether a hydrate will lose water under such storage.

#### *1.6.1.7 Absorbance Spectra*

Molecules with structural unsaturation can absorb light within a specific frequency range. The ultra-violet and visible spectra of compounds in solution are not highly specific; however, they are very suitable for quantitative analytical work and serve additional information for compound identification. The IR spectrum is highly specific for each chemical structure, with small structural differences resulting in significant spectral changes. After running a spectrum, significant peaks relating to major functional groups are identified; spectra of subsequent samples of the same compound are compared with the original. If IR spectral differences are found, the reason for and source of change should be investigated.

#### *1.6.1.8 Solubility*

Solubility is of prime importance for developing solutions that can be injected intravenously or intramuscularly.

#### *1.6.1.9 Solubility measurement*

The analytical method used in obtaining solubility measurements may vary according to the drug moiety. If the drugs structure has unsaturated conjugation, enabling it to absorb visible or ultraviolet light, spectrophotometric analysis can be performed. Solubility determination of compounds that don't absorb UV or visible light can be attempted by using gravimetric analysis

#### *1.6.1.10 pH – Solubility profile*

Compounds with acidic or basic functionality will show differences in solubility characteristics with changes in solution pH in accordance to their ionization constants. pH – solubility profiles can be established by running equilibrium solubility experiments within the range 3 – 4 pH units on both sides of the $pK_a$. The relationship between solubility of an acidic drug and pH can be defined with respect to its $pK_a$ using equation.

$pH = pK_a + \log (c_s/c_a)$ ------ Equation 1

### *1.6.2 Accelerated stability evaluation*

Various stress tests are performed on solid and solution samples to establish the effect of heat, light, oxygen and pH on drug substance stability.

#### *1.6.2.1 Heat stability*

Heat stability of a drug substance in solution will have a major influence on the marketable physical form of the Injectable product as well as processing parameters allowable. Drugs that

are not stable in solution require refrigerated storage or lyophilisation.

#### *1.6.2.2 Light stability*

The effect of light on physical and chemical stability of a drug is examined to determine whether light protection is required for the drug substance alone as well as in the final formulation. Stability changes can occur in the form of colour change, precipitation, pH shift, or decomposition. Although there are no proven methods for extrapolating light stability data to normal lighting conditions, the detrimental effect of light can usually be observed during such studies, particularly when the compound is very light sensitive.

#### *1.6.2.3 pH stability profile*

A pH stability profile experiment is performed with solution samples between pH 2 and 12 at selected elevated temperature. Analytically prepared solutions, close to the desired product concentration, are prepared using buffer solution within the selected pH range and filled into ampoules. The samples are placed into a constant temperature bath or oven and maintained at specific temperature between 55$^{o}$ C and 95$^{o}$C for 2 weeks. Here again the air headspace of ampoules is replaced with nitrogen or argon to avoid any oxidative effects. At preselected present intervals, samples are quenched and assayed. The data are plotted on rectilinear paper. The pH range of maximum stability is usually evident by inspection. This would be the range recommended for formulation development.

#### *1.6.2.4 Autoclaving Studies*

Since autoclaving is a preferred means of achieving the sterility of solutions, an early determination of stability to autoclaving should be made. Vials containing solutions at the optimum pH range previously established are exposed to autoclaving conditions of 121$^{o}$C at 30 psig for 20, 30, 45 and 90 minutes. The higher time points are used in an attempt to force degradation. Assay data are recorded together with evaluation of change in colour, pH and particulate matter content. [17]

#### *1.6.2.5 Effect of oxygen*

The need to monitor headspace oxygen levels in parenteral containers arises from the requirement to ensure the stability and potency of oxygen-sensitive product. Besides a loss of efficacy and reduction in shelf life, exposure of such products to oxygen can result in product discoloration, changes in dissolution rate and profile, and even toxicity or other pharmacological properties associated with negative side effects. [18]

### *1.6.2.6 Terminal Sterilization*

The modes of sterilization commonly used for parametric release are moist heat, ethylene oxide, and ionizing radiation sterilization

***Moist Heat Sterilization***

The most commonly used technique for terminal sterilization is autoclaving, which makes use of saturated steam. Moist heat sterilization of pharmaceutical products includes several types of sterilizing environments and sterilizing media. "Saturated steam, hot water spray, and submerged hot water processes are all considered as moist heat sterilizing environments. Different processes may be used to sterilize products by moist heat, and they include batch-type sterilizers and continuous-type sterilizers". [19]

### *1.6.2.7 Filtration*

Filtration may be used for the removal of particles, including microorganism, from the solution without the application of heat. The process depends upon the physical removal of organisms by passing through proof filter, which is used for the sterilization of thermo labile solutions, useful process for sterilization of large solution and gases including air. Membrane filters are compared of various type of cellulose and cellulose derivatives. A vast range of grades and pore size are available. They are very thin and should be handle carefully. The pore size most often used for sterilization of parenteral is 0.22 µm for the clarification sometimes 0.45 µm. [20]

## *1.6.3 Drug-Excipient Compatibility Studies*

Inappropriate excipients can also give rise to inadvertent and/or unintended effects which can affect the chemical nature, the stability and the bioavailability of the API, and consequently, their therapeutic efficacy and safety. Studies of drug-excipient compatibility represent an important phase in identifying interactions between potential formulation excipients and the API in the development stage of all dosage forms, three types of incompatibility:

- Physical incompatibility: We assess the change in the physical form of the formulation, like colour changes, dissolution, solubility, sedimentation rate, liquefaction, phase separation or immiscibility.
- Chemical incompatibility: We assess undesirable react between API and excipients to monitor if compounds undergo hydrolysis, oxidation, reduction, precipitation, decarboxylation, and racemization.
- Therapeutic incompatibility: We assess the interactions which are observed after administration of the medication. Examples of biopharmaceutical interactions are premature

breakdown of enteric coat, interactions due to adjunct therapy and increase in gastrointestinal motility. [21, 22]

## 1.7 General modes of drug degradation

Various functional groups within a molecule may be prone to a specific type of reactivity under appropriate conditions. The conditions necessary for degradation are generally more pronounced when drugs are in solution or suspension. The following reactions are commonly encountered.

1. Hydrolysis
2. Oxidation
3. Decarboxylation
4. Racemisation
5. Polymorphism
6. Solvate formation

## 1.8 Formulation development of small volume parenteral dosage form

The united states pharmacopoeia defines a small volume injectable (SVI) as “an injection that is packaged in containers labelled as containing 100 mL or less”. Therefore, all sterile products packaged in vials, ampoule, syringes, cartridges, bottles, or any other container that is 100 mL or less fall under this classification. SVIs can be sterilized terminally or by aseptic filtration and processing. [23]

### *1.8.1 Influence of the route of administration*

One of the most important considerations in formulating a parenteral product is the appropriate volume into which the drug should be incorporated. The intravenous route is the only route by which large volumes can be administered, although the rate of administration must be carefully controlled. Volume up to 10 ml can be administered intraspinally, while the intramuscular route is normally limited to 3 ml, subcutaneous to 2 ml and Intradermal to 0.2 ml.

The choice of the solvent system or vehicle is directly related to the intended route of administration of the product. Intravenous and Intraspinal injections are normally restricted to dilute aqueous solutions, whereas oily solutions, cosolvent solutions, suspensions and emulsions can be injected intramuscularly and subcutaneously.

### *1.8.2 Selection of the vehicle*

Most parenteral products are aqueous solutions. Chemically, the high dielectric constant of water makes it possible to dissolve ionisable electrolytes and its hydrogen bonding potential facilitates the solution of alcohols, aldehydes, ketones and amines. When it is not possible to use a wholly aqueous solution for physical or chemical reasons, the addition of solubilising agents or cosolvents may be necessary.

### *1.8.3 Solubility*

Various techniques as mentioned as follows can be employed to enhance the solubility of the drug.

- ***Methods of improving inherent solubility***

When a drug's inherently low water solubility does not meet the solution concentration, various approaches like salt formation, prodrug design, complexation, particle size reduction, addition of a cosolvent, and use of surface-active agents are used.

### *1.8.4 Salt formation*

To improve the aqueous solubility of the base, various organic acid salts were prepared. Results indicate that a significant increase in solubility can be achieved with a proper choice of salt form. This enhanced aqueous solubility was attributed in part to the decrease in the crystal lattice energy. If a particular salt form can't be isolated due to its very high solubility, the same end result can be achieved by insitu salt formulation.

### *1.8.5 Cosolvents*

solvents are used to solubilize a drug substance when its aqueous solubility alone is insufficient to achieve the desired level.

### *1.8.6 Prodrug approach*

The solubility characteristics of a drug can be altered via chemical modification. This has been referred to as prodrug approach.

### *1.8.7 Surfactants as solubilizers*

Drug solubility can be enhanced using surface-active agents such as Sorbitan monooleate and polyoxyethylene sorbitan monooleate. The surfactants are generally used in the range 0.05% to 0.5 %. They are effective solubilising agents because by virtue of their wetting properties and association tendencies, they are able to disperse water insoluble substances. Surfactants can be ionic or non-ionic type. With the exception of the non-ionic type, surfactants are not generally used in Parenteral because of destruction to biological membranes.

### *1.8.8 Alteration of $pK_a$*

The aqueous solubility of a base at given pH depends on the solubility of the free base, its $pK_a$ and the solubility of the salt species. If an analog of that base having a higher $pK_a$ value can be obtained without significantly decreasing the intrinsic solubility of the free-base form, then at given pH x, the solubility of the analog will also be higher.

### *1.8.9 Partition coefficient*

The partition coefficient, P, is a measure of lipophilicity of a compound. It is measured by determining the equilibrium concentration of a drug in an aqueous phase (generally water) and an oil phase (generally octanol or chloroform) held in contact with each other at constant temperature and is expressed as in equation

$P=[C_{oil}]/[C_{water}]$. ------ Equation 2

In case of parenteral emulsions values may provide an indication of the duration of activity that a drug is likely to achieve. If the partition coefficient is high, a depot effect can be expected for the drug dissolved in the oil phase.

### *1.8.10 Ionization constant*

The ionization constant provides information about the solubility dependence of the compound on the pH of the formulation.

### *1.8.11 Optical density*

Molecules capable of rotating a beam of plane polarized light are termed optically active. When working with optically active compound during preformulation studies, it is essential to monitor the optical rotation since the chemical assay alone will not always coincide with the biological activity.

### *1.8.12 Types of vehicles*

#### *1.8.12.1 Aqueous*

The vast majority of Injectable products are administered as aqueous solutions. The current USP has monographs for Purified water, Water for Injection, Sterile WFI and Sterile Water for Irrigation. WFI is the solvent of choice for making of Parenterals. It must be prepared fresh and be pyrogen free. Other USP requirements include no more than 10 parts per million of total solids, a pH of 5.0 to 7.0, absence of chloride, sulphate, calcium, ammonium ion and carbon dioxide and limits for heavy metals and organic material. The tests required for WFI are generally the same among the various pharmacopoeias, but differences do exist with regard to limits. For example, only the British Pharmacopoeia and the USP have standards for particulate matter.

WFI may be prepared by either distillation or reverse osmosis but the distillation method is by far the most common and accepted method. Prior to distillation, the water used as the source for WFI is usually subjected to chlorination, carbon treatment, and deionization and sometimes-reverse osmosis treatment. After distillation, it is filtered and then stored in a chemically resistant tank at a cold temperature around 5°C or at an elevated temperature between 65°C and 85°C to inhibit microbial growth and prevent pyrogen formation. Generally, the water is continually circulated during storage and usually filtered again prior to use in manufacturing. Sterile Water for Injection and bacteriostatic WFI are permitted to contain higher levels of solids than WFI because of the possible of the glass container constituents into the water during sterilization and storage.

| Type | Preparation | Pyrogen | Comments |
|---|---|---|---|
| **Purified water USP** | Distillation or ion exchange | No | Pharmaceutical solvent |
| **Water for injection USP** | Distillation or reverse osmosis Not sterile. | Yes[a] | Must be used within 24 Hours stored below $5^0$C or $80^0$C; used for manuf. of parenteral products to be sterilized |
| **Sterile water for injection USP** | Distillation or reverse osmosis | Yes[a] | Same as WFI; single-dose containers; also used to reconstitute sterile solids and dilute sterile solutions |
| **Bacteriostatic water for injection USP** | Distillation or reverse osmosis | Yes[a] | Multiple and single dose |
| **Sterile water for irrigation USP** | Distillation or reverse osmosis | Yes[a] | 1L or larger, wide mouth, does not meet particulate matter requirements for LVI; labelled ‘‘For Irrigation Only’’ |
| [a] ≤0.25 endotoxin units per ml | | | |

**Table 2: Various types of water for injection described in USP**

***1.8.12.2 Water Miscible:***

These cosolvents have already been discussed. Although water-miscible solvents are used in Parenterals, principally, to enhance drug solubility, it is important to mention that they also serve as stabilizers for those drugs that degrade by hydrolysis.

### *1.8.12.3 Nonaqueous:*

Drugs that are insoluble in aqueous systems are often incorporated into metabolizable oils as discussed before. [24]

## 1.9 Added substances

Added substances such as antioxidants, buffers, bulking agents, chelating agents, antimicrobial agents, solubilising agents, surfactants and tonicity adjusting agents must frequently be incorporated into parenteral formulae in order to provide safe, efficacious and elegant parenteral dosage forms.

Pharmacopoeias often specify the type and amount of additive substances that may be included in Injectable products. These requirements often vary from compendia to compendia. So, it is important to refer to the specific pharmacopoeia that applies to the product in question.

### *1.9.1 Buffers:*

Changes in the pH of a preparation may occur during storage because of degradation reactions within the product, interaction with container components and absorption or evolution of gases and vapours. To avoid these problems, buffers are added to many products to resist a change in pH. A suitable buffer system should have an adequate buffer capacity to maintain the pH of the product at a stable value during storage, while permitting the body fluids to adjust the pH easily to that of the blood following administration. Therefore, the ideal pH to select would be 7.4, the pH of blood. Extreme deviation from this can cause complications.

| **PH** | **Buffer system** | **Concentration (%) w/v** |
|---|---|---|
| **3.5–5.7** | Acetic acid–acetate | 1–2 |
| **2.5–6.0** | Citric acid–citrate | 1–5 |
| **6.0–8.2** | Phosphoric acid–phosphate | 0.8–2 |
| **8.2–10.2** | Glutamic acid–glutamate | 1–2 |

**Table 3: Buffers commonly used in Parenteral**

### *1.9.2 Antioxidants*

Many drugs in solution are subjected to oxidative degradation. Such reactions are mediated either by free radicals or by molecular oxygen and often involve the addition of oxygen and

removal of hydrogen. Oxidative decomposition is catalysed by metal, hydrogen and hydroxyl ions. By increasing the oxidation potential of the drug, oxidation can be minimized. Lowering the pH of the solution will increase the oxidation potential. This occurs according to the simplified version of the Nernst equation

$E=E_o+RT/nlog[H^+][Ox]/Rd]$ -------- Equation 3

An increase in hydrogen ion concentration causes the increase in the actual oxidation potential, E. In this equation, $E_o$ is the standard oxidation potential, R, is the gas constant, T is the absolute temperature and n represents the number of electrons taking part in the oxidation-reduction reaction. For products in which oxygen is directly involved in the degradation, protection can be afforded by displacing oxygen from the system. This is accomplished by bubbling nitrogen, argon or carbon dioxide through the solution prior to filling and sealing in the final container.

| Antioxidant | Concentration range (%) | Antioxidant | Concentration range (%) w/v |
|---|---|---|---|
| **Sodium bisulphite** | 0.05–1.0 | Butylated hydroxyanisole | 0.005–0.02 |
| **Sodium sulphite** | 0.01–0.2 | Butylated hydroxytoluene | 0.005–0.02 |
| **Sod. Metabisulfite** | 0.025–0.1 | L- and D-ascorbic acid | 0.02–1.0 |

**Table 4: Antioxidants used in SVPs**

### *1.9.3 Antimicrobials*

Agents with antimicrobial activity must be added to preparations packaged in multiple dose containers unless prohibited by the monograph or unless the drug itself is bacteriostatic. They are often added to unit-dose solutions that are not sterilized at the terminal stage of their manufacture. In the case of multiple-dose preparations, the anti-microbial agent is required as a bacteriostatic to inhibit any microbes accidentally introduced while withdrawing doses. The effectiveness of the antimicrobial agents can be tested by challenging the product with selected organisms to evaluate the bacteriostatic or bactericidal activity in a formulation.

| Antimicrobial Agents | MIC range | Amount most often used % |
|---|---|---|
| **Benzalkonium chloride** | 0.005-0.03 | 0.01 |
| **Benzethonium chloride** | 0.005-0.03 | 0.01 |
| **Benzyl alcohol** | 1.0 -10.00 | 1.0 |
| **Chlorobutol** | 0.2-0.8 | 0.5 |
| **Chlorocresol** | 0.1-0.3 | 0.1-0.25 |
| **Cresol** | 0.1-0.6 | 0.3 |
| **Parabens (methyl, ethyl, propyl, butyl esters)** | 0.05-0.25 methyl<br>0.005-0.03 other | 0.18<br>0.02 |
| **Phenol** | 0.1-0.8 | 0.5 |
| **Phenyl mercuric nitrate** | 0.001-0.05 | 0.002 |
| **Thiomersal** | 0.005-0.03 | 0.01 |

**Table 5: Antimicrobials used in Parenteral Products**

### *1.9.4 Tonicity contributors*

Isotonic solutions exert the same osmotic pressure as blood plasma. To minimize tissue damage and irritation, reduce haemolysis of cells and prevent electrolytic imbalance upon administration of small volume Parenteral the products should be isotonic, or nearly so. Sodium or Potassium chloride and dextrose are commonly added to adjust the hypotonic solutions. There are several methods available to calculate tonicity. The sodium chloride equivalent method is the most convenient.

If a solution is hypertonic, not much can be done with the formulation unless it can be diluted with water prior to administration. Administration of a hypertonic solution should be done slowly to permit dilution by the blood. Tonicity agents are dextrose, glycerine, mannitol, potassium and sodium chloride. [25]

## 1.10 Evaluation of parenteral

### *1.10.1 pH*

pH is the measure of the concentration of protons ($H^+$) in a solution that is the potential of hydrogen. It is the identification of a substance how it is acidic or alkaline by using a scale of acidity from 0 to 14. More the acidic solutions having lower pH, and more alkaline solutions

having higher pH values. pH value less than 7 are acids and pH of greater than 7 are alkaline. The neutral solutions that are the substances which are not acidic or alkaline have a pH value of 7.

### *1.10.2 Particulate matter*

In injections and parenteral infusions particulate matter is considered as, the mobile undissolved particles, other than the gas bubbles, unintentionally that present in the solutions. There are two procedures involved in the determination of the particulate matter.

| **Light Obscuration Particle Count Test** | | |
|---|---|---|
| | **> 10µm** | **> 25µm** |
| **Small volume injection** | 6000 | 600 per container |
| **Large volume injection** | 25 | 3 per ml |
| **Microscopic particular count test** | | |
| | **> 10µm** | **> 25µm** |
| **Small volume injection** | 3000 | 300 per container |
| **Large volume injection** | 12 | 2 per ml |

**Table 6: USP limit for particulate matter**

### *1.10.3 Sub-visible particles*

Method 1 (light obscuration particle count test)

Method 2 (microscopic particle count test)

Injections and parenteral infusions are examined for sub-visible particles usually method 1 is preferred mostly. Then also some preparations by light obscuration particle count test that followed by a microscopic particle count test are necessary to test. No, all parenteral are examined by method 1 such as preparations that reduced the clarity or increased viscosity, since these tests are carried out according to method 2. For example, colloids, emulsions. Particulate matter contamination is still having a potential cause to harm patients.

### *1.10.4 Sterility test*

Sterility testing is to identify the presence or absence of viable micro-organisms in the sample.

A. Immersion (Direct inoculation)

It requires the test article to be inoculated directly into test media.

B. Membrane Filtration

It requires the test article to pass through a size exclusion membrane capable of retaining micro-organisms. The filter should be rinsed. Then the membrane is transferred to the test medium.

Media types: mostly used Soya-bean casein digest (SCD) and Fluid thioglycolate media (FTM).

Incubation period: all test containers should incubate at temperatures as specified in the pharmacopoeial method, which is for each test media at least 14 days, depends on whether filtration or direct inoculation test is used.

### *1.10.5 Stability test*

Stability is defined as the capacity of a drug substance or drug product to remain within the established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating period. The objective of stability study is to determine the shelf life, namely the time period of storage at a specified condition within which the drug product still meets its established specifications. Stability testing also gives information about drug vulnerability to degrade by oxidation, hydrolysis, isomerization, polymerization, decarboxylation, moisture, heat, and light. Stability study is performed for a specific time at specific environmental conditions according to ICH guidelines.

### *1.10.6 Osmolality*

Osmotic pressure plays a critical role in all biological processes that involve diffusion of solutes or transfer of fluids through membranes. Osmosis occurs when solvent but not solute molecules across a semipermeable membrane from regions of lower to higher concentrations to produce equilibrium. The knowledge of osmotic pressures is important for practitioners in determining whether a parenteral solution is hypo-osmotic, iso-osmotic, or hyperosmotic. A quantitative measure of osmotic pressure facilitates the dilution required to render a solution iso-osmotic relative to whole blood.

The results suggest that the solution is slightly hyperosmotic because the osmolality of blood ranges between 285 and 310 mOsmol per kg.

**1.11 Sterilization:**

Sterilization of parenteral products should be done after sealing it to the final container that is called as terminal sterilization. It should do within as short time as that possible after the filling and sealing are fully completed. This is accomplished usually by the thermal process. Radiation sterilization also will do to the parenteral finished products in sometimes. The care should be taken in the effect of the elevated temperature on the stability of the products. The elevated temperature that required for the sterilization by thermal process is adversely affects in many products like both pharmaceutical and biological. Non thermal methods are used for the heat-labile products. These nonthermal methods include filtration through the bacteria retaining filters. Aseptic conditions should be strictly followed for all operations, and then only the contamination is not introduced into the filtrate. Dry-heat sterilization is performed for few dry solids that are not adversely affects by the high temperatures and that require long period of heating. For the sterilization of glassware and metal ware mostly performs the dry-heat sterilization process. After the sterilization process all the equipment will be sterile, dry and pyrogen-free. Autoclaving (saturated steam under pressure) is the most common method used for sterilization process. It is the most effective sterilization method that used for the aqueous liquids or substances, since it can be reached or penetrated by the steam. Radiation sterilization is a terminal sterilization method with an ionizing radiation (gamma rays). There is an advantage for the applying on drugs in their final container, that also without any rise in temperature. One of the disadvantages is the possible formation of radiolytic products which leads to a change in the color and odor of the product.

***1.11.1 Packaging materials for parenteral dosage form:***

A. Glass containers
B. Plastic containers
C. Rubber closures

***1.11.1.1 Glass containers***

In most of the small volume injections glass is used as the material choice for the containers. Principally glass containers are composed of silicon dioxide with varying amounts of various oxides such as potassium, sodium, calcium, magnesium, aluminum, boron, and iron. Silicon

oxide tetrahedron forms the basic structural network of glass. Boric oxide will enter into the basic structural network and the other oxides do not enter into this structure.

***1.11.1.2 Plastic containers***

Sterile preparation like large-volume parenteral, ophthalmic solutions and mainly in small volume parenteral uses thermoplastic polymers as packaging materials. The principal advantage of plastic while comparing with glass, it is not breakable easily and reduction in weight also. In large-volume intravenous fluids currently uses the flexible bags of PVC (polyvinyl chloride) or select polyolefin. This having major advantage that there is no requirement of air interchange.

***1.11.1.3 Rubber Closures***

Rubber closures are made up of using milling machines by multiple ingredients plasticized and mixed together at an elevated temperature. The allergenic proteins from the natural rubber vial closures or stoppers that release into aqueous pharmaceuticals induce some allergic reactions in individuals with latex allergy receiving medications from such vials. [26]

## 2. Literature review

1. **Jason P Hecht et al.,[27] (2019)** studied the Safety of high-dose intravenous labetalol in hypertensive crisis by assessing in adults. Patients were included if they received 300 mg of I.V. labetalol within a 24-hour period. Vital signs, adverse events and cumulative medication doses were obtained for up to 24 hours while on labetalol. The cumulative labetalol dose was not associated with adverse safety outcomes ($p$ = 0.428), although eighty-one patients (44.3%) experienced adverse events. Sixty-six patients (36.5%) developed bradycardia and 34 patients (18.6%) developed hypotension. Only five patients (2.7%) required a rescue agent for refractory adverse events, the study found that these events rarely caused clinically significant hemodynamic compromise and was not statistically associated with adverse events.

2. **F. A. Marzook et al.,[28] (2019)** studied the preparation of labetalol as a $\beta_1$-Adrenoceptor for Use in Nuclear Medicine. The reaction conditions (amounts of stannous chloride and substrate, pH, time) were optimized, and the radiochemical yield as high as 95 ± 0.4% was reached. Biodistribution in mice was studied. Labetalol showed excellent initial heart uptake and good retention. The heart/liver ratios were 5.16, 2.70, and 2.15 at 30, 60, and 120 min post injection, respectively.

3. **Fransway, Anthony F et al.,[29] (2019)** reviewed the paraben toxicity, the allergologic concerns regarding parabens raised during the past century are no longer a significant issue. To assert that parabens are safe for use as currently used in the pharmaceutical industries, all toxicological end points must be addressed. Parabens are involved in the genesis or propagation of these controversial and important health problems are premature. Haste to remove parabens from consumer products could result in their substitution with alternative, less proven, and potentially unsafe alternatives.

4. **Kabirdas Ghorpade and Sharda Shinde.,[30] (2019)** reviewed the particulate matter in parenteral. The presence of foreign visible and sub visible particulate matter in parenteral formulation affects its biological safety. Hence there is need to proper check on the sources of the particulate matters into the formulation. Various regulatory bodies established procedures and standards to ensure the quality of the parenteral. So,

manufacturers should be to continue to minimize the risk of the particulate and product should meet the specifications.

5. **Neha Tiwari et al.,[31] (2018)** investigated the development and validation of RP-HPLC method for simultaneous determination of paraben preservatives in pharmaceutical liquid dosage, that to allows the simultaneous estimation of the methyl paraben (MP) and propyl paraben (PP) in pharmaceutical liquid dosage form. The mobile phase was filtered and degassed mixture of buffer (pH 2.0) and acetonitrile (68: 32). The detector wavelength was set at 205 nm and flow rate was 1.0 mL/min. The method was successfully used for estimating both paraben preservatives in pharmaceutical liquid formulation.

6. **Mariani A.Ciciliati et al.,[32] (2018)** investigated by the thermal behaviour of labetalol hydrochloride, has been using by thermogravimetry (TG), differential thermal analysis (DTA), differential scanning calorimetry (DSC), thermogravimetry coupled to infrared spectroscopy (TG-FTIR), hot stage microscopy and gas chromatography coupled to mass spectrometry (GC–MS). DSC curves demonstrated that the sample melted at T = 180.8 °C, without recrystallization on cooling. Hot stage microscopy confirmed that labetalol melted and decomposed releasing water from its structure. GC–MS analysis allowed characterizing some intermediates of the drug degradation and the identification of other degradation products.

7. **Pablo Zambrano et al.,[33] (2018)** studied the α1-and β-adrenergic antagonist labetalol induces morphological changes in human erythrocytes, at low concentrations labetalol preferentially interacted with dimyristoylphosphatidylethanolamine (DMPE). On the other hand, results obtained by scanning electron microscopy (SEM) showed that labetalol alters the normal biconcave form of erythrocytes to stomatocytes and knizocytes. According to the bilayers couple hypothesis, this result implied that the drug inserted in the inner monolayer of the human erythrocyte membrane.

8. **Akter FA et al.,[34] (2017)** studied the response & side effects of injectable labetalol in pregnancy induced severe hypertension. They performed to assess the response & side effects of injectable Labetalol in the treatment of pregnancy induced severe hypertension. All patients were treated with intravenous Labetalol 20 mg & the dose was repeated at

sequential escalating dosages every 15 minutes until a therapeutic goal of systolic blood pressure less than 160 mm of Hg & diastolic blood pressure less than 105 mm of Hg were achieved. It was observed that majority patients' blood pressure was controlled by 1-2 doses. It was noticed that injection Labetalol controls blood pressure in 80% antenatal cases & 86% postnatal cases.

9. **Raju Sudhakar et al.,[35] (2016)** studied the intravenous labetalol with oral antihypertensive combination Nifedipine and Alpha-methyldopa in the acute management of high blood pressure in severe pregnancy-induced hypertension patients. Intravenous labetalol achieves adequate and faster blood pressure control with better heart rate maintenance than the routinely used oral antihypertensive combination of tablets alpha-methyldopa and nifedipine in the control of blood pressure in severe pregnancy-induced hypertension patients.

10. **Raju Prasad Tayung et al.,[36] (2016)** studied the Effect of intravenous labetalol in controlling the cardiovascular response to laryngoscopy and intubation. Laryngoscopy and intubation violate the patient's protective airway reflexes and lead to physiological changes involving various systems of the body. The present study compares the safe and clinically effective intravenous bolus dose of labetalol and lignocaine for controlling the cardiovascular response to laryngoscopy and intubation. Significant increase in heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure were observed in group I after laryngoscopy and intubation.

11. **Verma S et al.,[37] (2016)** studied the Inadvertent intra-thecal injection of labetalol. The use of labetalol to treat hypertension, especially in pregnant patients., and a report a case of a female patient who was given labetalol intrathecal administration in place of bupivacaine due to a similar appearance of ampoules which resulted in a drop-in blood pressure and pulse rate. The patient responded to fluid resuscitation and there occurred no neurological sequela.

12. **Thomas et al.,[38] (2011)** determined the efficacy and safety of labetalol for hypertensive crisis in children less than or equal to 24 months of age. Retrospective chart review. Statistical analysis utilized analysis of variance for continuous data, chi-square tests for

nominal data, and linear regression. A 737-bed pediatric teaching institution. Twenty-seven patients less than or equal to 24 months of age were treated with 37 intravenous infusions of labetalol, nicardipine, or nitroprusside for hypertensive crisis or hypertensive urgency, Continuous infusion of labetalol reduced mean systolic blood pressure by at least 20% in <8 hrs. This effect was similar to nicardipine and nitroprusside infusions.

13. **Samir Fahed et al.,[39] (2008)** studied the labetalol infusion for refractory hypertension causing severe hypotension and bradycardia. incremental doses of intravenous labetalol are safe and effective and, at times, such therapy may need to be augmented by a continuous infusion of labetalol to control severe hypertension. Continuous infusions of labetalol may exceed the recommended maximum daily dose of 300 mg on occasion. The labetalol infusion resulted in a profound cardiovascular compromise in this postoperative critically ill patient.

14. **Sofuoglu M et al.,[40] (2003)** investigated the effects of labetalol, on acute physiological and subjective effects of intravenous nicotine and on tobacco withdrawal symptoms. Following overnight abstinence from smoking, subjects were treated orally with a single 100- or 200-mg dose of labetalol in each of three experimental sessions. Two hours after the medication treatment, subjects received an intravenous injection of 15 microg/kg nicotine. No treatment effects were found for systolic or diastolic blood pressure changes. For the subjective effects of nicotine, treatment with both high and low doses of labetalol enhanced the ratings of "head rush" and "drug strength."

15. **S Oishi et al.,[41] (2002)** reviewed the Pharmacology, Pharmacokinetics, Clinical uses and Adverse effects, the uses of Parabens as preservatives in pharmaceuticals, that propyl paraben also adversely affects the hormonal secretion and the male reproductive functions. Propyl paraben was administered to 3-week-old rats with the AIN93G modified diet. At the end of 4 weeks, the rats were sacrificed by decapitation and the weights of testes, epididymites, prostates, seminal vesicles and preputial glands were determined. The exposure level at which this effect was observed is the same as the upper-limit acceptable daily intake (10 mg/kg body weight/day) of parabens.

16. **Yeleswaram K et al.,[42] (1993)** studied the transplacental and nonplacental clearances, metabolism and pharmacodynamics of labetalol in the fetal lamb after direct intravenous administration study describes the pharmacokinetics, metabolism and pharmacodynamics of labetalol in the fetal lamb after direct fetal IV bolus (4 mg) administration. The glucuronide conjugate of labetalol was found in the amniotic fluid at up to 20 times the free drug concentration. The calculated hind limb arteriovenous lactate flux showed a net output of lactic acid equal to 3.85 +/- 2.05 g from the hind limb over 24 h after labetalol administration.

17. **John A. Clark et al.,[43] (1990)** studies the hepatotoxicity of labetalol, the temporal circumstances strongly implicate labetalol; the conditions of nine patients improved after cessation of labetalol therapy, and one patient had a recurrence after therapy was restarted. The reported histologic changes were consistent with hepatocellular necrosis in four instances and chronic active hepatitis in one. The clinical presentation of the cases was most compatible with the mechanism of metabolic idiosyncrasy, but other pathogenetic explanations could not be entirely excluded.

18. **G.Krumpl et al., [44] (1990)** studied the antiarrhythmic efficacy of labetalol as assessed by programmed electrical stimulation. Labetalol was administered in cumulative doses (0.5, 1 and 3 mg/kg−1 90 min−1, IV). Compared to control the systolic blood pressure was significantly decreased 20 min after 0.5, 1 and 3mg/ kg−1 and up to 30 min after 3 mg/ kg−1 labetalol. The diastolic blood pressure was significantly decreased 20 and 30 min after 0.5 and 3 mg/kg−1 but was not significantly altered after 1 mg /kg−1 labetalol. Labetalol was active against arrhythmias induced by programmed electrical stimulation. This effect was already present after the lowest dose (0.5 mg /kg−1). Labetalol may be of potential benefit in controlling arrhythmias arising following myocardial infarction.

19. **James Huey et al.,[45] (1988)** investigated clinical evaluation of intravenous labetalol for the treatment of hypertensive urgency, the antihypertensive medications currently used in the treatment of hypertensive urgencies are limited due to deleterious side effects or requirements for sophisticated monitoring techniques. Labetalol HCl is a unique adrenergic blocking agent that can smoothly lower blood pressure following bolus

injection without increasing heart rate or cardiac output. This study evaluates the efficacy and safety of intravenous boluses of labetalol HCl in the treatment of patients presenting to the hospital with a diagnosis of hypertensive urgency (diastolic blood pressure ≥ 110 mm Hg).

20. **R.M. Bojar et al.,[46] (1988)** studied the intravenous labetalol for the control of hypertension following repair of coarctation of the aorta. An early phase of systolic hypertension has been ascribed to elevated levels of norepinephrine. Activation of the renin-angiotensin system from sympathetic stimulation has been implicated in a later phase of systolic and diastolic hypertension that can result in mesenteric arteritis. The use of a rapidly acting, titratable intravenous alpha and beta adrenergic blocker, such as labetalol hydrochloride, addresses both of these neurohormonal mechanisms. in the intravenous form, it would appear to be an excellent choice for the management of early postoperative hypertension and it can be converted to the oral form in cases of persistent hypertension.

21. **Blair P. Grubb et al.,[47] (1987)** studied the intravenous labetalol in acute aortic dissection acute, is one of the most common clinical catastrophes involving the aorta. The major advances have occurred in our ability to diagnose and treat this devastating disorder. Coupled with the strides made in surgical therapy, prompt medical therapy has dramatically improved survival. Medical therapy consists of lowering both the blood pressure (BP) and the velocity of left ventricular ejection (dv/dt). Labetalol hydrochloride is a traditional combination therapy. We describe herein the successful use of intravenous labetalol in the treatment of acute aortic dissection.

22. **M Lebel et al.,[48] (1985)** studied the labetalol infusion in hypertensive emergencies. The antihypertensive effects of labetalol infusion (2 mg/min; maximal dose 150 mg) were evaluated in 22 subjects requiring rapid lowering of blood pressure because of severe hypertension, a hypertensive crisis after surgery, or before angiographic examination. Overall systolic and diastolic blood pressures were reduced from 201 ± 4 to 164 ± 4 mm Hg and from 123 ± 3 to 107 ± 3 mm Hg, respectively, but one subject had a transitory hypotensive episode that did not require treatment. Intravenous labetalol appears effective and well tolerated in the control of blood pressure in hypertensive emergencies.

23. **Lund-Johansen P et al.,[49] (1984)** reviewed the pharmacology of combined alpha-beta-blockade and Haemodynamic effects of labetalol, the cardinal haemodynamic disturbance in established hypertension is an increased total peripheral resistance and a subnormal blood flow, particularly during exercise. The spontaneously occurring changes in central haemodynamic have been followed in young males with essential hypertension over a 17-year period: a gradual increase in total peripheral resistance and blood pressure, and a gradual fall in cardiac output and stroke volume, have been demonstrated. Labetalol is given in most series the average reduction in blood pressure was 17 to 22%, the reduction in total peripheral resistance 11 to 14%, and the reduction in cardiac output 2 to 10%.

24. **E. Paul MacCarthy et al.,[50] (1983)** studies have shown that the antihypertensive efficacy of labetalol is administered alone or with a diuretic is often effective when other antihypertensive regimens have failed. Studies have shown that labetalol is effective in the treatment of essential hypertension, renal hypertension, pheochromocytoma, pregnancy hypertension and hypertensive emergencies. In addition, preliminary studies indicate that labetalol may be of value in the management of ischemic heart disease.

25. **Pul-Ho C. Yuen et al.,[51] (1983)** studied the compatibility and stability of labetalol hydrochloride in commonly used intravenous solutions by adding labetalol hydrochloride injection to 11 large volume parenteral (LVP) at concentrations of 1.25, 2.50, and 3.75 mg/ml. The initial and 72-hour samples were analysed for drug concentration, pH, osmolarity, and visual changes. High-performance liquid chromatography (HPLC) was used for the assay. In the admixture with 5% sodium bicarbonate injection, a white precipitate formed within six hours. No haze, precipitate, or colour change occurred in the 10 admixtures. Labetalol hydrochloride was stable for 72 hours at 4°C and 25°C in all i.v. solutions studied except 5% sodium bicarbonate injection.

26. **John D. Wallin, et al.,[52] (1983)** studied the current research and therapeutic status of labetalol hydrochloride, is the prototype drug of a new class of antihypertensive agents. It possesses approximately one fourth of the β-blocking activity of propranolol hydrochloride and one half of the α-blocking activity of phentolamine. In intravenous

form to manage hypertensive emergencies. Overall, the drug appears to offer several advantages over pure β-blocking drugs in some patients and should expand the armamentarium of the practicing physician in the management of the difficult hypertensive patient.

27. **JA Kane.,[53] (1982)** reviewed the labetalol in general practice**.** The results of the treatment of 8573 hypertensive patients with labetalol in general practice, for periods between 1 month and 5 year, are reviewed. The withdrawal rate attributable to side effects was between 6% and 13%. Tiredness, dizziness, headache and upper gastro intestinal symptoms were the four principal side effects, but they were usually transient when labetalol treatment was continued. No drug interactions or adverse haematological or biochemical changes were seen.

28. **R. C. Dage et al.,[54] (1980)** studied the direct vasodilatation by labetalol in anaesthetized dogs. The effects of several doses of labetalol (0.03 to 1 mg/kg) given intravenously and into the vertebral artery were examined in anaesthetized dogs. Labetalol produced to immediate (5 min) change in blood pressure or heart rate when given by either route, with one exception. In adrenalectomized, vagotomised spinal dogs, both labetalol (0.1 to 1 mg/kg IV) and hydralazine (1 mg/kg IV) elicited a fall in blood pressure without changing heart rate or cardiac output.

29. **CA Michael et al.,[55] (1979)** studies have shown to treat severe hypertensive disease complicating pregnancy. Labetalol was estimated in the cord blood of the fetus at delivery as well as in the breast milk of mothers on day 3 post-partum. There were no adverse effects of the drug on the infants and significant hypotension did not occur. It is concluded that the efficient hypotensive action of labetalol, together with apparent freedom from maternal and fetal side effects, and consequent improved perinatal mortality, suggest that it is a suitable drug for use in pregnancy complicated by hypertension.

30. **L Kaufman et al.,[56] (1979)** studied the use of labetalol during hypotensive anaesthesia and in the management of pheochromocytoma in 88 patients undergoing plastic surgery, 8 patients with carcinoma of the breast, 10 with carcinoma in the head and neck, each anaesthetized twice. The use of labetalol intravenously produced hypotension and a bloodless operating field in patients undergoing plastic surgery and in those undergoing

radical surgery for the removal of carcinoma. Two patients with pheochromocytoma pre-treated with oral labetalol before anaesthesia, Labetalol seems to be simpler and safer than previous techniques involving drugs with separate alpha and beta adrenoceptor blocking effects.

## 3. Aim of the present formulation development

Parenteral formulation is widely used especially when an immediate physiology response is needed, in emergency conditions and administering those drugs that are destroyed in gastro intestinal tract. These are the drug delivery system of choice for non-cooperative, nauseous and unconscious patients. There are different dosage forms available for the administration of the drug. Parenteral route constitutes the major advantages over the other routes, since the drug does not pass through the GIT and first pass metabolism are bypassed. The drugs that are available as a parenteral dosage forms provide the complete bioavailability since the drug passes through the systemic circulation.

The aim of the present study to develop a pharmaceutically acceptable, stable and reproducible generic formulation of labetalol hydrochloride injection. The qualitative and quantitative composition of the proposed generic drug product would be exactly the same as that of RLD in order to have a pharmaceutically and therapeutically equivalent formulation. The formulation is to be developed, considering the appearance, clarity, pH, colour, chemical stability attributes which govern the quality of the product. In order to overcome these disadvantages and to facilitate its administration to non-cooperative patients in emergency, the present study is undertaken with an intention to develop a stable and effective parenteral formulation containing labetalol hydrochloride.

In order to formulate stable injection formulation, the order of addition of drug, and excipients to be determined for four batches. Container compatibility, tubing compatibility and filter compatibility also included in the study. The oxygen sensitivity study, pH extreme studies, freeze thaw study and photo stability study also planned for the present investigation. Stability study of the optimized formulation to be conducted as per standard protocol.

## 4. Plan of work

**Step 1: Literature review**

**Step 2: Preformulation development studies**

- Raw material analysis of drug
- Evaluation study for Containers and closure
- Determination of interaction by Fourier Transform Infra-red Spectroscopy

**Step 3: Formulation development of labetalol hydrochloride injection**

- Determination of drug solubility in vehicle
- Determination of order of mixing
- Excess fill volume

**Step 4: Formulation process of labetalol hydrochloride injection**

**Step 5: Selection of sterilization method**

**Step 6: Stress study for labetalol hydrochloride injection**

- pH extreme studies
- Oxygen sensitivity studies
- Freeze thaw study
- Photo stability study

**Step 7: Hold time compatibility study for labetalol hydrochloride injection**

- Stainless steel vessel compatibility study
- Tubing compatibility
- Filter compatibility

**Step 8: Quality control study for labetalol hydrochloride injection**

**Step 9: Stability study for final development batch of labetalol hydrochloride injection**

## 5. Drug profile

### 5.1 Labetalol hydrochloride[57, 58]

**IUPAC name** : 2-hydroxy-5-{1-hydroxy-2-[(4-phenylbutan-2-yl)amino]ethyl}benzamide

**Synonyms** : 3-carboxamindo-4-hydroxy-alpha-((1-methyl-3-phenlpropylamino) methyl)benzyl alcohol

5-(1-Hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl)salicylamide

Labétalol, Labetalol, Labetalolum, Labetolol

**Molecular weight** : 364.87 g/mol

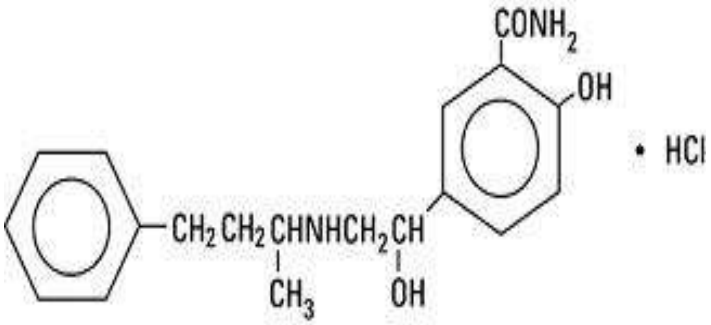
**Molecular formula** : $C_{19}H_{24}N_2O_3$

**CAS Number** : 32780-64-6

**Therapeutic group** : Blocker of both alpha- and beta-adrenergic receptors that is used as an antihypertensive

**Recommended dose:** 300 mg/day

**Structure** :



### 5.2 Physical and Chemical parameters

**Appearance** : white or off-white crystalline powder

**Solubility** : soluble in water and alcohol, it is insoluble in ether and chloroform

**pH** : 3.0 to 4.5

**pKa** : 8.05 to 9.8

**Melting point** : 180 $^{0}$C

### 5.3 Pharmacokinetics:

**Absorption** : Completely absorbed (100%)

**Protein binding** : 50%

**Metabolism** : Primarily hepatic, undergoes significant first pass metabolism

**Route of elimination** : Metabolites are present in plasma and are excreted in the urine, and via the bile, into the faeces

**Half-life** : 5.5 hours

**Renal clearance** : 33 mL/min/kg.

### 5.4 Mechanism of Action

Labetalol HCl combines both selective, competitive, alpha-1-adrenergic blocking and nonselective, competitive, beta-adrenergic blocking activity in a single substance. In man, the ratios of alpha- to beta- blockade have been estimated to be approximately 1:3 and 1:7 following oral and intravenous (IV) administration, respectively. The principal physiologic action of labetalol is to competitively block adrenergic stimulation of β-receptors within the myocardium (β1-receptors) and within a bronchial and vascular smooth muscle (β2-receptors), and α1-receptors within the vascular smooth muscle. This causes a decrease in systemic arterial blood pressure and systemic vascular resistance without a substantial reduction in resting heart rate, cardiac output, or stroke volume, apparently because of its combined α- and β-adrenergic blocking activity.

### 5.5 Indication

For the management of hypertension (alone or in combination with other classes of antihypertensive agents), as well as chronic stable angina pectoris and sympathetic overactivity syndrome associated with severe tetanus. Labetalol is used parenterally for an immediate reduction in blood pressure in severe hypertension or in hypertensive crises when considered an emergency, for the control of blood pressure in patients with pheochromocytoma and pregnant women with preeclampsia, and to produce controlled hypotension during anaesthesia to reduce bleeding resulting from surgical procedures.

### 5.6 Pharmacodynamics

Labetalol is a selective alpha-1 and non-selective beta-adrenergic blocker used to treat high blood pressure. It works by blocking these adrenergic receptors, which slows sinus heart rate, decreases peripheral vascular resistance, and decreases cardiac output. Labetalol has two asymmetric centres and therefore, exists as a molecular complex of two diastereoisomeric pairs. Dilevalol, the R,R' stereoisomer, makes up 25% of racemic labetalol.

### 5.7 Contraindication

Labetalol HCl injection is contraindicated in bronchial asthma, overt cardiac failure, greater than first-degree heart block, cardiogenic shock, severe bradycardia, other conditions associated with severe and prolonged hypotension, and in patients with a history of hypersensitivity to any component of the product.
Beta-blockers, even those with apparent cardioselectivity, should not be used in patients with a history of obstructive airway disease, including asthma.

### 5.8 Drug-drug interaction

Labetalol HCl in combination with tricyclic antidepressants experienced tremor. The contribution of each of the treatments to this adverse reaction is unknown, but the possibility of a drug interaction cannot be excluded.

Cimetidine has been shown to increase the bioavailability of labetalol HCl. Since this could be explained either by enhanced absorption or by an alteration of hepatic metabolism of labetalol HCl, special care should be used in establishing the dose required for blood pressure control in such patients.

Labetalol HCl blunts the reflex tachycardia produced by nitroglycerin without preventing its hypotensive effect. If labetalol HCl is used with nitroglycerin in patients with angina pectoris, additional antihypertensive effects may occur.

Both digitalis glycosides and beta-blockers slow atrioventricular conduction and decrease heart rate. Concomitant use can increase the risk of bradycardia.

### 5.9 Adverse drug reaction

Labetalol injection is usually well tolerated. Most adverse effects have been mild and transient,

- **Cardiovascular System:** Ventricular arrhythmia.
- **Central and Peripheral Nervous Systems:** Dizziness; tingling of the scalp/skin; hypoesthesia (numbness) and vertigo,
- **Gastrointestinal System:** Nausea; vomiting; dyspepsia and taste distortion,
- **Metabolic Disorders:** Transient increases in blood urea nitrogen and serum creatinine levels, these were associated with drops in blood pressure, generally in patients with prior renal insufficiency.
- **Psychiatric Disorders:** Somnolence/yawning
- **Respiratory System:** Wheezing
- **Skin:** Pruritus

## 6. Excipients profile

### 6.1 Methyl paraben:[59]

**Non-proprietary Names:**

- BP: - Methyl Hydroxybenzoate
- JP: - Methyl Parahydroxybenzoate
- PhEur: - Methyl Parahydroxybenzoate
- USP-NF - Methylparaben

**Synonyms**:

Aseptoform M; CoSept M; E218; 4-hydroxybenzoic acid methyl ester; metagin; Methyl Chemosept; methylis parahydroxybenzoas; methyl p-hydroxybenzoate; Methyl Parasept; Nipagin M; Solbrol M; Tegosept M; Uniphen P-23.

**Description:**

**Appearance** **:** colorless crystals or a white crystalline powder.

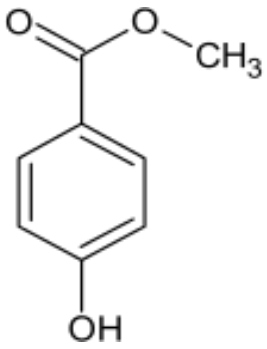
**Odour** **:** odorless

**Chemical Name and CAS Registry Number:** Methyl-4-hydroxybenzoate [99-76-3]

**Empirical Formula:** $C_8H_8O_3$

**Molecular Weight**: 152.15

**Structural Formula:**



**Functional Category:**

Antimicrobial preservative.

**Typical Properties:**

- Density (true) :1.352 g/cm$^3$
- Dissociation constant pKa : 8.4 at 22$^o$ C
- Melting point :125–128$^o$ C

**Antimicrobial activity**:

Methylparaben exhibits antimicrobial activity of pH 4 – 8. Preservative efficacy decreases with increasing pH owing to the formation of the phenolate anion. Parabens are more active against yeasts and molds than against bacteria. They are also more active against gram positive bacteria than against gram negative bacteria. Methylparaben is the least active of the parabens; antimicrobial activity increases with increasing chain length of the alkyl moiety. Activity may be improved by using combinations of parabens as synergistic effects occur. Therefore, combinations of methyl-, ethyl-, propyl-, and butylparaben are often used together.

**Solubility:**

| Solvent | Solubility at 20°C |
|---|---|
| **Ethanol** | 1 in 2 |
| **Ethanol (95%)** | 1 in 3 |
| **Ethanol (50%)** | 1 in 6 |
| **Ether** | 1 in 10 |
| **Glycerine** | 1 in 60 |
| **Mineral oil** | Practically insoluble |
| **Water** | 1 in 400 |

**Table 7: solubility of Methylparaben**

**Stability and Storage Conditions:**

Aqueous solutions of Methylparaben at pH 3 – 6 may be sterilized by autoclaving at 120$^o$ C for 20 minutes, without decomposition. Aqueous solutions at pH 3 – 6 are stable (less than 10%

decomposition) for up to about 4 years at room temperature, while aqueous solutions at pH 8 or above are subject to rapid hydrolysis

**Incompatibilities:**

The antimicrobial activity of methylparaben and other parabens is considerably reduced in the presence of non-ionic surfactants, such as polysorbate 80, as a result of micellization. However, propylene glycol (10%) has been shown to potentiate the antimicrobial activity of the parabens in the presence of non-ionic surfactants and prevents the interaction between methylparaben and polysorbate 80. Incompatibilities with other substances, such as bentonite, magnesium trisilicate, talc, tragacanth, sodium alginate, essential oils, sorbitol, and atropine, have been reported. It also reacts with various sugars and related sugar alcohols. Absorption of methylparaben by plastics has also been reported; the amount absorbed is dependent upon the type of plastic and the vehicle. It has been claimed that low-density and high-density polyethylene bottles do not absorb methylparaben. Methylparaben is discoloured in the presence of iron and is subject to hydrolysis by weak alkalis and strong acids.

**Safety**:

Methylparaben and other parabens are widely used as antimicrobial preservatives in cosmetics and oral and topical pharmaceutical formulations. Although parabens have also been used as preservatives in injections and ophthalmic preparations, they are now generally regarded as being unsuitable for these types of formulations owing to the irritant potential of the parabens. These experiences may depend on immune responses to enzymatically formed metabolites of the parabens in the skin. Parabens are nonmutagenic, nonteratogenic, and noncarcinogenic. Sensitization to the parabens is rare, and these compounds do not exhibit significant levels of photo contact sensitization or phototoxicity.

**Applications in Pharmaceutical Formulation or Technology:**

Methylparaben is widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations. It may be used either alone or in combination with other M Methylparaben 441 parabens or with other antimicrobial agents. In cosmetics, methylparaben is the most frequently used antimicrobial preservative. The parabens are effective over a wide pH range and have a broad spectrum of antimicrobial activity, although they are most effective against yeasts and molds. Antimicrobial activity increases as the chain length of the alkyl moiety is increased, but aqueous solubility decreases; therefore, a mixture of parabens is frequently used

to provide effective preservation. Preservative efficacy is also improved by the addition of propylene glycol (2–5%), or by using parabens in combination with other antimicrobial agents such as imidurea; see Section 10. Owing to the poor solubility of the parabens, paraben salts (particularly the sodium salt) are more frequently used in formulations. However, this raises the pH of poorly buffered formulations.

### 6.2 Propylparaben:[60]

**Non-proprietary Names**

- BP - Propyl Hydroxybenzoate
- JP - Propyl Parahydroxybenzoate
- PhEur - Propyl Parahydroxybenzoate
- USP-NF - Propylparaben

**Synonyms**:

Aseptoform P; CoSept P; E216; 4-hydroxybenzoic acid propyl ester; Nipagin P; Nipasol M; propagin; Propyl Aseptoform; propyl butex; Propyl Chemosept; propylis parahydroxybenzoas; propyl phydroxybenzoate; Propyl Parasept; Solbrol P; Tegosept P; Uniphen P-23.

**Description:**

**Appearance** **:** colorless crystals or a white crystalline powder.

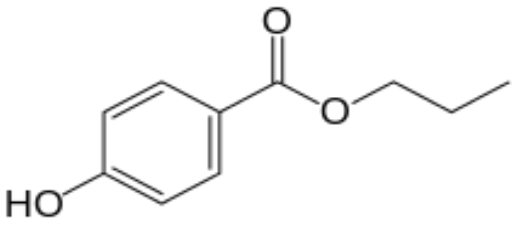
**Odour** **:** odorless

**Chemical Name and CAS Registry Number:** Propyl 4-hydroxybenzoate [94-13-3]

**Empirical Formula:** $C_{10}H_{12}O_3$

**Molecular Weight**: 180.20

**Structural Formula:**



**Functional Category:**

Antimicrobial preservative.

**Typical Properties:**

- Boiling point : 295° C
- Density (bulk) : 0.426 g/cm3
- Density (tapped) : 0.706 g/cm3
- Density(true) : 1.288 g/cm3
- Dissociation constant pKa: 8.4 at 228° C
- Flash point : 140° C

**Antimicrobial activity**:

Propylparaben exhibits antimicrobial activity between pH 4 – 8. Preservative efficacy decreases with increasing pH owing to the formation of the phenolate anion. Parabens are more active against yeasts and molds than against bacteria. They are also more active against Gram-positive than against Gram-negative bacteria. The activity of the parabens increases with increasing chain length of the alkyl moiety; however, solubility decreases. Activity may be improved by using combinations of parabens, as additive effects occur. Propylparaben has been used with methylparaben in parenteral preparations, and is used in combination with other parabens in topical and oral formulations. Activity has also been reported to be improved by the addition of other excipients with Methylparaben.

**Solubility:**

| Solvent | Solubility at 20°C |
|---|---|
| **Acetone** | Freely soluble |
| **Ethanol (95%)** | 1 in 1.1 |
| **Ethanol (50%)** | 1 in 5.6 |
| **Propylene glycol** | 1 in 3.9 |
| **Ether** | Freely soluble |
| **Glycerin** | 1 in 250 |
| **Water** | 1 in 4350 |

**Table 8: solubility of propyl paraben**

**Stability and Storage Conditions:**

Aqueous propylparaben solutions at pH 3 – 6 can be sterilized by autoclaving, without decomposition. At pH 3 – 6, aqueous solutions are stable (less than 10 % decomposition) for up to about 4 years at room temperature, while solutions at pH 8 or above are subject to rapid hydrolysis

**Incompatibilities:**

The antimicrobial activity of propylparaben is reduced considerably in the presence of nonionic surfactants as a result of micellization. Absorption of propylparaben by plastics has been reported, with the amount absorbed dependent upon the type of plastic and the vehicle. Magnesium aluminium silicate, magnesium trisilicate, yellow iron oxide, and ultramarine blue have also been reported to absorb propylparaben, thereby reducing preservative efficacy. Propylparaben is discoloured in the presence of iron and is subject to hydrolysis by weak alkalis and strong acids.

**Safety**:

Propylparaben and other parabens are widely used as antimicrobial preservatives in cosmetics, food products, and oral and topical pharmaceutical formulations. Propylparaben and methylparaben have been used as preservatives in injections and ophthalmic preparations; however, they are now generally regarded as being unsuitable for these types of formulations owing to the irritant potential of the parabens

**Applications in Pharmaceutical Formulation or Technology:**

Propylparaben is widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations. It may be used alone, in combination with other paraben esters, or with other antimicrobial agents. It is one of the most frequently used preservatives in cosmetics. The parabens are effective over a wide pH range and have a broad spectrum of antimicrobial activity, although they are most effective against yeasts and molds; Owing to the poor solubility of the parabens, the paraben salts, particularly the sodium salt, are frequently used in formulations. This may cause the pH of poorly buffered formulations to become more alkaline. Propylparaben (0.02% w/v) together with methylparaben (0.18% w/v) has been used for the preservation of various parenteral pharmaceutical formulations

### 6.3 D-glucose:[61]

**Non-proprietary Names**:

- BP: Glucose
- JP: Glucose
- PhEur: Glucose Monohydrate
- USP: Dextrose

**Synonyms**:

Blood sugar; Caridex; corn sugar; C*PharmDex; Dextrofin; D-(þ)- glucopyranose monohydrate; glucosum monohydricum; grape sugar; Lycadex PF; Roferose; starch sugar; Tabfine D-100.

**Description:**

**Appearance** : colorless crystals or as a white crystalline or granular powder.

**Odour** : odorless

**Taste** : sweet taste

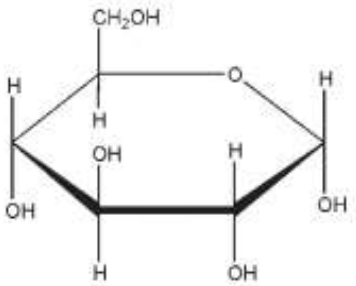
**Chemical Name and CAS Registry Number:**

D-(þ)-Glucose monohydrate [5996-10-1]

**Empirical Formula:** $C_6H_{12}O_6H_2O$

**Molecular Weight**: 198.17

**Structural Formula:**



**Functional Category:**

Diluent; therapeutic agent; tonicity agent; sweetening agent.

**Typical Properties:**

- Acidity/alkalinity: pH = 3.5–5.5 (20% w/v aqueous solution)
- Density (bulk): 0.826 g/cm3
- Density (tapped): 1.020 g/cm3
- Density (true): 1.54 g/cm3
- Heat of solution: 105.4 J/g (25.2 cal/g)
- Melting point: 83º C
- Moisture content: Dextrose anhydrous absorbs significant amounts of moisture at 25º C and a relative humidity of about 85% to form the monohydrate. The monohydrate similarly only absorbs moisture at around 85% relative humidity and 25º C
- Osmolarity A: 5.51% w/v aqueous solution is isosmotic with serum. However, it is not isotonic since dextrose can pass through the membrane of red cells and cause haemolysis.

**Solubility:**

| Solvent | Solubility at 20°C |
|---|---|
| **Chloroform** | Practically insoluble |
| **Ethanol (95%)** | 1 in 60 |
| **Ether** | 1 in 10 |
| **Glycerine** | 1 in 60 |
| **Water** | 1 in 1 |

**Table 9: solubility of Dextrose**

**Stability and Storage Conditions:**

Dextrose has good stability under dry storage conditions. Aqueous solutions may be sterilized by autoclaving. However, excessive heating can cause a reduction in pH and caramelization of solutions. The bulk material should be stored in a well-closed container in a cool, dry place.

**Incompatibilities:**

Dextrose solutions are incompatible with a number of drugs such as cyanocobalamin, kanamycin sulfate, novobiocin sodium, and warfarin sodium. Erythromycin gluceptate is unstable in dextrose solutions at a pH less than 5.05. Decomposition of B-complex vitamins may occur if they are warmed with dextrose. In the aldehyde form, dextrose can react with amines, amides, amino acids, peptides, and proteins. Brown coloration and decomposition occur with strong alkalis. Dextrose may cause browning of tablets containing amines

**Safety**:

Dextrose is rapidly absorbed from the gastrointestinal tract. It is metabolized to carbon dioxide and water with the release of energy. Concentrated dextrose solutions given by mouth may cause nausea and vomiting. Dextrose solutions of concentration greater than 5% w/v are hyperosmotic and are liable to cause local vein irritation following intravenous administration. Thrombophlebitis has been observed following the intravenous infusion of isosmotic dextrose solution with low pH, probably owing to the presence of degradation products formed by overheating during sterilization. The incidence of phlebitis may be reduced by adding sufficient sodium bicarbonate to raise the pH of the infusion above pH 7.

**Applications in Pharmaceutical Formulation or Technology:**

Dextrose is widely used in solutions to adjust tonicity and as a sweetening agent. Dextrose is also used as a wet granulation diluent and binder, and as a direct-compression tablet diluent and binder, primarily in chewable tablets. Although dextrose is comparable as a tablet diluent to lactose, tablets produced with dextrose monohydrate require more lubrication, are less friable, and have a tendency to harden. The mildly reducing properties of dextrose may be used when tableting to improve the stability of active materials that are sensitive to oxidation. Dextrose is also used therapeutically and is the preferred source of carbohydrate in parenteral nutrition regimens.

## 7. Materials and Instruments

### *7.1 Drugs, excipients and materials used in the experiment are listed in Table*

| S.No | Name of Material | Manufacture |
|---|---|---|
| 1 | **Labetalol Hydrochloride** | Procos S.P.A, Italy |
| 2 | **Dextrose Anhydrous** | Merck Ltd, Germany |
| 3 | **Edetate disodium** | Merck Ltd, Germany |
| 4 | **Methylparaben** | Merck Ltd, Germany |
| 5 | **Propylparaben** | Merck Ltd, Germany |
| 6 | **Citric acid anhydrous** | Merck Ltd, Germany |
| 7 | **Sodium hydroxide** | Merck Ltd, Germany |
| 8 | **Water for injection** | In House |
| 9 | **20mL/20 mm clear moulded glass vial [USP type-I]** | Schott-Kaisha private limited, Germany |
| 10 | **20 mm bromobutyl stoppers** | West pharmaceutical service, Pennsylvania |
| 11 | **20 mm Aluminium flip off seals** | Adit pharma, Maharashtra |
| 12 | **0.2 µ Filters (PES)** | mdi Membrane Technologies, Germany |

***Table 10: List of material used in formulation process***

*7.2 Instruments used in the experiment for the formulation are listed in the table*

| S.No | Instruments | Company Name |
|---|---|---|
| 1 | **Electronic balance** | LCGC Radwag, Hyderabad |
| 2 | **pH Meter** | Mettler Toledo, United states |
| 3 | **Magnetic stirrer** | Sartorius, Germany |
| 4 | **Osmometer (Model 3250)** | Advanced Instruments, Inc. united states |
| 5 | **Autoclave** | Systec, Germany |
| 6 | **Hot Air Oven** | Labman scientific, Chennai |
| 7 | **Seal crimper** | Kebby power crimper, Indiana |
| 8 | **Stability Chambers** | Ai si li test equipment co ltd, china |
| 9 | **Laminar Air Flow Chamber** | Fabtech technologies, Mumbai |
| 10 | **HPLC** | Agilent, California |
| 11 | **Headspace oxygen analyser** | Lighthouse Instruments, Virginia |
| 12 | **Pressure vessel** | Millipore, Germeny |
| 13 | **Lux meter** | PCE instruments, USA |
| 14 | **DO meter** | Mettler Toledo, United states |

***Table 11: Instruments used in formulation process***

## 8. Methods

### 8.1 Raw Material Analysis of Labetalol hydrochloride:[62]

#### *8.1.1 Description:*

White or almost white powder

#### *8.1.2 Melting range:*

The melting point of labetalol hydrochloride was determined by capillary method.

### 8.2 Identification test for labetalol hydrochloride:

#### *8.2.1 Loss on drying:*

About 1g of the drug was accurately weighed and dried at 105° for 4 hours.

#### *8.2.2 Residue on ignition:*

Not more than 0.1%

#### *8.2.3 Test for chloride*:

To 10 mL of solution, 1 mL of 2 N nitric acid and 1 mL of silver nitrate was added.

#### *8.2.4 Percentage purity:*

#### *8.2.4.1 Assay:*

About 0.2 g of labetalol hydrochloride was weighed and dissolved in 10 mL of anhydrous formic acid and 40 mL of acetic anhydride, and titrated with 0.1 M Perchloric acid, and the end point was determined potentiometrically. Blank titration was performed. Each mL of 0.1M Perchloric acid is equivalent to 0.0369 g of $C_{19}H_{24}N_2O_3$.HCl.

### 8.3 Preformulation study:

#### *8.3.1 Evaluation study for Containers and closure:*

#### *8.3.1.1 Evaluation of container*

- ***Surface glass test* (*hydrolytic resistance of the inner surfaces of glass containers):***

  6 containers at random from the sample lot were taken, and the containers were cleaned, any debris or dust is removed. Before the test the containers were carefully rinsed for three times with Purified Water and allowed to drain. The containers were filled with

Purified Water up to the filling volume. Each container was loosely caped with an inert material, sufficient number of containers were selected to completely fill the tray within the autoclave chamber. The end of the calibrated resistance thermometer or calibrated thermocouple was inserted into a filled container through a hole in the closure having approximately the same diameter as the probe and it was connected to the external measuring device. Using the calibrated thermocouple measuring device, the deviations from the holding temperature of 121 ± 1° were ensured within the tolerance limit. At the end of the cycle, the hot samples were removed from the autoclave and cooled to room temperature within 30 min. within 1 hr of the removal of the containers from the autoclave, the titrations were carried out. The liquids obtained from the containers were combined, and mixed. 25 mL volume of samples was introduced into a conical flask; same volume of purified water (30 mL) was added, and used as a blank, into a second similar flask. 0.05 mL of Methyl red solution was added for each 25 mL of liquid. The blank was titrated with 0.01 M hydrochloric acid. The test solution was titrated with the same acid until the colour of the resulting solution is the same as that obtained for the blank. The value founded for the blank titration was subtracted from that founded for the test solution and the results were expressed in millilitres of 0.01 M hydrochloric acid per 100 mL of test solution. Alternatively, an auto titrator was used. Titration values of less than 1.0 mL were expressed to two decimal places; titration values of greater than or equal to 1.0 mL were expressed to one decimal place.

- ***Glass grains test (hydrolytic resistance of glass grains):***

  The containers to be tested were rinsed with Purified Water and dried in the oven. Three of the glass articles were wrapped in clean paper, and crushed to produce two samples of about 100 g each in pieces NMT 30 mm across. 30 g of the pieces were placed in the motor between 10 and 30 mm across taken from one of the samples, insert the pestle, and struck heavily. Alternatively, samples were transferred into a ball mill-breaker, the balls were added, and the glasses were crushed. The contents of the mortar or ball mill were transferred to the coarsest sieve (No. 25) of the set. The set of sieves were shaken for a short time, and the glass that remains on sieves was removed. These portions were submitted to further fracture, repeating the operation until about 10 g of glass remains on sieve No. 25. This portion and the portion that passes through sieve No. 50 were rejected. The set of sieves were Reassembled, and shaken for 5 min. the glass grains that passed through sieve No. 40 and are retained on sieve No. 50 was transferred to a

weighing bottle. the crushing and sieving procedure were Repeated with the second glass sample until two samples of grains are obtained, each of which weighs more than 10 g. the grains were dried, first by putting the beaker on a warm plate, then by heating at 140° for 20 min in a drying oven. The dried grains were transferred from each beaker into separate weighing bottles; the stoppers were inserted, and cooled in a desiccator.

10.00 g of the cleaned and dried grains Weighed and added into two separate conical flasks. 50 mL of Purified Water were pipetted into each of the conical flasks (test solutions). 50 mL of Purified Water were pipetted into a third conical flask that served as a blank. The grains were distributed evenly over the flat bases of the flasks by shaking gently. All three flasks were placed in the autoclave containing the water at ambient temperature, and ensured that they are held above the level of the water in the vessel.

To each of the three flasks 0.05 mL of Methyl red solution was added. The blank solution was titrated immediately with 0.02 M hydrochloric acid, and then the test solutions were titrated until the colour matches that obtained with the blank solution. The titration volume for the blank solution was subtracted from that for the test solutions. The mean value of the results in mL of 0.02 M hydrochloric acid per gram of the sample was calculated.

- ***Surface etching test***:

The containers were rinsed twice with Purified Water, mixture of 1 volume of hydrofluoric acid and 9 volumes of hydrochloric acid was filled to the brim-full point, and allowed to stand for 10 min. the containers were Emptied, and rinsed carefully five times with Purified Water. Before the test, once again the container was rinsed with Purified Water. These containers were submitted to the same autoclaving and determination procedure as described in the Surface Glass Test. Test results were shown in **table 15.** [63]

### *8.3.1.2 Evaluation test for closure*

- ***Penetrability test:***

10 suitable vials were filled to the nominal volume with water, the closures to be examined was fitted, and secured with a cap. Using a new hypodermic needle, pierce the closure with the needle perpendicular to the surface.

- ***Fragmentation test:***

  12 clean vials were filled with water to 4 mL less than the nominal capacity. The closures to be examined were fitted and secured with a cap, and allowed to stand for 16 hours. Using a hypodermic needle as described above fitted to a clean syringe, into each vial 1 mL of water were injected while removing 1 mL of air. This procedure was repeated four times for closure, pierced each time at a different site. A new needle was used for each closure, checked that it was not blunted during the test. The total volume of liquid in all the vials was filtered through a single filter with a nominal pore size no greater than 0.5 mm. The rubber fragments on the surface of the filter visible to the naked eye were counted.

- ***Self-Sealing Capacity test***:

  10 suitable vials were filled with water to the nominal volume. The closures that are to be examined were fitted, and caped. With a new hypodermic needle pierced each closure 10 times, pierced each time at a different site. The 10 vials were immersed in a solution of 0.1% (1 g per L) methylene blue, and reduced the external pressure by 27 kPa for 10 minutes. Restored to atmospheric pressure and left the vials immersed for 30 minutes. The outside of the vials was rinsed. results were shown in **table 16.** [64]

### *8.3.2 Drug Excipients compatibility study by FT-IR:*

Fourier Transform Infra-Red Spectroscopy (FTIR) is a reliable method of infrared spectroscopy. FTIR can provide significant amounts of information, and is used to identify an unknown material, the quality or consistency of a sample, the number of components in a mixture. The normal instrumental components of a Fourier Transform Infrared Spectrometer consist of a source, an interferometer, a sample compartment, a detector and a computer.

The physiochemical compatibility between the Labetalol hydrochloride and the excipients used in the formulation was tested by FT-IR spectroscopic method. One mg of the drug (Labetalol hydrochloride) was mixed with 100 mg of potassium bromide and compressed to form a KBr disc. The sample was scanned at 4000– 400 $cm^{-1}$. The compatibility of the drug substance with that of excipients was studied. The results were shown in the **fig 6 - 10.** [65]

### *8.4* Formulation development of labetalol hydrochloride injection:

#### *8.4.1 Determination of drug solubility:*

Proposed Labetalol hydrochloride injection is aqueous parenteral product. Hence water for injection is selected as a vehicle. Solubility study of the API was checked in water (vehicle). This study was completed by differing time for solubilization, different volume of WFI at room temperature.

The label claim of drug product is 5mg/mL, the study was planned to determine the qualitative solubility of API differing the volume of WFI. Dissolution of 0.5 g of Active substance (Labetalol hydrochloride) in 100ml will provide the target concentration.

Accurately weighed quantity of 0.1gm of Labetalol hydrochloride was taken in a glass beaker and stirred at different speed on a magnetic stirrer and also the quantity of water varied. Time taken for the solubilisation of drug was noted and the results were shown in the **table 17.**

#### *8.4.2 Order of mixing of the ingredients in labetalol hydrochloride injection formulation:*

Labetalol hydrochloride injection formulation contains Labetalol hydrochloride as the active pharmaceutical ingredient (API), and Ethylene diamine tetra acidic acid (EDTA) (Chelating agent), Propylparaben (Preservative), Methylparaben (Preservative), anhydrous dextrose (Tonicity agent) as the excipients. Four trials were taken for the optimization process. The order of addition for different trials gives the time taken for solubility of the drug along with the excipients was noted. The order of mixing in which the solubility of the drug and other excipients was at minimal time was taken as a trial batch for product formulation, and that trial batch was analysed for physical parameters, pH, Assay of drug content, excipient content and related substances. The results were shown in the **table 18.**

**Order of addition: (API – Labetalol hydrochloride)**

**Trail 1: Methylparaben + Propylparaben »» Dextrose »» Edetate disodium »» API**

**Trail 2: Methylparaben + Propylparaben »» API »» Dextrose »» Edetate disodium**

**Trail 3: Methylparaben + Propylparaben »» Edetate disodium »» Dextrose »» API**

**Trail 4: Edetate disodium »» Methylparaben + Propylparaben »» API »» Dextrose**

- In the first trial, the methylparaben and propylparaben was added to the water for injection and dextrose was added followed by edetate disodium and API (Labetalol hydrochloride) was added.
- In second trial, the methylparaben and propylparaben was added to the water for injection and API was added followed by the addition of dextrose and edetate disodium.
- In the third trial, the methylparaben and propylparaben was added to the water for injection followed by edetate disodium then dextrose and API was added.
- In the final trial, edetate disodium was added to the water for injection followed by the addition of methylparaben and propylparaben and then API and dextrose was added.

### *8.4.3 Fill volume Determination:*

Each container of an Injection is filled with a volume in slight excess of the labelled ‘‘size’’ or that volume that is to be withdrawn. The excess volumes recommended in the accompanying table are usually sufficient to permit withdrawal and administration of the labelled volumes.

It was determined for innovator’s labetalol hydrochloride Injection. The volume in the innovator’s labetalol hydrochloride Injection, Single Use Vial Packs was determined by means of opening them and emptying the contents directly into the graduated cylinder.

| Labelled size | For mobile liquids | For viscous liquids |
|---|---|---|
| **0.5 mL** | 0.10 mL | 0.12 mL |
| **1.0 mL** | 0.10 mL | 0.15 mL |
| **2.0 mL** | 0.15 mL | 0.25 mL |
| **5.0 mL** | 0.30 mL | 0.50 mL |
| **10.0 mL** | 0.50 mL | 0.70 mL |
| **20.0 mL** | 0.60 mL | 0.90 mL |
| **30.0 mL** | 0.80 mL | 1.20 mL |
| **50.0 mL or more** | 2 % | 3 % |

**Table 13: Recommended excess volume**

## 8.5 Formulation process of labetalol hydrochloride injection:

### *8.5.1 Preparation of 1 % citric acid solution:*

1.0 g of citric acid was added in approximately 80 ml of water for injection and mixed till a clear solution is observed. Then the volume was made up to 100 ml with water for injection and mixed well.

### *8.5.2 Preparation of 1 N Sodium hydroxide solutions:*

4.0 g of sodium hydroxide was added in approximately 80 ml of water for injection and stirred till a clear colorless solution was observed. Then the volume was made up to 100 ml with water for injection and mixed well.

### *8.5.3 Preparation of labetalol hydrochloride injection:*

i. 110 % batch size of water for injection was collected in glass duran bottle and nitrogen was purged for 1 hour.
ii. Approximately 70% batch size of nitrogen purged water for injection (WFI) was taken from the duran bottle into clean and dried glass beaker (pH 5.92)
iii. Collected WFI was heated up to $80^{o}$ C and dispensed quantity of methylparaben and propylparaben were added, stirred for 10 minutes at 600 rpm. Clear colorless solution was observed. Solution cooled to $25^{o}$ C. pH was checked (pH 5.12) and continued the nitrogen purging.
iv. Dextrose anhydrous was added to the beaker, stirred for 5 minutes at 600 rpm, clear colorless solution was observed, pH was checked (pH 5.10)
v. Dispensed quantity of edetate disodium was added to the beaker, stirred for 5 minutes at 600 rpm, clear colorless solution was observed, pH was checked (pH 4.95)
vi. The calculated quantity of labetalol hydrochloride was added to the beaker, stirred for 15 minutes at 600 rpm, clear colorless solution was observed, pH was checked (pH 4.54). pH was adjusted to 3.83 with approx. 4 mL of 1 % citric acid solution under stirring.
vii. Volume was made up to 100 % (1500 mL) with nitrogen purged water for injection, stirred at 600 rpm for 5 minutes, clear colorless solution was observed. Find pH was checked as 3.89 (pH limit: 3.0 to 4.5)

viii. 1500 mL of bulk solution was filtered through 0.22 µm PES. filtered solution was filled into 20 mL/ 20 mm clear moulded glass vials with 20 mL fill volume and stopped with 20 mm bromobutyl stopper. Sealed with vials.

ix. 75 vials (1500 mL) were autoclaved at 121$^{o}$ C for 20 minutes, then loaded in stability chamber.

Above procedure was followed for all the batches.

| Ingredients USP grade | Quantity mg/mL | Quantity per 1500 mL |
|---|---|---|
| **Labetalol Hydrochloride** | 5.0 | 7.569 g |
| **Dextrose Anhydrous** | 45.0 | 67.500 g |
| **Edetate disodium** | 0.1 | 150.00 mg |
| **Methylparaben** | 0.8 | 1.200 g |
| **Propylparaben** | 0.1 | 150.00 mg |
| **Citric acid anhydrous** | q.s to adjust pH | q.s to adjust pH |
| **Sodium hydroxide** | q.s to adjust pH | q.s to adjust pH |
| **Water for injection** | q.s to 1 ml | q.s to 1.5 Litres |

**Table 12**: **Drug product formula for labetalol hydrochloride injection**

### 8.5.3 Process Development

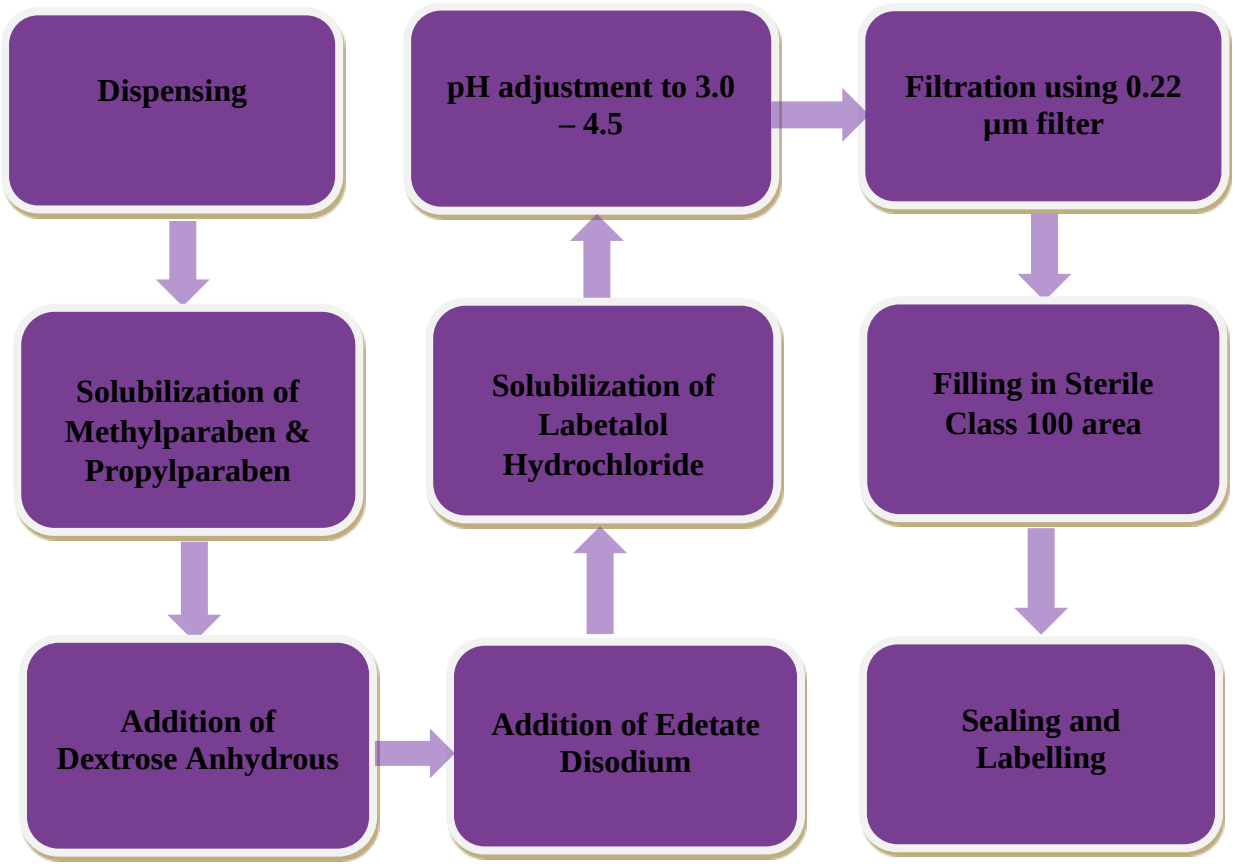



**Fig 5: manufacturing schematic representation of labetalol hydrochloride**

***8.6 Selection of sterilization method:***

Labetalol hydrochloride injection was prepared by the standard manufacturing process and was evaluated for feasibility of terminal sterilization by autoclaving in the proposed container-closure system. The samples were subjected to terminal sterilization by autoclaving at 121°C for 20 and 30 minutes time intervals to study the sensitivity of the product. The results are tabulated in the **table 19 and fig 13**.

**8.7 Hold time compatibility study for labetalol hydrochloride injection:**

***8.7.1 compatibility with stainless steel:***

Stainless steel is an integral component of the drug product compounding and filling equipment. The bulk solution of labetalol hydrochloride injection was prepared by

manufacturing procedure and solution was held in a closed stainless-steel container; the bulk solution was maintained at 20-30º C throughout the study. The hold time study with stainless steel vessel was performed with before filtration and after filtration of product bulk solution. Samples were collected at predetermined intervals i.e. initial, after 48 hours and 72 hours and analysed. The results are summarized in **table 20-21 and fig 14-15**.

### *8.7.2 Tubing compatibility study:*

Transfer tubing is a processing aid required for fluid transfer during filtration and filling. Therefore, the study was undertaken to select suitable tubing for labetalol hydrochloride injection. The bulk solution was prepared by standard manufacturing procedure and solutions were filled in the tubing's like tube A (sanitech) and Tube B (pharmapure) tubing's; maintained at 20-30º C. The solutions were sampled at predetermined time intervals i.e. initial, after 12 hours, 24 hours, 48 hours and tested. Then the solution is assayed for drug content, excipients content, and impurities at the end of the study. Results were presented in **table 22-23 and fig 16-17.**

### *8.7.3 Compatibility with filters:*

Aseptic filtration is an integral part of processing of parenteral formulations. Compatibility of labetalol hydrochloride injection was studied with 47mm, 0.2-micron filters-PVDF (polyvinylidene difluoride) and PES (Polyethersulfone) filters. The bulk solution of labetalol hydrochloride injection was prepared by standard manufacturing procedure. Filters were soaked in labetalol hydrochloride injection. The samples were collected at different predetermined time and points i.e. initial, after 48 hours and 72 hours and analysed. The solution was assayed for drug content, excipients content, and impurities at the end of the study. The results are summarized in **table 24-25 and fig 18-19.**

## 8.8 Stress studies:

### *8.8.1 Oxygen sensitivity study:*

The study was performed to evaluate the effect of oxygen in the formulation. Labetalol hydrochloride injection, USP 5.0mg/mL was manufactured with and without nitrogen as per standard manufacturing process. The samples were autoclaved at 121º C for 20 minutes. The product vials were loaded to stability chamber for stability studies. The labetalol hydrochloride

injection was filled in 10 mL clear tubular vials with nitrogen and without nitrogen and the drug samples was loaded for both accelerated stability study (40ºC±2°C/75%±5% RH for 3 months) and long term stability study (25ºC±2°C /60%±5% RH for 6 months) in an inverted and upright conditions and the solution was assayed for drug content, excipients content, and impurities at the end of the study. Analytical results were presented in **table 26- 27 and fig 20-21.**

***8.8.2 pH extreme study:***

Labetalol hydrochloride injection pH extreme studies were carried based on USP product monograph range i.e. in the range of 3.0-4.5. The standard manufacturing process was used to prepare the drug product solution, and then the pH was adjusted as per the study requirement i.e. around pH 3.0 and around 4.5 by using Citric acid anhydrous or sodium hydroxide solution. The samples were filled in the 10ml clear tubular vial with the fill volume of 10 ml and autoclaved at 121° C for 20 minutes. The product vials were loaded for both stability studies for accelerated stability study (40ºC±2°C /75%±5% RH for 3 months) and long term stability study (25ºC±2°C /60%±5% RH for 6 months) in an inverted and upright conditions and the solution was assayed for drug content, excipients content, and impurities at the end of the study and analytical results are presented in **table 28-29 and fig 22-23.**

***8.8.3 Freeze thaw study:***

The freeze thaw studies were undertaken to understand the stability characteristics of the product when subjected to extreme temperature conditions that may be encountered during the drug product distribution process. The product in its final container was subjected to a temperature cycle of 20º C ±5ºC for 2 days followed by 40ºC±2ºC/ 75%±5% RH for 2 days, the study constituted three such cycles. A set of product samples were analysed at the end of third cycle, and the solution was assayed for drug content, excipients content, and impurities at the end of the study. The results are presented in **table 30 and fig 24**

**8.8.4** ***Photo stability study:***

Labetalol hydrochloride injection USP product solution filled into glass vials were subjected to a photo-stability study. Samples wrapped in aluminium foil placed alongside the test sample served as controls. Vials packed in cartons were also studied to simulate the actual market pack of the product and the light protection that the secondary pack would offer to the product. The total light exposure would provide an overall illumination of 1.2 million lux hours and an

integrated near ultraviolet energy of not less than 200-watt hours/ square meter. The samples were analysed at the end of the light exposure. Results of the testing at the end of the light exposure are shown in **table 31 and fig 25.**

### 8.9 Quality control study for labetalol hydrochloride injection

#### 8.9.1 Description:

The prepared labetalol hydrochloride injection was analysed for the physical appearance of the drug.

#### 8.9.2 pH:

pH of the labetalol hydrochloride injection was observed for the prepared injection solution with the pH meter.

#### *8.9.3 Clarity test:*

Clarity is tested by conducting a visual inspection of containers under light and viewed against a black and white background. The instrumental method of evaluation is based on the light scattering principle, electrical resistance and light absorption which are used to count particle and particle size distribution. The visual inspection of a product container is usually done by individual human inspection of each externally clean container under a good light, baffled reflection into the eyes and viewed against a black and white background, with the contents set in motion with a swirling action. For monitoring particulate matter, Light obscuration particle count test was performed, and the suspended particles (black or white particles) were counted.

#### *8.9.4 Leaker test:*

Leaker test was performed to determine whether any capillary pores or tiny cracks are present on the vials which may lead to microbes or other dangerous contaminants to enter the formulation or may lead to leakage. This may lead to contamination of the content or spoilage of the package. This test was used to detect incompletely sealed vials so that they can be discarded in order to maintain the sterile conditions of the preparation. The test was conducted by placing the vials filled with prepared labetalol hydrochloride injection in a vacuum chamber and completely submerged in deeply colored dye solution of about 0.5 to 1% methylene blue. A negative pressure is applied within the sample making the dye to penetrate through any opening or pores if present on the vials which will be visible after the washing of the vials.

### *8.9.5 Bacterial endotoxin test or LAL test*:

The LAL (limulus amoebocyte lysate) testing, also known as bacterial endotoxin testing, is an *in vitro* assay used to detect the presence and concentration of bacterial endotoxin in drugs and biological products, and is an important part of pharmaceutical microbiology. Endotoxins, which are a type of pyrogen, are lipopolysaccharides present in the cell walls of gram-negative bacteria. Pyrogens as a class are fever-inducing substances that can be harmful or even fatal if administered to humans above certain concentrations.

EL= K/M ---- equation 3

### 8.9.6 Sterility test:

**Membrane Filtration method:**

The injection sample was filtered through membrane filters of porosity 0.22 micron and Diameter 47mm with hydrophobic characteristics. The filtration is assisted under Vacuum, after filtration completion the membrane was made into 2 halves and one halve was placed in two test tubes containing FTM, SCDM medium and incubated for 14 days. During the incubation period the media was viewed for microbial growth.

### 8.9.7 Assay of labetalol hydrochloride injection

About 50 mg of labetalol hydrochloride is mixed with 100 mL of water. The 10 mL of solution is added to 10 mL of 0.05 M sulphuric acid and dilute to 100 mL of water. The absorbance was measured of the resulting solution at the maximum at 302 nm. The content of $C_{19}H_{24}N_2O_3$.HCl is calculated is taking 86 as the specific absorbance at the maximum at 302 nm. [66]

## 8.10 Stability study for final development batch of labetalol hydrochloride injection:

Final development batch of labetalol hydrochloride injection, USP manufactured using standard manufacturing process with pH approximately 3.8 in the selected packaging components and autoclaved at 121º C for 20 minutes. Then the Samples were loaded in to the stability chamber. The temperature is maintained at 40ºC±2°C and 75%±5% RH for 3 months for accelerated stability study and 25ºC±2°C and 60%±5% RH for 3 months for long term stability study. The stability data are presented in **table 33 and fig 26-27.**

# 9. Results

## 9.1 Raw material analysis of labetalol hydrochloride:

Labetalol hydrochloride drug sample was analysed for various physical and analytical characterizations and was found to comply with USP.

### *9.1.1 Physiochemical characters of Labetalol hydrochloride:*

| S.No | Test | Results |
|---|---|---|
| 1 | **Appearance** | White or almost white powder |
| 2 | **Melting range** | About 180$^{o}$ C |
| 3 | **Solubility**<br>**Sparingly soluble**<br>**Insoluble** | in water and in ethanol (96%)<br>in ether and in methylene chloride |

**Table 14: physiochemical characteristics of Labetalol hydrochloride**

## 9.2 Identification test:

***9.2.1 Loss on drying*** **:** Not more than 0.13

***9.2.2 Residue on ignition*** **:** Not more than 0.04%

***9.2.3 Test for chloride*** **:** No opalescence produced immediately

***9.2.4 Optical Rotation*** **:** 0.00

***9.2.5 Assay*** **:** 99.2 %

## 9.3 Preformulation study

### *9.3.1 Drug Excipients compatibility study by FT-IR:*

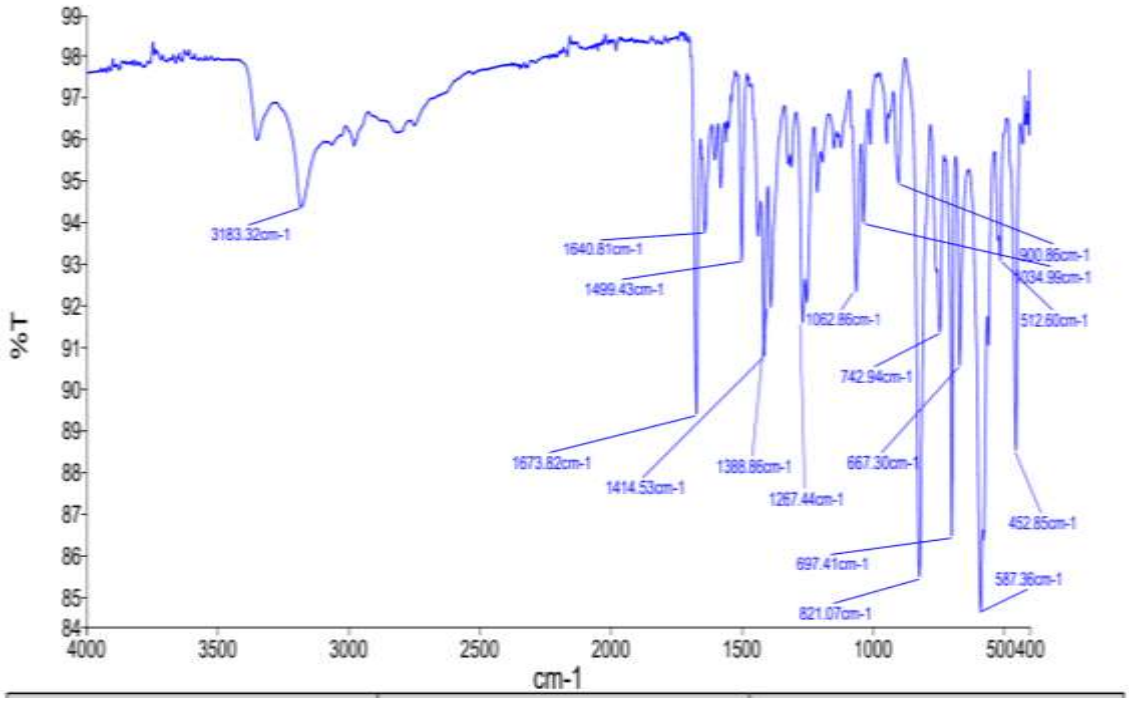



**Fig 6: IR *spectrum of pure Labetalol hydrochloride drug sample***

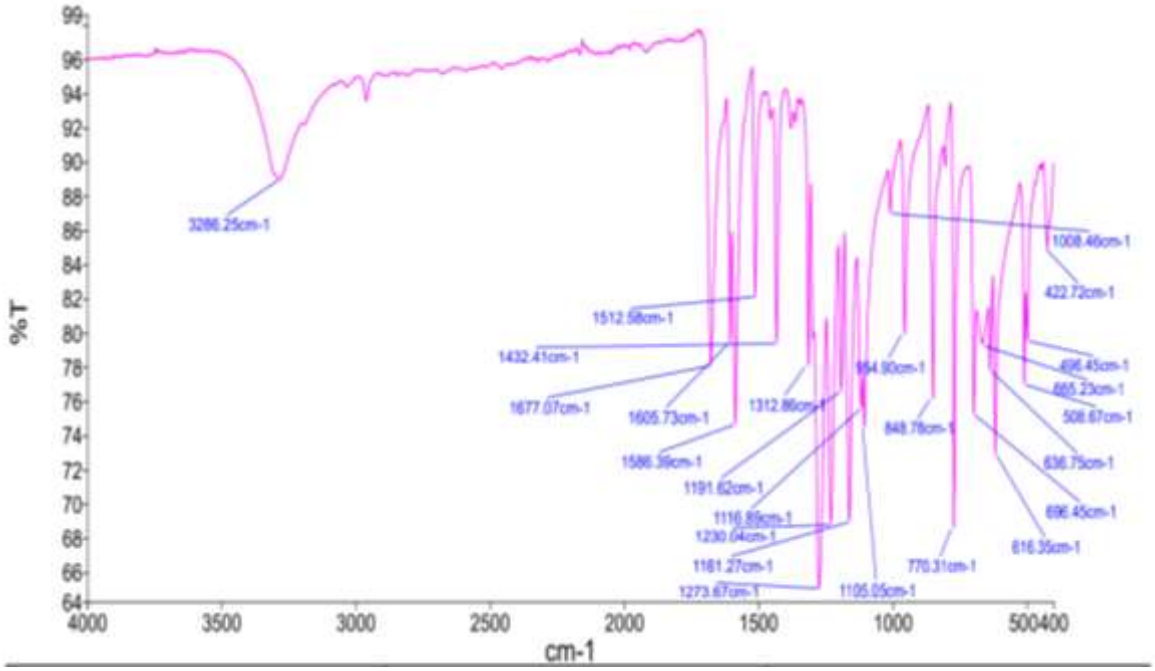



***Fig 7: IR spectrum of Methyl paraben***

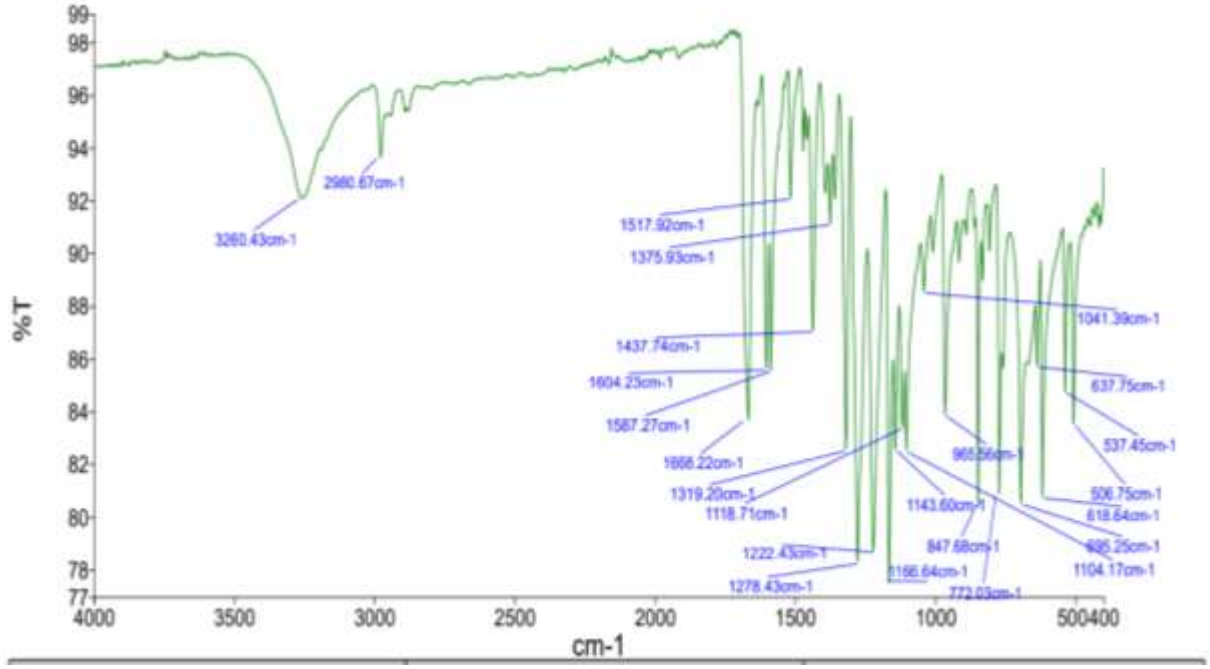



*Fig 8: IR Spectrum of Propyl paraben*

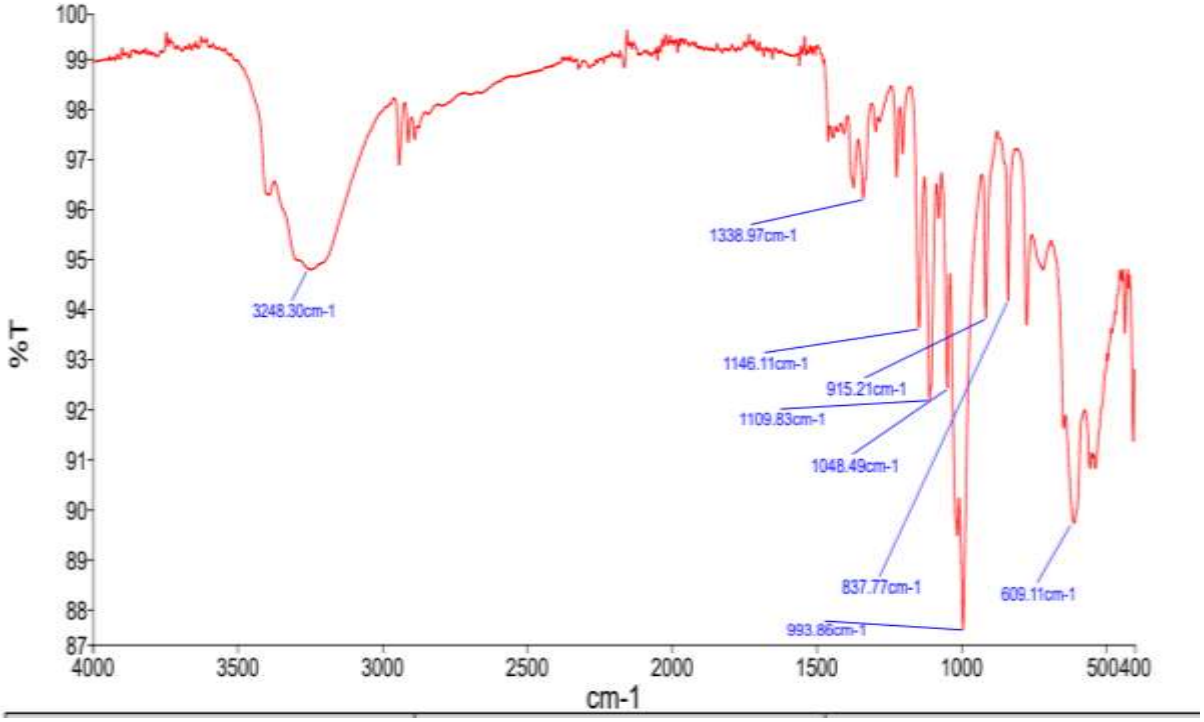



*Fig 9: IR Spectrum of D - glucose*

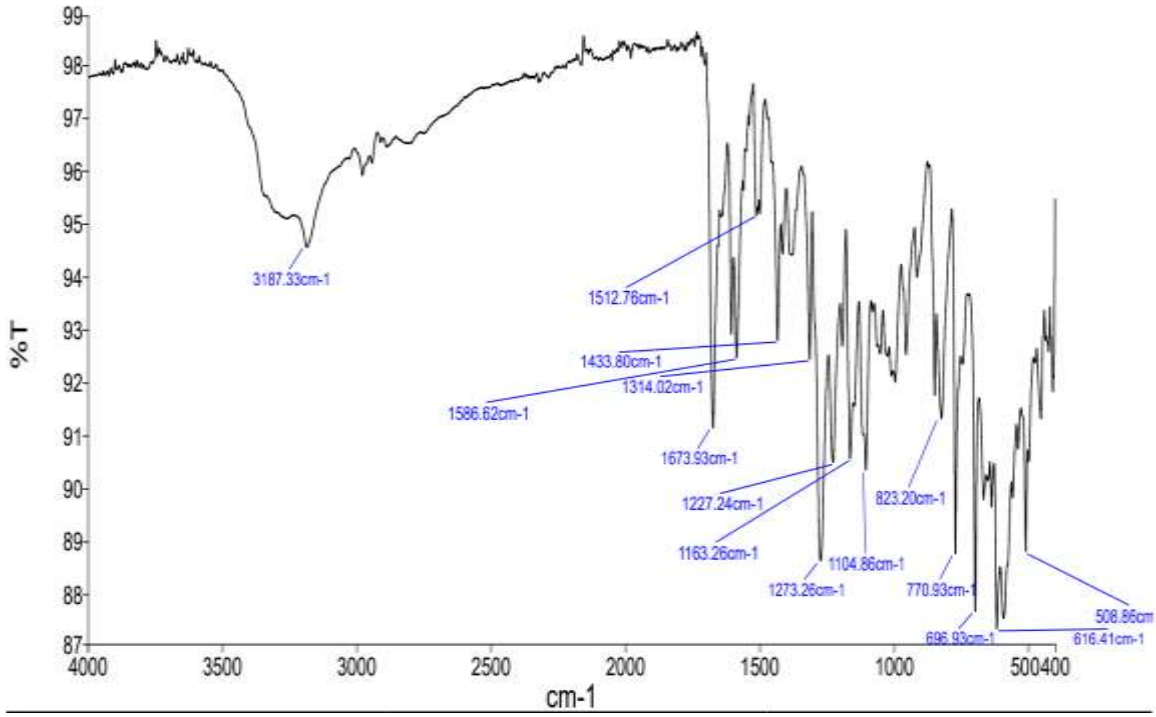



***Fig 10: IR spectrum of labetalol hydrochloride (API) + Methyl paraben + Propyl paraben + D - glucose***

### *9.4 Evaluation study for Containers and closure:*

### *9.4.1 Evaluation of container:*

| S.No | Test | Maximum limit of 0.01 M HCl (mL) | Observed value of HCl (mL) |
|---|---|---|---|
| **1.** | **Surface glass test** | 0.80 | 0.56 |
| **2.** | **Glass grains test** | 0.1 | 0.07 |
| **3.** | **Surface itching test** | 0.80 | 0.47 |

**Table 15: results for evaluation of containers.**

### **9.4.2** *Evaluation test for closure:*

| S.No | Evaluation test | Result |
|---|---|---|
| **1** | **Penetrability test** | The force required for piercing was found not exceeded 10N (1 kgf) |
| **2** | **Fragmentation test** | No fragments found |
| **3** | **Self-Sealing Capacity test** | None of the vials contains any the trace of coloured solution |

**Table 16: results for evaluation of containers**

## *9.5* Formulation development of labetalol hydrochloride injection

### *9.5.1 Drug solubility at different RPM:*

| S.No | Volume of WFI | RPM / Time taken for solubilization | | | | |
|---|---|---|---|---|---|---|
| | | **400 rpm** | **450 rpm** | **500 rpm** | **550 rpm** | **600 rpm** |
| 1 | 70 mL | 20.10 min | 19.38 min | 17.50 min | 16.00 min | **15.22 min** |
| 2 | 80 mL | 19.45 min | 19.10 min | 17.02 min | 15.33 min | **15.05 min** |
| 3 | 90 mL | 19.30 min | 18.05 min | 16.50 min | 15.25 min | **14.41 min** |
| 4 | 100 mL | 18.46min | 17.20 min | 16.10 min | 14.59 min | **13.40 min** |

**Table 17: Drug solubility at different RPM**

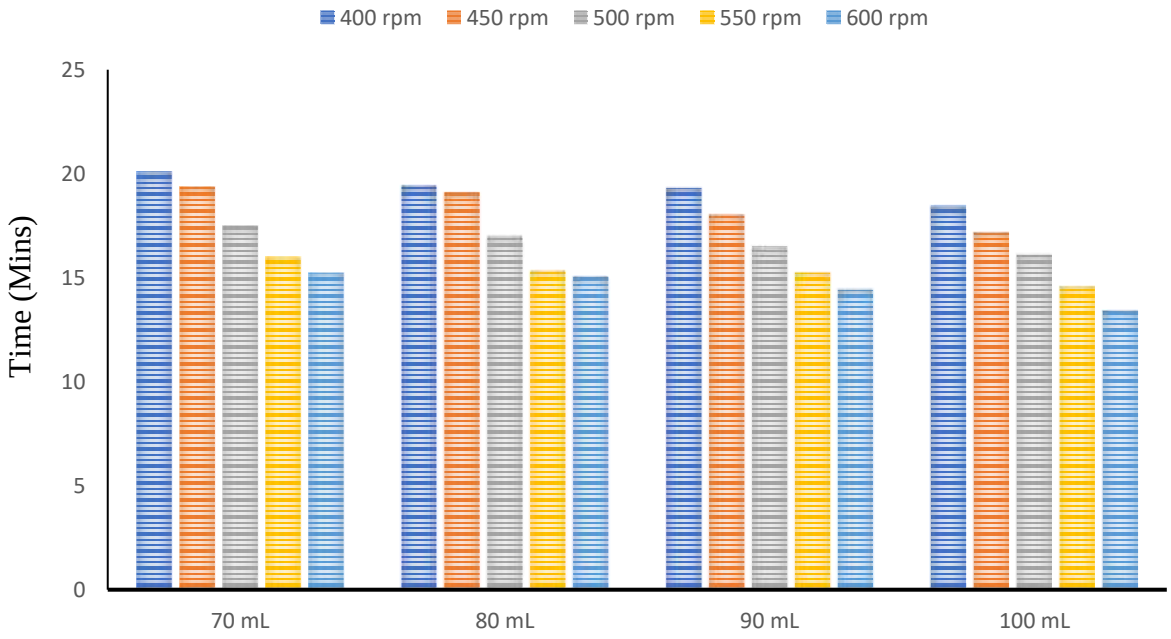



**Fig 11: drug solubility at different RPM**

### 9.5.2 *Order of mixing for labetalol hydrochloride injection:*

| *S.No* | Trials | API (A) | Methylparaben + Propylparaben (B) | Dextrose anhydrous (C) | EDTA (D) |
|---|---|---|---|---|---|
| **1** | **Trial 1(BCDA)** | **14.00 min** | **9.30 min** | **4.05 min** | **5.10 min** |
| **2** | **Trial 2(BACD)** | 13.55 min | 10.05 min | 4.45 min | 5.45 min |
| **3** | **Trial 3(BDCA)** | 14.30 min | 9.40 min | 4.30 min | 5.20 min |
| **4** | **Trial 4(DBAC)** | 14.10 min | 10.20 min | 4.50 min | 5.05 min |

**Table 18: Solubility of the drug and excipients at different order of mixing**

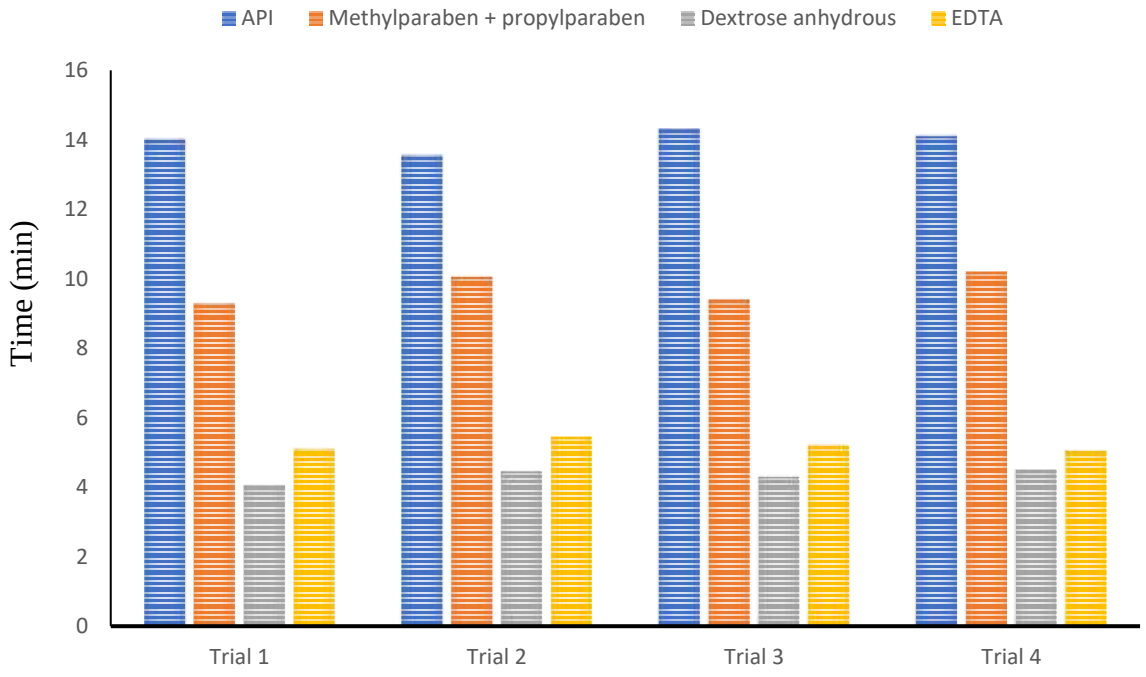



**Fig 12: order of mixing**

### *9.5.3 Determination of fill volume*

The excess fill volume of labetalol hydrochloride injection was **0.60 mL** than the labelled volume of the labetalol hydrochloride injection for 20 mL fill volume.

## 9.6 Calculation of API (Labetalol hydrochloride)

$$\frac{\textbf{Label claim} \times 100 \times 100 \times \textbf{batch size}}{\textbf{Assay (100 – LOD)}} \quad \textbf{-------- Equation 5}$$

Label claim = 5 mg/mL

Assay value = 99.2

Loss on drying = 0.13

Batch size = 1.5 liters

$$= \frac{5 \times 100 \times 100 \times 1500}{99.2\ (100 - 0.13)\ 1000}$$

$$= \frac{5.040 \times 100 \times 15}{99.87 \times 10}$$

$$= \frac{7560}{998.7} = 7.569$$

= 7.569 gram of API / 1500 mL

### 9.7 Selection of sterilization method results:

| Test parameters | Specification Limit | Un-autoclave | Autoclaved at 121°C | |
|---|---|---|---|---|
| | | | 20 min | 30 min |
| **Description** | Clear colourless solution | Complies | Complies | Complies |
| **pH** | 3.0 – 4.5 | 3.78 | 3.67 | 3.60 |
| **Assay of Labetalol** | 95.0 -105 % | 100.8 | 101.3 | 101.5 |
| **Assay of Methylparaben** | 90.0 -110 % | 100.5 | 100.1 | 100.2 |
| **Assay of Propylparaben** | 90.0 -110 % | 98.8 | 99.2 | 99.5 |
| **Assay of EDTA** | 90.0 -110 % | 99.7 | 100.0 | 100.3 |
| **Related substances (% w/w, by HPLC)** | NMT 1.0 | 0.05 | 0.18 | 0.10 |
| **Particulate matter ≥10µm** | NMT 6000 | 12 | 90 | 312 |
| **Particulate matter ≥25µm** | NMT 600 | 3 | 18 | 57 |

**Table 19: Selection of sterilization method**

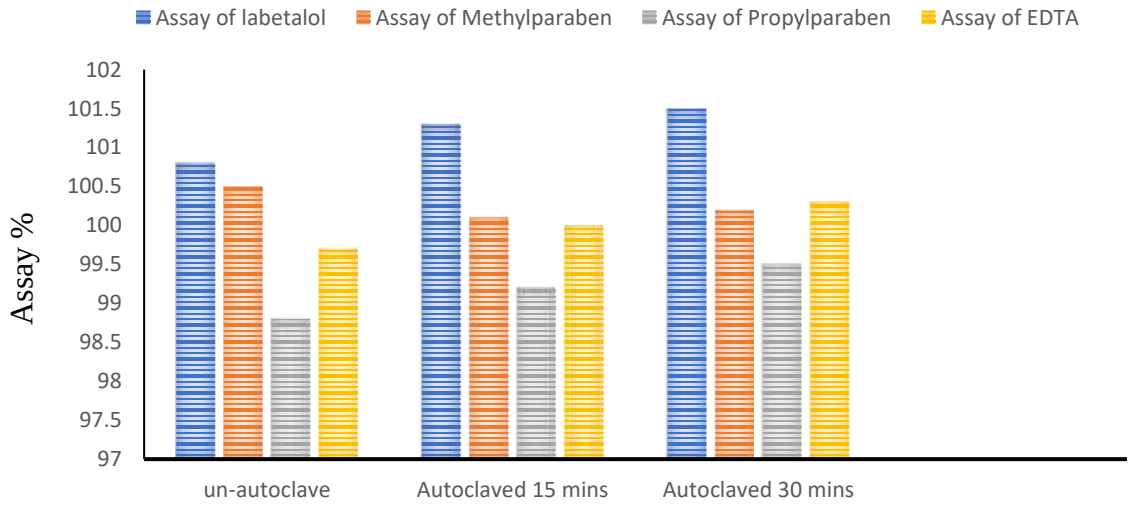



***Fig 13: Assay (%) for un-autoclaved and autoclaved samples***

## 9.8 Hold time compatibility study for labetalol hydrochloride injection:

### *9.8.1 Compatibility with stainless steel:*

| Test parameters | Limit | Initial | 48 hrs | 72 hrs |
|---|---|---|---|---|
| **Description** | Clear colourless solution | Complies | Complies | Complies |
| **pH** | 3.0 – 4.5 | 3.80 | 3.84 | 3.81 |
| **Assay of Labetalol** | 95.0-105 % | 100.7 | 100.8 | 103.7 |
| **Assay of methylparaben** | 90.0-110 % | 100.1 | 99.8 | 100.2 |
| **Assay of propylparaben** | 90.0-110 % | 98.7 | 96.8 | 96.5 |
| **Assay of EDTA** | 90.0-110 % | 101.5 | 100.6 | 100.9 |
| **Impurity A** | NMT 0.1 | ND | BQL | BQL |
| **Unspecified impurity** | NMT 0.1 | ND | ND | ND |
| **Total impurities** | NMT 0.4 | ND | ND | ND |

**Table 20: Compatibility evaluation with Stainless steel (before filtration)**

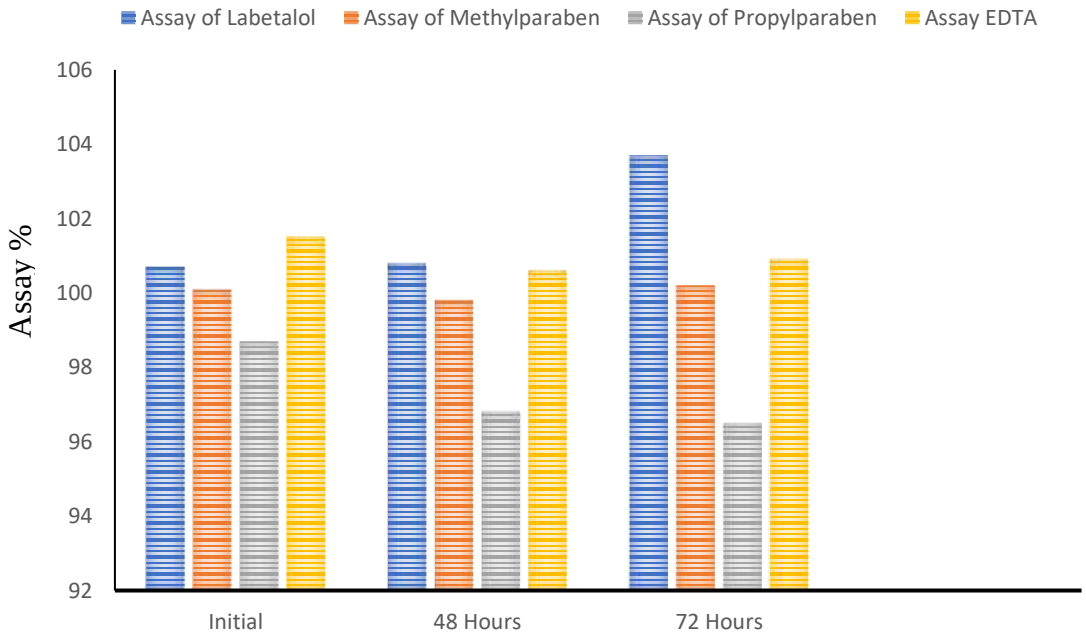



**Fig 14: Compatibility evaluation before filtration**

| Test parameters | Limit | Initial | 48 Hours | 72 Hours |
|---|---|---|---|---|
| **Description** | Clear colourless solution | Complies | Complies | Complies |
| **pH** | 3.0 – 4.5 | 3.74 | 3.82 | 3.85 |
| **Assay of labetalol** | 95.0-105 % | 100.1 | 100.6 | 100.2 |
| **Assay of methylparaben** | 90.0-110 % | 101.44 | 100.1 | 101.01 |
| **Assay of propylparaben** | 90.0-110 % | 98.6 | 98.3 | 99.2 |
| **Assay of EDTA** | 90.0-110 % | 101.4 | 100.4 | 101.3 |
| **Impurity A** | NMT 0.1 | ND | BDL | ND |
| **Unspecified impurity** | NMT 0.1 | ND | ND | ND |
| **Total impurities** | NMT 0.4 | ND | ND | ND |

**Table 21: compatibility evaluation with stainless steel (after filtration)**

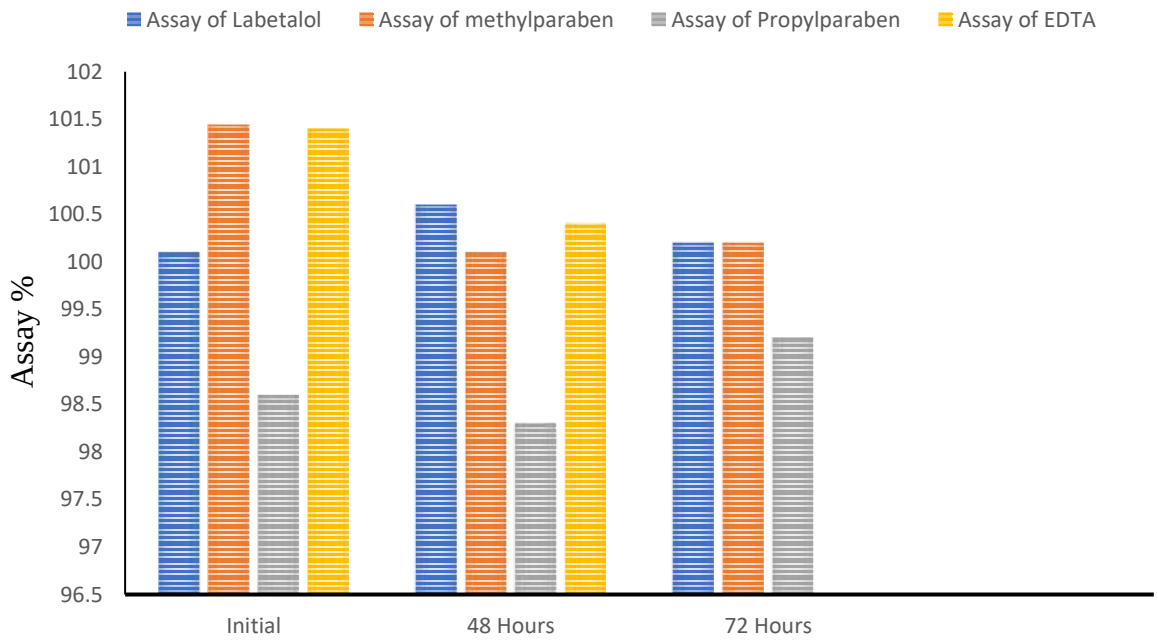



**Fig 15: compatibility evaluation after filtration**

### *9.8.2 Tubing compatibility study:*

| Test parameters | Limit | Initial | 48 Hours | 72 Hours |
|---|---|---|---|---|
| **Description** | Clear colourless solution | Complies | Complies | Complies |
| **pH** | 3.0 – 4.5 | 3.91 | 4.01 | 4.11 |
| **Assay of Labetalol** | 95.0-105 % | 101.6 | 101.8 | 102.5 |
| **Assay of Methylparaben** | 90.0-110 % | 100.1 | 99.4 | 98.9 |
| **Assay of Propylparaben** | 90.0-110 % | 99.1 | 94.4 | 92.2 |
| **Assay of ETDA** | 90.0-110 % | 101.3 | 100.4 | 100.9 |
| **Impurity A** | NMT 0.1 | ND | BDL | BDL |
| **Unspecified impurity** | NMT 0.1 | ND | ND | ND |
| **Total impurities** | NMT 0.4 | ND | ND | ND |

**Table 22: Compatibility evaluation with tube A (Sanitech)**

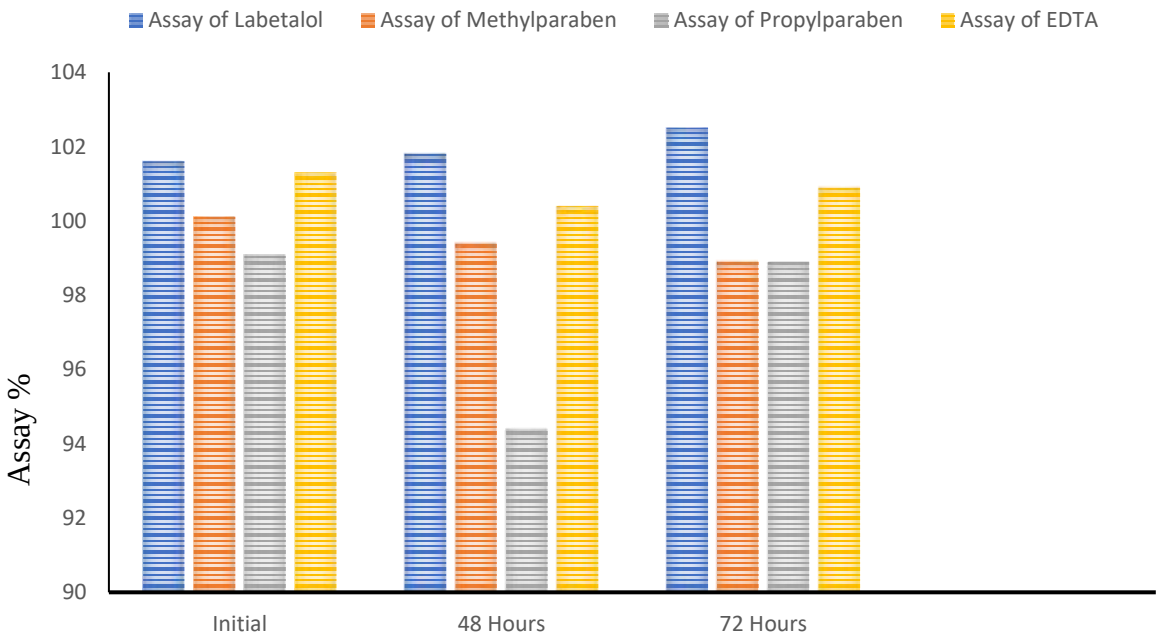



***Fig 16:*** **compatibility evaluation with tube A (Sanitech)**

| Test parameters | Limit | Initial | 48 Hours | 72 Hours |
|---|---|---|---|---|
| **Description** | Clear colourless solution | Complies | Complies | Complies |
| **Ph** | 3.0 – 4.5 | 3.84 | 3.92 | 4.02 |
| **Assay of Labetalol** | 95.0-105 % | 100.5 | 100.8 | 101.5 |
| **Assay of Methylparaben** | 90.0-110 % | 100.5 | 100.0 | 99.4 |
| **Assay of Propylparaben** | 90.0-110 % | 99.4 | 96.4 | 94.2 |
| **Assay of ETDA** | 90.0-110 % | 101.5 | 99.8 | 100.4 |
| **Impurity A** | NMT 0.1 | ND | BDL | BDL |
| **Unspecified impurity** | NMT 0.1 | ND | ND | ND |
| **Total impurities** | NMT 0.4 | ND | ND | ND |

**Table 23: Compatibility evaluation tube B (Pharmapure)**

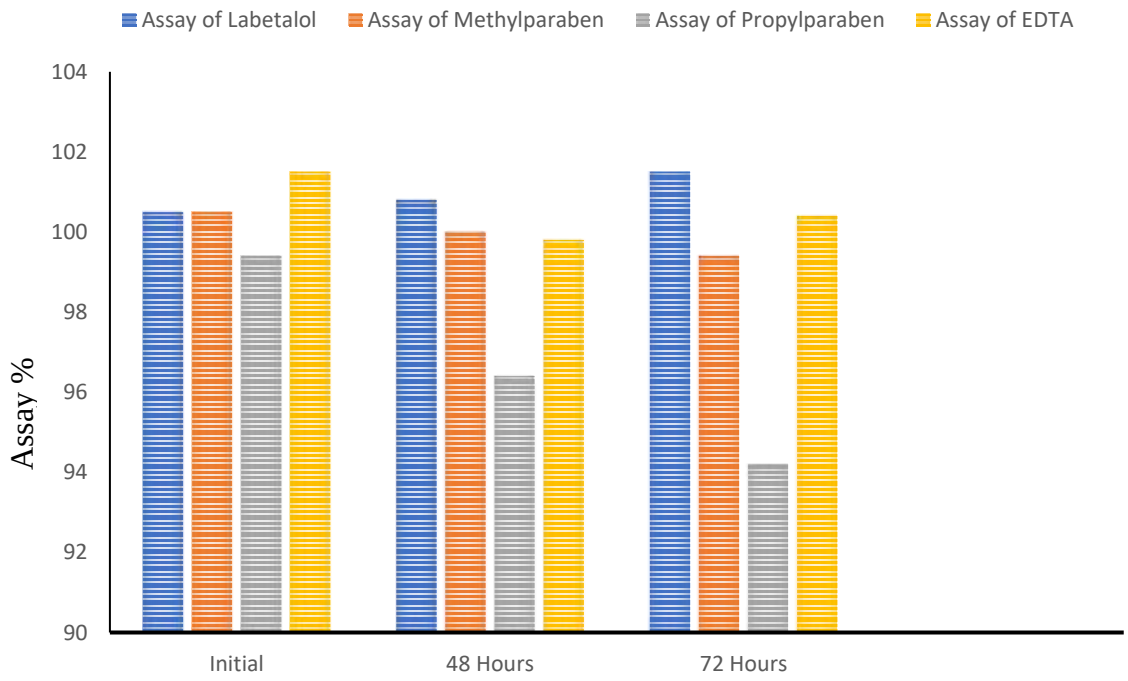



***Fig 17:*** **compatibility evaluation with tube B (Pharmapure)**

### *9.8.3 Compatibility with filters:*

| Test parameters | Limit | Initial | 48 Hours | 72 Hours |
|---|---|---|---|---|
| **Description** | Clear colourless solution | Complies | Complies | Complies |
| **pH** | 3.0 – 4.5 | 3.79 | 3.65 | 3.61 |
| **Assay of Labetalol** | 95.0-105 % | 100.9 | 100.8 | 101.5 |
| **Assay of Methylparaben** | 90.0-110 % | 100.1 | 98.0 | 97.1 |
| **Assay of Propylparaben** | 90.0-110 % | 99.4 | 94.9 | 93.0 |
| **Assay of Disodium edetate** | 90.0-110 % | 101.0 | 101.1 | 102.8 |
| **Impurity A** | NMT 0.1 | BQL | BQL | BQL |
| **Unspecified impurity** | NMT 0.1 | 0.05 | 0.06 | 0.05 |
| **Total impurities** | NMT 0.4 | 0.12 | 0.13 | 0.12 |

**Table 24: Compatibility evaluation with membrane filters (PVDF) filter 0.22 µm.**

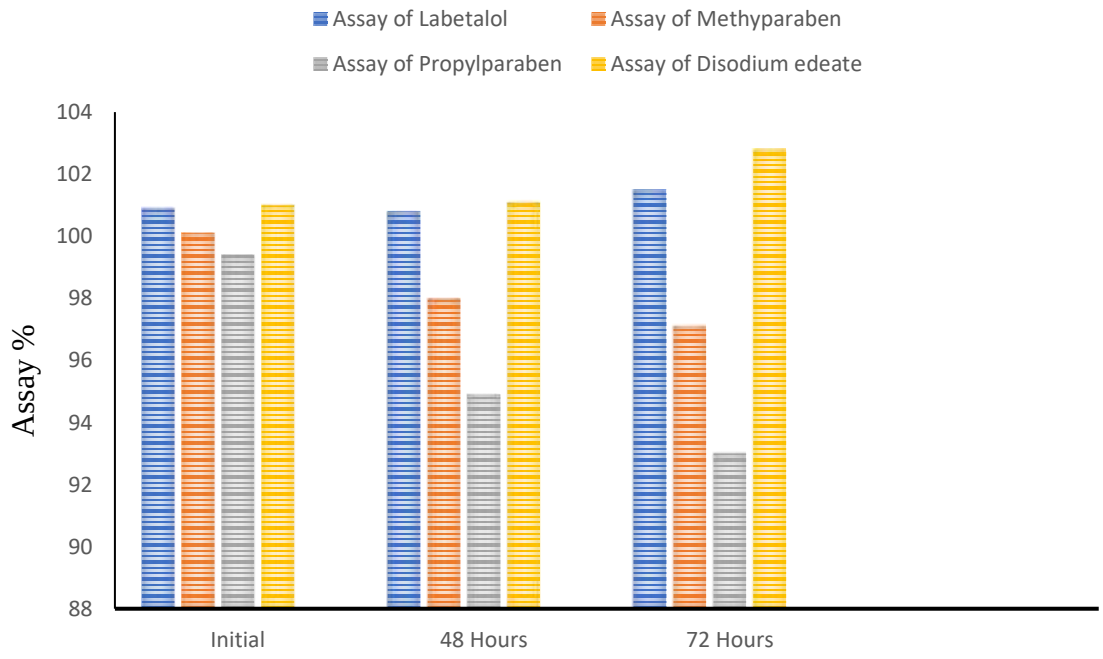



**Fig 18: Compatibility evaluation with PVDF filter.**

| Test parameters | Limit | Initial | 48 hrs | 72 hrs |
|---|---|---|---|---|
| **Description** | Clear colourless solution | Complies | Complies | Complies |
| **pH** | 3.0 – 4.5 | 3.79 | 3.64 | 3.60 |
| **Assay of Labetalol** | 95.0-105 % | 100.9 | 101.7 | 101.2 |
| **Assay of Methylparaben** | 90.0-110 % | 100.1 | 100.9 | 100.2 |
| **Assay of Propylparaben** | 90.0-110 % | 99.4 | 98.3 | 99.0 |
| **Assay of Disodium edetate** | 90.0-110 % | 101.0 | 101.4 | 101.7 |
| **Impurity A** | NMT 0.1 | BQL | BQL | BQL |
| **Unspecified impurity** | NMT 0.1 | 0.05 | 0.06 | 0.05 |
| **Total impurities** | NMT 0.4 | 0.12 | 0.13 | 0.12 |

**Table 25: Compatibility evaluation with membrane (PES) filter 0.22 µm.**

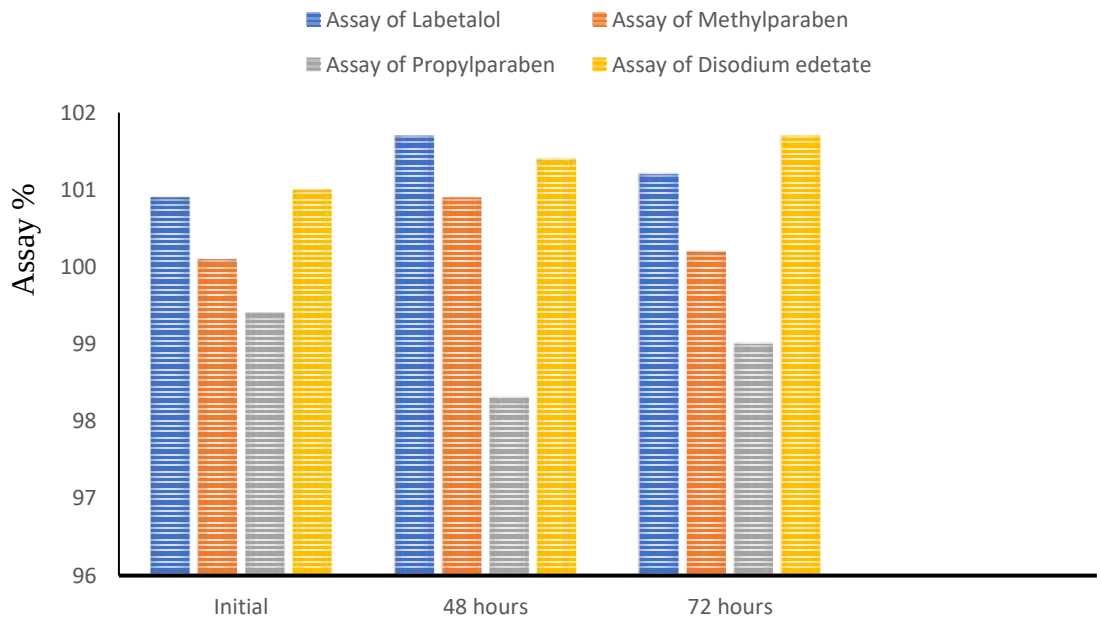



**Fig 19: Compatibility evaluation with membrane (PES) filter.**

**9.9 Stress studies:**

**9.9.1** ***Oxygen sensitivity study:***

| Test parameters | Without Nitrogen purging in Headspace | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | 40 ± 2°C, 75 ± 5% RH | | | | 25°C± 2°C /60± 5% RH | |
| | | 3 M (I) | 3 M (U) | 6M (I) | 6 M (U) | 3 M (I) | 6 M (I) |
| **Description** | Complies | Complies | Complies | Complies | Complies | Complies | Complies |
| **pH** | 3.66 | 3.68 | 3.66 | 3.67 | 3.64 | 3.66 | 3.69 |
| **Assay of Labetalol** | 101.4 | 100.3 | 100.8 | 99.3 | 99.7 | 100.1 | 99.5 |
| **Assay of Methylparaben** | 100.5 | 100.2 | 99.8 | 99.5 | 100.0 | 99.2 | 100.2 |
| **Assay of Propylparaben** | 99.3 | 98.9 | 97.2 | 98.5 | 99.5 | 99.2 | 99.8 |
| **Assay of EDTA** | 101.6 | 101.3 | 101.3 | 100.5 | 100.6 | 101.4 | 100.8 |
| **Impurity A** | 0.11 | 0.15 | 0.15 | 0.17 | 0.16 | 0.13 | 0.12 |
| **Unspecified Impurity** | BQL | ND | ND | BQL | BQL | ND | BQL |
| **Total Impurities** | 0.11 | 0.15 | 0.15 | 0.17 | 0.16 | 0.13 | 0.12 |
| **Particulate matter ≥10µm** | 90 | 108 | NP | 426 | NP | NP | 164 |
| **Particulate matter ≥25µm** | 2 | 4 | NP | 14 | NP | NP | 2 |

**Table 26: Oxygen sensitivity study of Labetalol hydrochloride injection (without nitrogen)**

| Test parameters | With Nitrogen purging in Headspace | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | 40 ± 2°C, 75 ± 5% RH | | | | 25°C± 2°C /60± 5% RH | |
| | | 3 M (I) | 3 M (U) | 6M (I) | 6 M (U) | 3 M (I) | 6 M (I) |
| **Description** | Complies | Complies | Complies | Complies | Complies | Complies | Complies |
| **pH** | 3.66 | 3.68 | 3.68 | 3.67 | 3.63 | 3.68 | 3.19 |
| **Assay of Labetalol** | 100.9 | 100.9 | 100.2 | 99.4 | 99.0 | 100.7 | 99.1 |
| **Assay of Methylparaben** | 100.3 | 99.6 | 99.8 | 99.2 | 99.3 | 99.9 | 99.3 |
| **Assay of Propylparaben** | 99.6 | 98.3 | 98.7 | 99.4 | 99.4 | 99.7 | 99.3 |
| **Assay of EDTA** | 101.2 | 100.5 | 100.8 | 100.3 | 100.6 | 100.2 | 100.0 |
| **Impurity A** | 0.10 | 0.14 | 0.13 | 0.14 | 0.14 | 0.12 | 0.11 |
| **Unspecified Impurity** | 0.05 | ND | ND | 0.05 | BQL | ND | 0.05 |
| **Total Impurities** | 0.15 | 0.14 | 0.13 | 0.19 | 0.14 | 0.12 | 0.16 |
| **Particulate matter ≥10µm** | 68 | 48 | NP | 54 | NP | NP | 64 |
| **Particulate matter ≥25µm** | 4 | 2 | NP | 2 | NP | NP | 0 |

**Table 27: Oxygen sensitivity study of Labetalol hydrochloride injection (with nitrogen)**

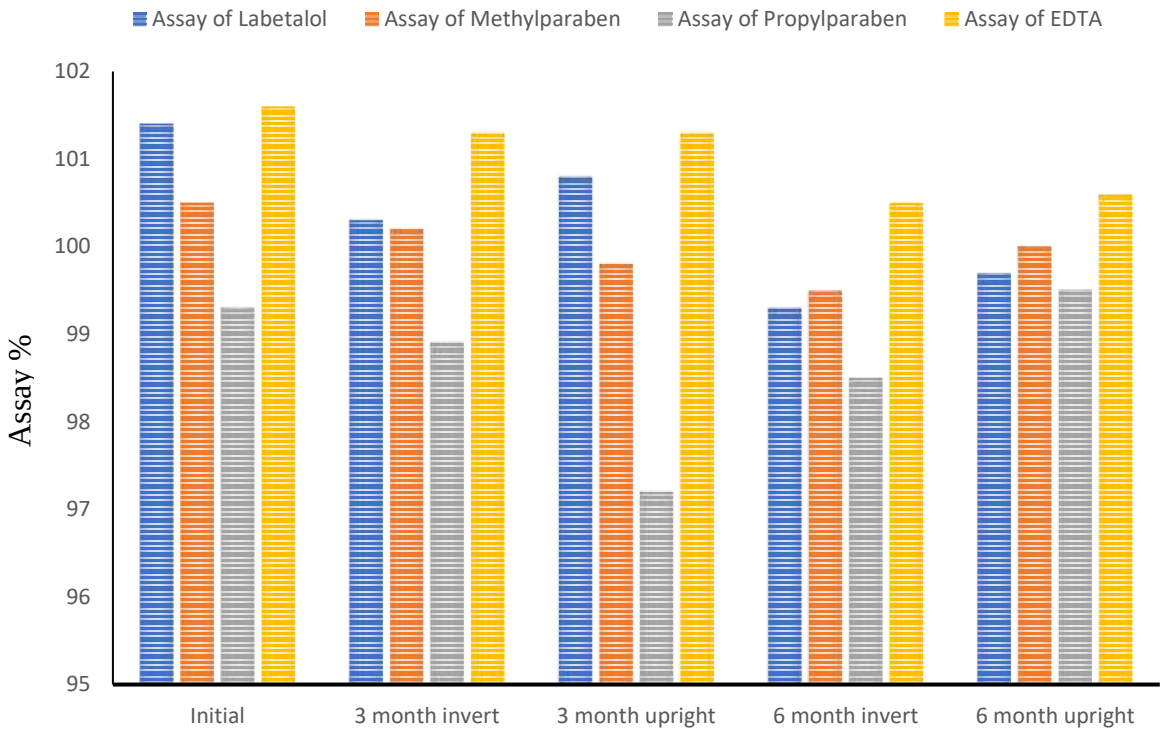



**Fig 20: Compatibility evaluation study without Nitrogen purging in Headspace**

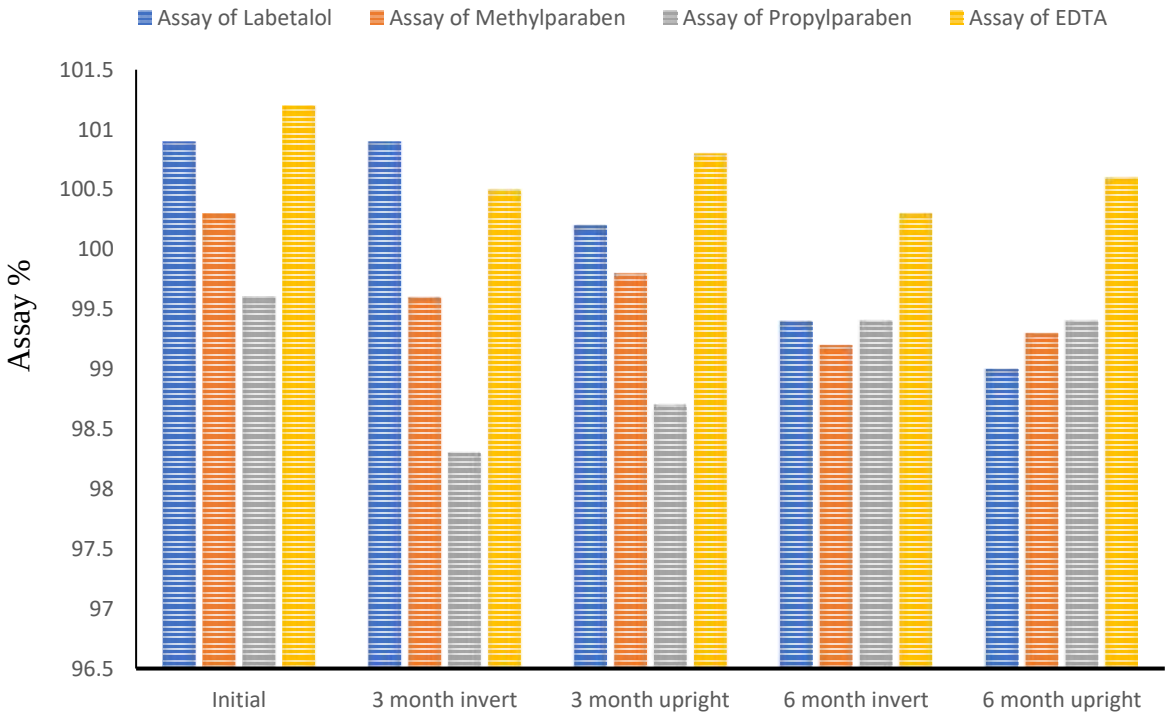



**Fig 21: Compatibility evaluation study with Nitrogen purging in Headspace**

*9.9.2 pH extreme study:*

| Test parameters | With lower extremes of pH range (pH 3.10) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | 40 ± 2°C, 75 ± 5% RH | | | | 25°C± 2°C /60± 5% RH | |
| | | 3 M (I) | 3 M (U) | 6M (I) | 6 M (U) | 3 M (I) | 6 M (I) |
| **Description** | Complies | Complies | Complies | Complies | Complies | Complies | Complies |
| **pH** | 3.10 | 3.07 | 3.09 | 3.06 | 3.12 | 3.09 | 3.10 |
| **Assay of Labetalol** | 100.6 | 99.9 | 99.7 | 99.3 | 99.4 | 100.3 | 99.9 |
| **Assay of Methylparaben** | 101.2 | 100.9 | 100.8 | 101.1 | 101 | 100.2 | 100.5 |
| **Assay of Propylparaben** | 97.5 | 96.9 | 93.8 | 96.8 | 98.7 | 99.6 | 99.1 |
| **Assay of EDTA** | 101.6 | 101.4 | 101.3 | 100.9 | 101.1 | 100.8 | 101 |
| **Impurity A** | 0.05 | 0.06 | 0.07 | 0.06 | 0.08 | 0.06 | 0.05 |
| **Unspecified Impurity** | 0.06 | ND | ND | ND | ND | ND | ND |
| **Total Impurities** | 0.17 | 0.06 | 0.07 | 0.06 | 0.08 | 0.06 | 0.05 |
| **Particulate matter ≥10µm** | 7 | 18 | NP | 22 | NP | NP | 20 |
| **Particulate matter ≥25µm** | 0 | 0 | NP | 0 | NP | NP | 0 |

**Table 28: Stability results of Labetalol hydrochloride injection adjusted to lower extremes of pH range.**

| Test parameters | With higher extremes of pH range (pH 4.4) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | 40 ± 2°C, 75 ± 5% RH | | | | 25°C± 2°C /60± 5% RH | |
| | | 3 M (I) | 3 M (U) | 6M (I) | 6 M (U) | 3 M (I) | 6 M (I) |
| **Description** | Complies | Complies | Complies | Complies | Complies | Complies | Complies |
| **Ph** | 4.40 | 4.42 | 4.52 | 4.34 | 4.38 | 4.46 | 4.49 |
| **Assay of Labetalol** | 99.5 | 99.6 | 98.4 | 99.8 | 99.7 | 100.1 | 99.2 |
| **Assay of Methylparaben** | 100.2 | 98.7 | 98.8 | 99.4 | 99.7 | 99.9 | 99.2 |
| **Assay of Propylparaben** | 99.3 | 98.3 | 98.4 | 99.2 | 99.9 | 99.8 | 99.4 |
| **Assay of EDTA** | 101.4 | 101.2 | 100.8 | 99.6 | 99.8 | 100.9 | 100.2 |
| **Impurity A** | 0.06 | 0.08 | 0.06 | 0.05 | 0.06 | 0.04 | 0.04 |
| **Unspecified Impurity** | 0.07 | ND | ND | ND | ND | ND | ND |
| **Total Impurities** | 0.13 | 0.08 | 0.06 | 0.05 | 0.06 | 0.04 | 0.04 |
| **Particulate matter ≥10µm** | 40 | 80 | NP | 156 | NP | NP | 90 |
| **Particulate matter ≥25µm** | 0 | 10 | NP | 2 | NP | NP | 8 |

**Table 29: Stability results of Labetalol hydrochloride injection adjusted to higher extremes of pH range.**

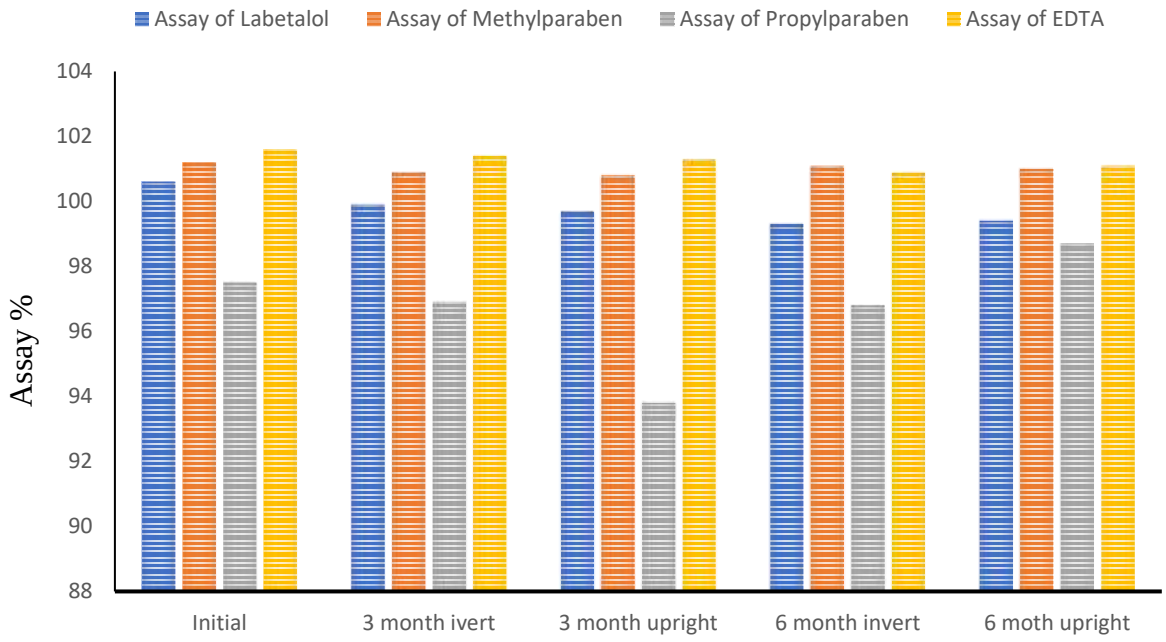



**Fig 22: Compatibility evaluation study with lower extremes of pH range**

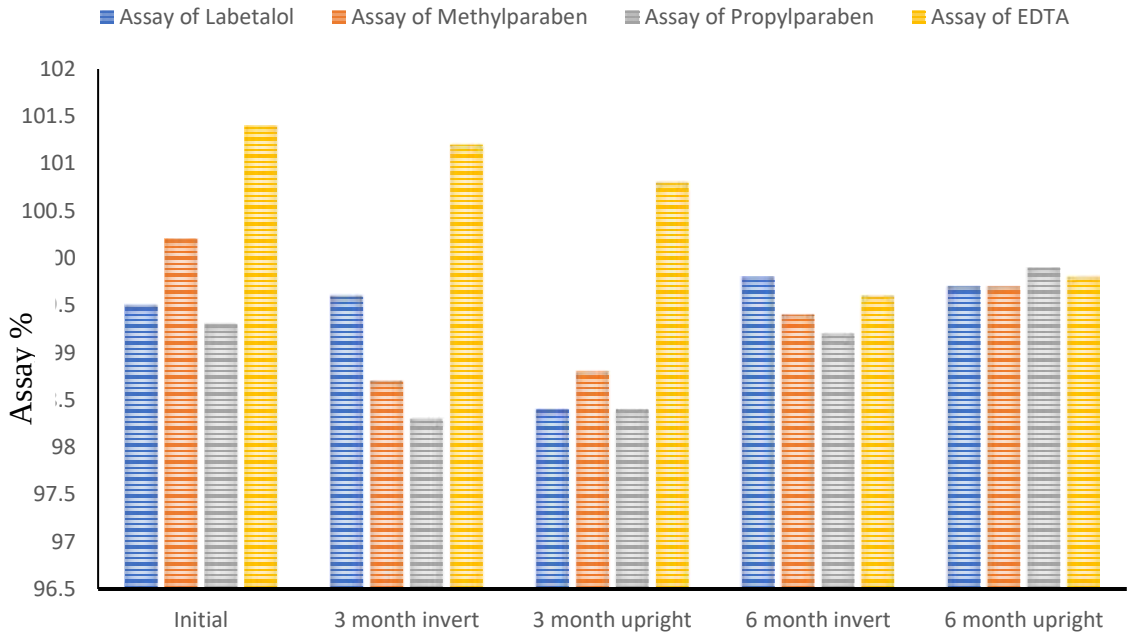



**Fig 23: Compatibility evaluation study with higher extremes of pH range**

### *9.9.3 Freeze thaw study:*

| Test parameters | Limit | Initial | After 1st cycle | After 2nd cycle | After 3rd cycle |
|---|---|---|---|---|---|
| **Description** | Clear colourless solution | Complies | Complies | Complies | Complies |
| **pH** | 3.0 – 4.5 | 3.66 | 3.67 | 3.64 | 3.68 |
| **Assay of Labetalol** | 90.0-120 % | 100.4 | 99.7 | 99.5 | 99.9 |
| **Assay of Methylparaben** | 80.0-120 % | 99.8 | 100.2 | 100.5 | 100.3 |
| **Assay of Propylparaben** | 80.0-120 % | 96.6 | 97.3 | 97.5 | 97.8 |
| **Assay of EDTA** | 80.0-120 % | 100.3 | 100.3 | 100.9 | 101.1 |
| **Osmolality (mOsm/kg of $H_2O$)** | Report the Value | 304 | 301 | 305 | 304 |
| **Impurity A** | NMT 1.0 | 0.09 | 0.06 | 0.06 | 0.06 |
| **Unspecified impurity** | NMT 0.4 | BQL | ND | ND | ND |
| **Total impurities** | NMT 2.0 | 0.08 | 0.06 | 0.06 | 0.06 |
| **Particulate matter ≥10µm** | NMT 6000 | 458 | 528 | 470 | 605 |
| **Particulate matter ≥25µm** | NMT 600 | 28 | 7 | 28 | 23 |

**Table 30: Freeze thaw study results of Labetalol hydrochloride injection**

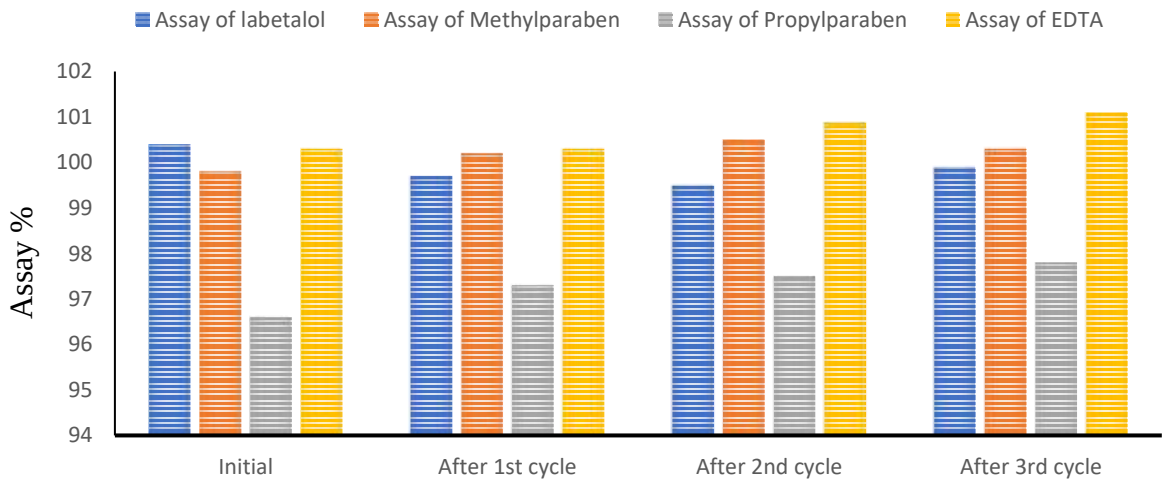



**Fig 24: Bar chart for Freeze thaw study results**

### 9.9.4 *Photo stability study:*

| Test parameters | Limit | Dark control | Primary pack | Secondary pack |
|---|---|---|---|---|
| **Description** | Clear colourless solution | Complies | Complies | Complies |
| **Ph** | 3.0 – 4.5 | 3.81 | 3.85 | 3.90 |
| **Assay of Labetalol** | 90.0-110 % | 100.9 | 90.1 | 90.8 |
| **Assay of Methylparaben** | 80.0-120 % | 100.1 | 99.5 | 98.9 |
| **Assay of Propylparaben** | 80.0-120 % | 98.4 | 97.6 | 95.9 |
| **Assay of EDTA** | 80.0-120 % | 100.9 | 100.9 | 100.8 |
| **Osmolality (mOsm/Kg)** | Report the Value | 301 | 297 | 295 |
| **Impurity A** | NMT 0.1 | 0.03 | 0.05 | 0.06 |
| **Unspecified impurity** | NMT 0.1 | BQL | ND | BQL |
| **Total impurities** | NMT 0.4 | 0.03 | 0.05 | 0.06 |

**Table 31: Photo stability results of Labetalol hydrochloride injection**

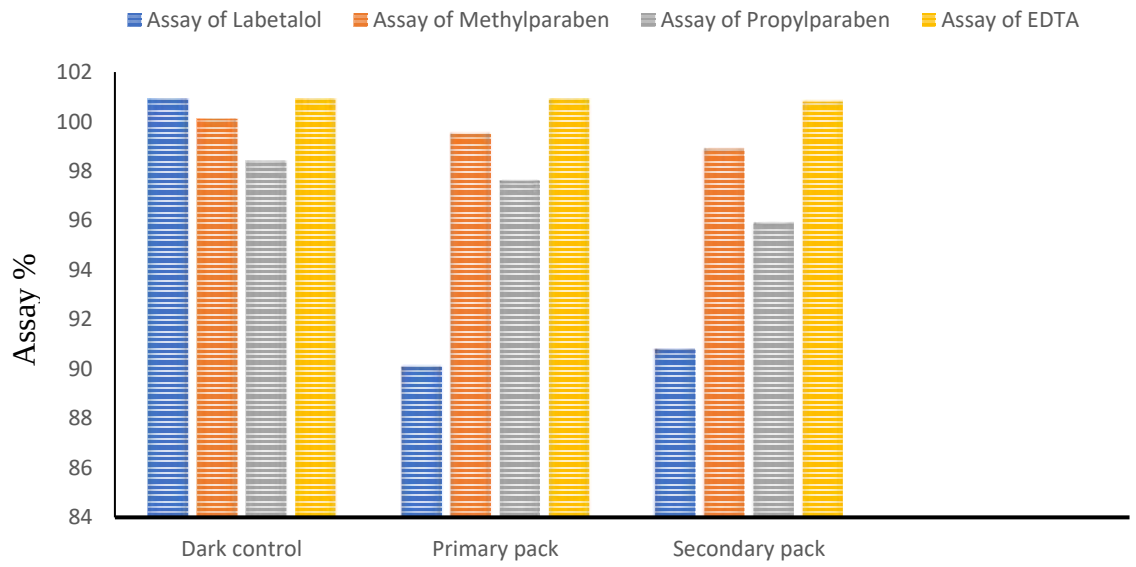



**Fig 25: Photo stability study of labetalol hydrochloride**

### 9.10 Quality control study for labetalol hydrochloride injection as per USP

| *S.No* | *Evaluation parameter* | *Results* |
|---|---|---|
| *1* | ***Description*** | clear colorless solution |
| *2* | ***pH*** | 3.89 |
| *3* | ***Sterility Test*** | No microbial growth |
| *4* | ***Particulate matter ≥10 µm*** | 04 particles per container |
| *5* | ***Particulate matter ≥25 µm*** | 01 particle per container |
| *6* | ***Leak test*** | No color change of the solution |
| *7* | ***Bacterial Endotoxin Test (LAL Test)*** | 1.2 EU/ mg |
| *8* | ***Assay of Labetalol hydrochloride*** | 100.5 |

***Table 32:*** **Quality control study for Labetalol hydrochloride**

*9.11* **Stability study for final development batch of labetalol hydrochloride injection:**

| **Product name** | | **Labetalol hydrochloride injection –batch size-1500 mL** | | | | |
|---|---|---|---|---|---|---|
| **Test parameters** | **Limit** | **Initial** | **40 ± 2°C, 75 ± 5% RH** | | **25°C± 2°C /60± 5% RH** | |
| | | | **3 M (I)** | **3 M (U)** | **3 M (I)** | **3M (U)** |
| **Description** | Clear colourless solution | Complies | Complies | Complies | Complies | Complies |
| **pH** | 3 - 4.5 | 3.93 | 4.01 | 4.05 | 3.99 | 4.02 |
| **Osmolality (mOsm/Kg)** | Report the Value | 299 | 302 | 301 | 302 | 300 |
| **Assay of Labetalol** | 90 %-110 % | 99.3 | 99.7 | 98.5 | 100.1 | 99.1 |
| **Assay of Methylparaben** | 80 %-120 % | 100.1 | 98.8 | 98.9 | 99.9 | 99.4 |
| **Assay of Propylparaben** | 80 %-120 % | 99.1 | 95.3 | 94.4 | 97.9 | 95.4 |
| **Assay of EDTA** | 80 %-120 % | 101.5 | 101.3 | 100.4 | 100.9 | 100.0 |
| **Impurity A** | NMT 0.2 | BQL | 0.07 | 0.08 | 0.05 | BQL |
| **Unspecified Impurity** | NMT 0.2 | BQL | 0.06 | 0.06 | 0.05 | 0.05 |
| **Total Impurities** | NMT 1.0 | NIL | 0.18 | 0.14 | 0.10 | 0.05 |
| **Particulate matter ≥10µm** | NMT 6000 | NA | NA | NA | NA | NA |
| **Particulate matter ≥25µm** | NMT 600 | NA | NA | NA | NA | NA |

**Table 33: Stability evaluation of Labetalol hydrochloride injection**

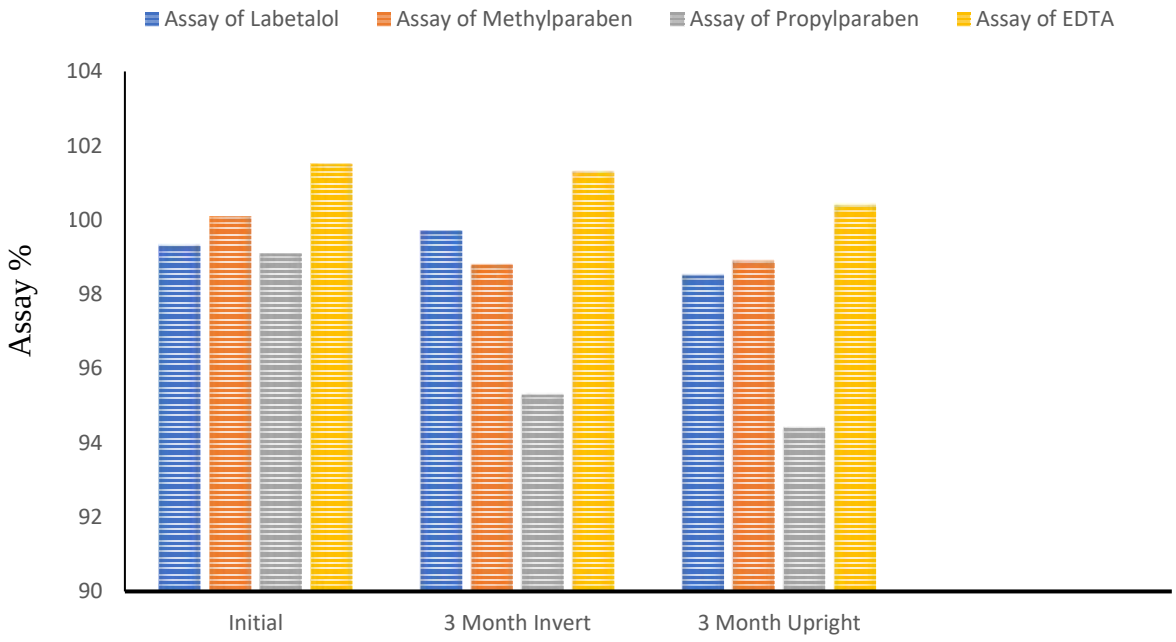



**Fig. 26: Stability evaluation study(accelerated conditions)**

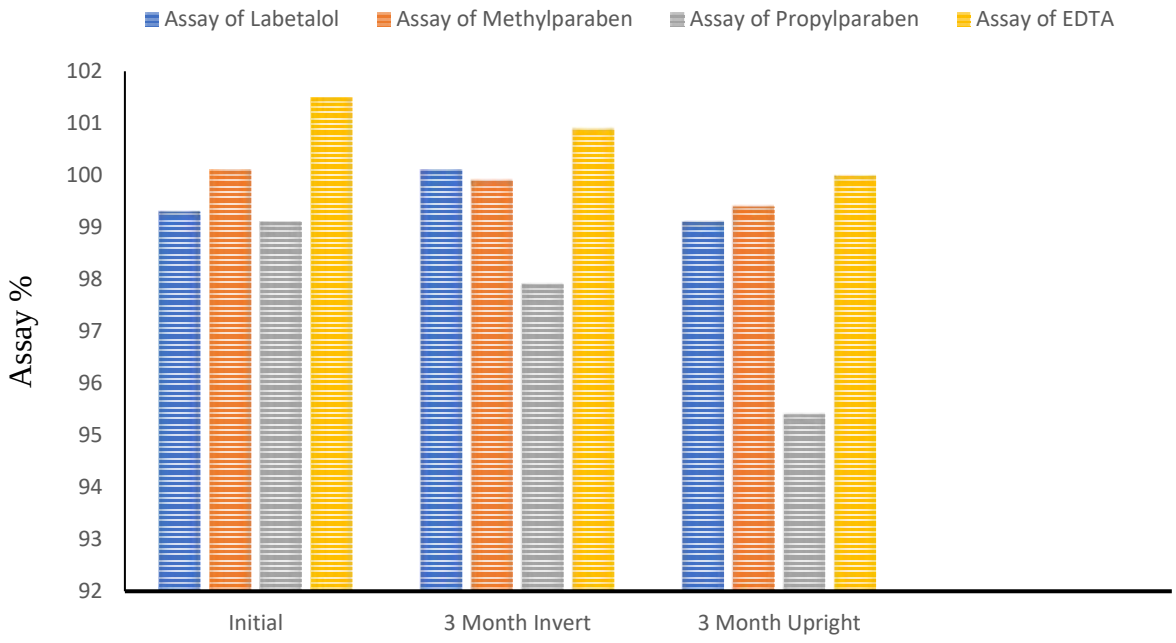



**Fig. 27: Stability evaluation study( real time conditions)**

## 10. Discussion

### 10.1 Reason for selection of Labetalol hydrochloride as injection dosage form:

Labetalol Hydrochloride Injection USP (labetalol hydrochloride) is an adrenergic receptor blocking agent possessing both alpha1 (post-synaptic) and beta-receptor blocking activity. Its action on beta-receptors is four times stronger than that on alpha-receptors. It antagonizes beta1- and beta2-receptors equally.

- The ratios of alpha- to beta-blockade differ depending on the route of administration estimated to be 1:3 (oral) and 1:7 (IV)
- Onset of action- Oral: 20 minutes to 2 hours; IV: Within 5 minutes
- Peak effect: Oral: 2 to 4 hours; IV: 5 to 15 minutes
- Duration of action- Oral: 8 to 12 hours (dose dependent), IV: Average: 16 to 18 hours (dose dependent)
- Half-Life Elimination -Oral: 6 to 8 hours; IV: ~5.5 hours

The parenteral route of Labetalol hydrochloride is considered as the best choice of route than compared to the oral route.

### 10.2 Raw material analysis of Labetalol hydrochloride:

#### *10.2.1 Physiochemical characters of Labetalol hydrochloride:*

The physiochemical characters of the active pharmaceutical ingredient Labetalol hydrochloride were found to comply with USP. The melting range of the Labetalol hydrochloride was found to be 180° C. The results were shown in the **Table 14,** and the drug was sparingly soluble in water and in ethanol (96%) and insoluble in ether and in methylene chloride

#### *10.2.2 Identification test:*

The test for Loss on drying was found to be not more than 0.13%**,** Residue on ignition was found to be not more than 0.04%, Test for chloride, and assay was done to identify the Labetalol hydrochloride and the results were found to comply with USP. The percentage purity was found to be 99.2%**.**

## 10.3 Incompatibility study:

### *10.3.1 Drug Excipients compatibility study by FT-IR:*

The pure drug of Labetalol hydrochloride and Labetalol hydrochloride along with the excipients used in the formulation were analysed by FTIR spectroscopic method. The FTIR spectra of Labetalol hydrochloride was shown in **Fig 6**. The functional peak at **3183.32 $cm^{-1}$** band was due to O-H stretching vibrations of chelate compounds. The peak **1673.82 $cm^{-1}$** was assigned to stretching vibrations of C=O, another peak at **1640.81 $cm^{-1}$** was due to C=O stretching vibrations of amide group.

The peaks **3187.33 $cm^{-1}$**, **1673.93 $cm^{-1}$**, and **1586.62 $cm^{-1}$** of Labetalol hydrochloride with excipients was shown in **Fig 10.** From the results it was clear that there is no interaction between Labetalol hydrochloride and excipients in the formulation and the drug was found to be compatible with the excipients.

### *10.3.2 Evaluation study for Containers and closure:*

#### *10.3.2.1 Evaluation of containers:*

Type I glass containers were preferred for the formulation and hence evaluation test has been carried out for the same glass containers. The glass container test results are found to be within the specification limits and the preferred glass passes all the test for containers (surface glass test, glass grains test and surface itching test) and hence the type I glass were used for the entire formulation and development process of Labetalol hydrochloride injection. The results were tabulated in **Table 15**.

#### *10.3.2.2 Evaluation of closures:*

Evaluation of closure was done with the rubber closure (bromobutyl rubber). The results show that the preferred closure passes the entire test for closure and there was no penetration, no fragments was seen after the tests, and none of the vials contains any the trace of coloured solution. The closure passes the evaluation test (functionality test) and the results were shown in **Table 16** and that rubber closure was used for the entire formulation and development process of Labetalol hydrochloride injection.

## 10.4 Preformulation study:

### *10.4.1. Determination of RPM:*

The API (Labetalol hydrochloride) was found to be solubilised easily at 600 rpm. The results were shown in **Table 17 & Fig 11.** From the above results it was observed that there is no significant difference in the solubilisation time at 550 rpm and 600 rpm. Based on the result, it was decided to take 80% of water for batch preparation and to stir at 600 rpm.

### *10.4.2 Order of mixing of the ingredients in Labetalol hydrochloride injection formulation:*

Labetalol hydrochloride and other excipients in this formulation were completely solubilised in water for injection in all the trials. The results were shown in the **Table 18 & Fig. 12** The trial 1 (BCDA) were selected as a preferred order of addition because of the lesser solubility time of the API and other excipients and the solution was found to yield a clear, colourless solution at this trial when compared to other trials of order of mixing. The pH of the solution at this stage was observed around 4.54. The pH of this solution was adjusted to 3.8 by using 1% citric acid.

From the above trial it was found that the API was completely soluble in the proposed concentration. The order of addition and pH were satisfactory. The product physiochemical parameters comply within the specification limits. The same procedure is finalized for Labetalol hydrochloride injection.

## 10.5 Selection of sterilization method:

The study was conducted to evaluate the thermal stability of the product during autoclave. From the result the product was found stable up to 30 minutes autoclave at 121°C. The physiochemical results of 20 and 30 minutes autoclaved samples at 121°C and un-autoclaved samples are shown in **Table 19 & Fig 13.** From the results the assay value that are autoclaved in sterilization at 121° C was found to be stable when compared to unautoclaved sample. Therefore, moist heat sterilization method by using autoclave was selected for the process development.

## 10.6 Process compatibility study for Labetalol hydrochloride injection:

### *10.6.1 Compatibility with stainless steel:*

The hold time compatibility with stainless steel vessel was performed with before filtration and after filtration of the product solution. The analytical results were found to be within the proposed specification limit and no significant changes were observed during the total contact duration of about 48 hours. The results were shown in the **Table20-21 & Fig 14-15.** The result data indicates that the Labetalol hydrochloride injection solution was compatible with stainless steel vessel (SS316L).

### *10.6.2 Tubing compatibility study:*

The tubing compatibility was studied with 2 tubings tubing A (Sanitech) and tubing B (pharmapure). Both the tubings did not show any physical changes or discoloration at the end of the study. There were no significant changes were observed in other parameters analysed in both the tubings. The results were shown in the **Table 22-23 & Fig 16-17.** From the analytical results it was concluded that the product is stable with both tubings, but it was found to be more compatible with tubing B (pharmapure) than tubing A (sanitech) and therefore tube B is selected for the entire process of formulation development of labetalol hydrochloride injection.

### *10.7 Filter compatibility study:*

The filters used for this formulation were analysed for physiochemical tests and both the filters did not show any physical changes or discoloration at the end of the study. The results were shown in **Table 24-25 & Fig 18-19.** It was found that the filters did not show shredding or fibre generation. All the results for PES and PVDF filters were found within the specification limit. Assay for drug content was found to be more stable and compatible with PES filter than PVDF and hence PES filter was selected for the Labetalol hydrochloride injection.

## 10.8 Stress studies:

### *10.8.1 Oxygen sensitivity study:*

The oxygen sensitivity of the Labetalol hydrochloride injection was performed and the results of product with nitrogen and without nitrogen were shown in the **Table 26-27 & Fig 20-21.** The physiochemical results of the sample without nitrogen at stability were found to decrease when compared to the samples with nitrogen. From the results the injection sample was found

to be sensitive towards oxidation. And it was concluded that the formulations batches should be taken with nitrogen purging throughout the process.

***10.8.2 pH extreme study:***

The Labetalol hydrochloride injection solution was adjusted to pH around 3.0- 4.5 and the results were shown in the **Table 28-29 & Fig 22-23**. The results of the Labetalol hydrochloride injection solution with higher extremes and lower extremes of pH complies with all specification limits of the product. Based on the results the product pH limit shall be proposed between 3.0 and 4.5 during shelf-life of the product.

***10.8.3 Freeze thaw study:***

The freeze thaw study analytical results of Labetalol hydrochloride were shown in the **Table 30 & Fig 24**. And the results indicated that the product stability was not affected by the extreme temperature conditions of the drug product. The labetalol hydrochloride injection was stable at different temperatures. The product characteristics at the end of freeze thaw stress were found to be within the specification limits**.**

***10.8.4 Photo stability study:***

The assay of Labetalol hydrochloride drug content when exposed to light was found to be decreased when compared with injection packed along with the aluminium foil wrapped control vials and the vials placed in the carton. The results were shown in the **Table 31 & Fig 25**. And from the results, the Labetalol hydrochloride injection is sensitive towards light, and therefore the batches were finalized to formulate with dark control (monochromatic light) throughout the completion of formulation process.

**10.9 Quality control study for Labetalol hydrochloride injection:**

Finished product quality control study has been carried out for the Labetalol hydrochloride injection. The quality control determinations like description, pH, Particulate matters, sterility test, Clarity test, bacterial endotoxin test (LAL), assay of labetalol hydrochloride injection were carried out as per USP. The prepared formulation of Labetalol hydrochloride injection passes all the quality control tests and complies with USP standard and the results were tabulated in **Table 32.**

**10.10 Stability study:**

Labetalol hydrochloride injection did not show any significant change in the physiochemical parameters during stability studies up to 3 months at accelerated (40°C ±2°C/75%RH±5%RH) and in real time (25°C ±2°C/60%RH±5%RH), stability conditions. The results were shown in the **Table 33 & Fig 26 - 27.** The impurity profile of the drug product complies with the product specification limits. All the critical attributes for the drug products were satisfactory; the drug product was found comparable to the reference listed drug (RLD) Product. No additional peaks were observed in the product stored in contact with the stoppers.

There are no high-risk formulation aspects affecting the quality of the product, the pH of the formulation has been optimized and studied over the proposed pH range. The drug substance and excipients characteristics have been finalized to provide compliance to applicable guidelines.

# 11. Summary

**Development of Labetalol hydrochloride as a parenteral dosage form:**

Labetalol Hydrochloride Injection USP (Labetalol hydrochloride) is an adrenergic receptor blocking agent possessing both alpha1 (post-synaptic) and beta-receptor blocking activity. Its action on beta-receptors is four times stronger than that on alpha-receptors. It antagonizes beta1- and beta2-receptors equally. The ratios of alpha- to beta-blockade differ depending on the route of administration estimated to be 1:3 (oral) and 1:7 (IV). Onset of action- Oral: 20 minutes to 2 hours; IV: Within 5 minutes. Peak effect: Oral: 2 to 4 hours; IV: 5 to 15 minutes. Duration of action- Oral: 8 to 12 hours (dose dependent), IV: Average: 16 to 18 hours (dose dependent). Half-Life Elimination -Oral: 6 to 8 hours; IV: ~5.5 hours. The parenteral route of Labetalol hydrochloride is considered as the best choice of route than compared to the oral route.

In this introduction chapter discussed about parenteral dosage form, its significance, hypertension, mechanism of alpha beta blockers, advantages and disadvantages, different routes of administration, formulation of parenteral dosage form, evaluation of parenteral along with sterilization technique. And also briefly discussed about Preformulation studies of parenteral medications.

The literature related to this work was surveyed and a brief discussion had been given on each literature in this chapter.

The objective of the present formulation development was to develop a pharmaceutically acceptable, stable and reproducible generic formulation of Labetalol hydrochloride injection. The development studies were aimed at developing a drug product formulation matching the RLD (Reference listed drug) drug product characteristics and complying to the product characteristics listed in the USP monograph for of Labetalol hydrochloride injection.

The method chapter covers the details of experimental methods, including Preformulation study, compatibility study, stress study along with evaluation study and finally stability study.

The result chapter depicts the results for the all tests indicated in the method chapter. The results for all the parameter to be evaluated for the prepared of Labetalol hydrochloride injection and the Stability of the prepared formulation were given in this chapter.

The discussion chapters deal with the optimization of the process and four formulation trials were taken for the optimization of process variables. The best trial was considered for further batches. The tests included in the study were performed with optimized batch and for each test separate batches were taken and study was conducted. The compatibility study with the containers, filters and tubings were tabulated. The prepared formulation was subjected to stress study with oxygen sensitivity, pH extremities, freeze thaw and photo stability and the results were found to be within the specification limits.

Evaluation is the necessary step for parenteral and the solution to be injected should be free from any particulate matter to provide the sterile dosage form. The prepared injection provides all the compatibility for the quality control tests and found to be sterile. The prepared of Labetalol hydrochloride injection was assured for stability and it passes the stability criteria for that particular injection.

The samples were analysed after withdrawal of the sample from stability chamber and all the test parameters was carried out accordingly and the sample passes all the test criteria and the results was found to be within specification.

## 12. Conclusion

Labetalol is a unique parenteral that competitively blocks alpha- and beta-adrenergic receptors. The main objective of the study was to formulate a safe and stable Labetalol Hydrochloride Injection USP (labetalol hydrochloride) with the dose of 5 mg/ mL.

Drug, methyl paraben, propyl paraben, EDTA, dextrose, order of mixing was determined in pre-formulation development. Based on D-value, moist-heat sterilization method (121°C for 20 mins) is chosen for the developed injection formulation. The Process compatibility study reveals that injection potency and purity did not affected when exposed to stainless steel and process tubing. The filter compatibility study demonstrates that the Labetalol Hydrochloride Injection passes through filter without having drug loss due to binding of the drug to the membrane. Stability study of developed formulation conducted at Nitrogen purged environment, different pH, and various temperatures are tested over time for the amount of drug, methylparaben, propylparaben, EDTA, impurities, and particulate matter clearly indicated the drug product was stable. Labetalol Hydrochloride injection passes the entire quality control release test and there were no mechanical issues during the process. Thus, the product can be manufactured at a large scale.

## 13. Bibliography


1. Neha Gulati, Himanshu Gupta. Parenteral Drug Delivery: A Review. Recent Patents on Drug Delivery & Formulation ,2011 5(2), pp. 133-145.
2. Suresh Ramnath Naik. Advances in formulation and technology of sterile products: Nirali Prakashan. 2019. pp. 35
3. Schou. Subcutaneous and Intramuscular Injection of Drugs. Concepts in Biochemical Pharmacology, 1971. (28:47), pp. 67-72.
4. Sinny Delacroix. Hypertension: Pathophysiology and Treatment. Journal of Neurology & Neurophysiology November 25, 2014. pp.35
5. Lackland, DT; Weber, MA. Global burden of cardiovascular disease and stroke. Can J Cardiol. May 2015;31(5):569-71
6. Stella S. Daskalopoulou. Hypertension at the core. The Canadian journal of cardiology. May 2015.Vol 31 (5), pp 549–568
7. Mendis S, Puska P, Norrving B. Global Atlas on Cardiovascular disease and stroke: hypertension at the core. Can j Cardiol 2015 May:31 (5): 569-571.
8. Poulter NR, Prabhakaran D, Caulfield M. Hypertension. Lancet. August 2015; 386(9995):801-812.
9. James PA. Evidence-Based Guideline for the Management of High Blood Pressure in Adult: report from the Panel Members Appointed to the Eight Joint National Committee (JNC 8). JAMA. Dec 2013; Vol 311(5).507-520.
10. Law M,Wald N. Lowering blood pressure to prevent myocardial infraction and stroke: a new preventive strategy. Health technol assess 2003; 7(31).1-94
11. Ostchega Y, Dillon CF, Hughes JP, Carroll M, Yoon S. Trends in hypertension prevalence, awareness, treatment, and control in order U.S. adults: data from the National Health and Nutrition Examination Survey 1988 to 2004. J Am Geriatr soc 2007 July; 55(7): 1056-65.
12. Gavin WK Wong and Alexandra Laugerotte, Blood pressure lowering efficacy of dual alpha and beta blockers for primary hypertension, Cochrane Database Syst Rev; 2015 vol 5(8).

13. Indian pharmacopoeia. vol 2. (ed.8) Ministry of Health & Family welfare, Ghaziabad: Parenteral preparations, 2018. pp. 2365.

14. The United States pharmacopeia. National formulary- Vol 1. Rockville (MD): United States Pharmacopeial convention, 2006. Injections; pp.895.

15. Avella Specialty Pharmacy infusion, Available at: www.avella.com, (Accessed: Jan 2019)

16. PV Swamy, P Sushma, G Chirag, K Prasad, M Younus Ali, SA Raju. Parenteral formulation of zopiclone. Ind J Pharm sci 2008:70(10:99- 102).

17. R Gopinath. Pharmaceutical Preformulation Studies – Current Review. International Journal of Pharmaceutical & Biological Archives 2011; 2(5):1391-1400.

18. Lighthouse Instruments (2019) in-process monitoring of headspace oxygen levels in parenteral containers., Available at: https://www.pharmaceuticalonline.com, (Accessed: Aug 2019).

19. The United States pharmacopeia. National formulary- Vol 1. Rockville (MD): United States Pharmacopeial convention, 2005. Terminally Sterilized Pharmaceutical Products; pp. 2746.

20. Michael J.Akers. Excipient–drug interactions in parenteral formulations. Journal of pharmaceutical sciences, Nov 2002; vol 91(11), 2283-2300.

21. Patel P, Ahir K, Patel V. Drug-Excipient compatibility studies: First step for dosage form development". The Pharma Innovation Journal 4(5): 14-20 (2015).

22. Thorsteinn Loftsson. Drug Degradation, International Journal of Pharmaceutics. Vol 480 (1-2), 1 March 2015, pp 48-54.

23. Anu Kaushik, Vivek Chauhan. Recent review on parenteral products- sterile dosage form. IJARIIE. 2017. Vol-3 (1) 2395-4396.

24. Herbert A Lieberman, Lachman L. Pharmaceutical dosage forms: Parenteral medications, 3rd ed. Marcel Decker. 2010

25. The United States pharmacopeia. National formulary- Vol 1. Rockville (MD): United States Pharmacopeial convention, 2018. Pharmaceutical compounding of sterile preparations.; pp. 2536.

*26.* Thomas Sneha. Formulation and evaluation of an Injectable solution as a dosage form. Journal of Drug Delivery & Therapeutics. 2018; 8(5):81-87

27. Jason P Hecht, Safety of high-dose intravenous labetalol in hypertensive crisis. American Journal of Health-System Pharmacy, 1 March 2019. 76(5), pp 286–292.
28. F. A. Marzook, Preparation of $^{188}$Re-Labetalol as a $\beta_1$-Adrenoceptor for Use in Nuclear Medicine. Springer link, March 2019.61 (2), pp 238–243.
29. Fransway, Anthony F. Paraben toxicity, Dermatitis. 2019 Jan/Feb;30(1):32-45.
30. Kabirdas Ghorpade and Sharda Shinde, Particulate matter in parenterals: a review, World Journal of Pharmaceutical Research, 2019. 8(7), pp 394- 398,
31. Neha Tiwari, Development and validation of RP-HPLC method for simultaneous determination of paraben preservatives in pharmaceutical liquid dosage, Journal of pharmacognosy and phytochemistry, 2018.7(2). pp 1243-1245.
32. Mariani A.Ciciliati.Investigation of the thermal behaviour of Labetalol. Science direct, 10 October 2018. 668(10), pp 33-39,
33. PabloZambrano, α1-and β-adrenergic antagonist labetalol induces morphological changes in human erythrocytes, Biochemical and Biophysical Research Communications, 3 September 2018. 503(1), pp 209-214,
34. Akter FA. Response & Side Effects of Injectable Labetalol in Pregnancy Induced Severe Hypertension. Mymensingh Med J. 2017 Jul;26(3):608-613.
35. Raju Sudhakar, A comparative study of intravenous labetalol with oral antihypertensive combination nifedipine and alpha-methyldopa in the acute management of high blood pressure in severe pregnancy-induced hypertension patients, Journal of Evolution of Medical and Dental Sciences, Sept. 1, 20165 (70), pp 638 - 645.
36. Raju Prasad Tayung, Effect of intravenous labetalol in controlling the cardiovascular response to laryngoscopy and intubation: a comparison with intravenous lignocaine. Journal of Evolution of Medical and Dental Sciences, Aug 8 2016.Vol. 4 (2), pp 104 – 111.
37. Verma S. Inadvertent intrathecal injection of labetalol. Pubmed. Saudi J Anaesth. 2016 Jul-Sep; Vol.10(3) pp.345–346.
38. Thomas, Safety and efficacy of intravenous labetalol for hypertensive crisis in infants and small children, Pediatric Critical Care Medicine. January 2011.Vol 12 (1) - pp 28-32.

39. Samir Fahed, Labetalol infusion for refractory hypertension causing severe hypotension and bradycardia: an issue of patient safety, Patient Safety Surgery. 2008 May. Vol. 27. 2(13), pp 231 – 245.

40. Sofuoglu M. Labetalol treatment enhances the attenuation of tobacco withdrawal symptoms by nicotine in abstinent smokers. Nicotine Tob Res. 2003 Dec. Vol.5(6):947-53.

41. SOishi. Effects of propyl paraben on the male reproductive system, Food and Chemical Toxicology, Food Chem Toxicol. 2002 Dec. Vol.40(12):1807-13.

42. Yeleswaram K. Transplacental and nonplacental clearances, metabolism and pharmacodynamics of labetalol in the fetal lamb after direct intravenous administration. J Pharmacol Exp Ther, Oct 1993. Vol.267(1): pp 425-31.

*43.* John A. Clark, Labetalol Hepatotoxicity, Annals of Internal Medicine*;* 1990. Vol.113(3): pp 210-213.

44. G.Krumpl, Antiarrhythmic efficacy of labetalol as assessed by programmed electrical stimulation, British Pharmacological Society,1990.Vol.100(4), pp 855 – 861.

45. James Huey, Clinical Evaluation of Intravenous Labetalol for the Treatment of Hypertensive Urgency. American Journal of Hypertension, July 1988 1(3), Pages 284S–289S.

46. R. M. Bojar M.D., Intravenous Labetalol for the Control of Hypertension Following Repair of Coarctation of the Aorta, Clin Cardiol. 1988 Sep;11(9):639-41.

47. Blair P. Grubb, Intravenous Labetalol in Acute Aortic Dissection, JAMA networks1987. Vol. 258(1): pp 78-79.

48. M Lebel , Labetalol infusion in hypertensive emergencies, American Society for Clinical Pharmacology and Therapeutics, June 1985.Vol. 37(6), pp 615 – 618.

49. Lund-Johansen P, Pharmacology of combined alpha-beta-blockade. II. Haemodynamic effects of labetalol. Drugs. 1984. Vol.28(2). pp:35-50..

50. E. Paul MacCarthy, Labetalol: A Review of Its Pharmacology, Pharmacokinetics, Clinical Uses and Adverse Effects, American College of Clinical Pharmacy, July-August 1983.Vol.3(4), pp 193 – 217.

51. Pul-Ho C. Yuen, Compatibility and Stability of Labetalol Hydrochloride in Commonly Used Intravenous Solutions, American Journal of Hospital Pharmacy, 01 June 1983.Vol. 40(6), pp 1007–1009.

52. John D. Wallin, Labetalol, Current Research and Therapeutic Status, Arch Intern Med. 1983. Vol.143(3); pp 485-490.

53. JA Kane. Labetalol in general practice: a review, The British Pharmacological Society, February 1982. vol 13. Pp. 59S-63S,.

54. R. C. Dage. Direct vasodilatation by Labetalol in anaesthetized dogs. Br J Pharmacol, Oct 1980. vol 2, pp. 287–293.

55. CA Michael, Use of labetalol in the treatment of severe hypertension during pregnancy. British pharmacology society, April 1979 Vol.8(S2), pp 211S – 215S.

56. L Kaufman. Use of labetalol during hypotensive anaesthesia and in the management of pheochromocytoma. The British Pharmacological Society. April 1979. Vol8(S2), pp 229S-232S.

57. The National Centre for Biotechnology Information. Labetalol hydrochloride, Available at https://pubchem.ncbi.nlm.nih.gov (Accessed:15th Feb 2019).

58. Drugs.com. Labetalol hydrochloride, Available at: https://www.drugs.com/. (Accessed: 23rd Feb.2019).

59. Raymond C Rowe, Paul J Sheskey, Marian E Quinn (2009) Handbook of pharmaceutical Excipients, 6 edn., London: Pharmaceutical press, pp 546.

60. Raymond C Rowe, Paul J Sheskey, Marian E Quinn (2009) Handbook of pharmaceutical Excipients, 6 edn., London: Pharmaceutical press, pp 632.

61. Raymond C Rowe, Paul J Sheskey, Marian E Quinn (2009) Handbook of pharmaceutical Excipients, 6 edn., London: Pharmaceutical press, pp 369.

62. The United States pharmacopeia. National formulary- Vol 2. Rockville (MD): United States Pharmacopeial convention, 2016. Labetalol hydrochloride; pp. 4268.

63. The United States pharmacopeia. National formulary- Vol 1. Rockville (MD): United States Pharmacopeial convention, 2018. Containers-glass; pp. 554.

64. The United States pharmacopeia. National formulary- Vol 1. Rockville (MD): United States Pharmacopeial convention, 2018. Elastomeric closures used in Injectable dosage forms; pp. 326.

65. AR. Shabaraya, K. Aiswarya. Formulation and Evaluation of Mucoadhesive Bi-layer Buccal Tablets of Labetalol Hydrochloride Using Natural Polymers. International journal of advances in pharmacy. Biology and chemistry, Sep, 2012. Vol. 1(3), pp. 308.

66. The United States pharmacopeia. National formulary- Vol 1. Rockville (MD): United States Pharmacopeial convention, 2016. Injection and implanted drug products - product quality test; pp. 455.