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FORMULATION DEVELOPMENT OF ANTIHYPERTENSIVE DRUGLABETALOL HCl INJECTION

H. Padmalatha*, G. Shirisha, P.S. Vasahi Laxmi, K. Manasa, M. Sravan Kumar, Y. Madhu and N. Jyothi Reddy

Gyana Jyothi College of Pharmacy Gyana Jyothi Nagar, Uppal, Hyderabad, Telangana.

***Corresponding Author: H. Padmalatha**

Gyana Jyothi College of Pharmacy Gyana Jyothi Nagar, Uppal, Hyderabad, Telangana.

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ABSTRACT

The aim of the present study to develop a pharmaceutically acceptable, stable and reproducible generic formulation of labetalol hydrochloride injection. 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.

INTRODUCTION

Long-acting formulations (LAFs) are used for pharmacotherapy as sustained-release medications over a period of several days, weeks, or even months. Compared to conventional preparations, LAFs have many distinguished advantages related to its long-lasting curative effect, as well as its reduced toxicity, dosage and frequency of administration. These outstanding features of LAFs have encouraged researchers to pursue their further development to fulfill the unmet need for long-term treatments of chronic diseases or other prevalent diseases that threaten human health.

Patients are often forced to take daily prescriptions of medicine for years to treat chronic diseases or other serious diseases such as HIV/AIDS, psychiatric illnesses, cancer, and diabetes. However, most patients often cannot adhere to a frequent and prolonged dosing schedule. For the treatment of these conditions, long-acting parenteral formulations (LAPFs) are preferred over conventional formulations. The release duration of drug delivery conferred by LAPFs may be able to improve patient adherence and consequently improve treatment outcomes. The replacement of daily oral regimens with antiviral LAPFs could successfully improve HIV/AIDS prevention and treatment. A single subcutaneous (sc) implantation of antiviral LAPFs can provide a protective level of drug concentration for months, a year or even longer.[1] Besides AIDS medication, long-acting innovative anti-infective drugs have proven effective to eliminate the hepatitis B virus and chronic hepatitis C virus.[2,3] Similarly, long-acting antipsychotics formulations can effectively reduce relapse and re-admission to psychiatric institutions and improve long-term prognosis due to their consistent plasma drug concentration.[4,5] Furthermore, as the prevalence of diabetes is a growing problem worldwide, reaching the target blood glucose level is critical for the vast majority of diabetic patients, as their improper management of glycemia can lead to life-threatening adverse effects.[6] Currently, clinicians have many long-acting basal insulins to select from,[7] but there are still some unmet needs and challenges. Daily variability, low patient compliance and progressive micro and macrovascular risks require further research to formulate novel LAPFs for diabetic patients.[6] In addition to the treatment of above-mentioned diseases, LAPFs also have proven effective for contraception. Long-acting reversible contraceptives are recommended as first-line contraceptives for adolescents and young women.[8] Long-acting hormonal contraceptives with great efficacy and relative ease of use are widely accepted by millions of women with a variety of options9, 10, 11. Although great progress has been made in the development of

LAPFs to meet clinical needs, it is still far from ideal. All of these indicate that LAPFs are very promising and have market demand.

Presently, small molecules share almost 90% of the global pharmaceutical market and are still a leading in new drug approvals. However, some small molecular drugs require multiple doses in a day owing to its short biological half-life, which is due to its structure, as well as other characteristics. Therefore, the major issues of these drugs are their large fluctuations in blood concentration and the risk of potential side effects. These factors limit their efficacy and clinical use. So far, many efforts have been made to improve the pharmacokinetics of these drugs and some progress has been made, such as PEGylation of methotrexate[12] or making it into albumin-based nanomedicines[13,14] and making vincristine into injectable sulfate loaded dextran microspheres amalgamated with chitosan-*β*-glycerophosphate gel to achieve long-term effectiveness[15,16] Furthermore, combining small molecular drugs with ligands (such as long-chain fatty acids) or albumin prolongs the half-life of the drug. Currently, a greater number of researchers are turning their attention to macromolecular drugs that exert specific therapeutic effects and occupy an increasing market share. However, most of these drugs express poor stability, easy enzymes degradation, and rapid kidney clearance. To achieve therapeutic effects, long-term, frequent, or high-dose administrations are required through injection, which are accompanied by injection-associated pain, discomfort, certain psychological and economic burden. Encouragingly, significant attention and effort have been dedicated to the design and development of LAPFs of macromolecular drugs to keep them active for longer time in the body and improve their pharmacokinetics and therapeutic efficacy.[17,18]

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 excipientsto 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.

MATERIALS

Table 1: Drugs, excipients and materials used in the experiment.

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

METHODS

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 $C_{19}H_{24}N_2O_3.HCl$.

Preformulation study

Evaluation study for Containers and closure

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 thesame 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 servedas 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 thevessel.

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.

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 for16 hours. Using a hypodermic needle as described above fitted to a clean syringe, intoeach 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 tobe 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 asolution 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.

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 withthat of excipients was studied.

Formulation development of labetalol hydrochloride injection

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. Timetaken for the solubilisation of drug was noted and the results were shown in the **table 17.**

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 trialbatch was analysed for physical parameters, pH, Assay of drug content, excipient content andrelated substances. The results were shown in the **table 18.**

Order of addition: (API – Labetalol hydrochloride)

Trail 1: Methylparaben + Propylparaben »» Dextrose »» Edetate disodium »» APITrail 2: Methylparaben + Propylparaben »» API »» Dextrose »» Edetate disodiumTrail 3: Methylparaben + Propylparaben »» Edetate disodium »» Dextrose »» APITrail 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 edetatedisodium.
- 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.

Fill volume determination

Each container of an Injection is filled with a volume in slight excess of the labelled ‘‘size’’ orthat 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 meansof opening them and emptying the contents directly into the graduated cylinder.

Table 2: Recommended excess volume.

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 %

Formulation process of labetalol hydrochloride injection

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.

Preparation of 1 N Sodium hydroxide solutions

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

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 minutesat 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 for15 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 with20 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.

Table 3: Drug product formula for labetalol hydrochloride injection.

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

Process development

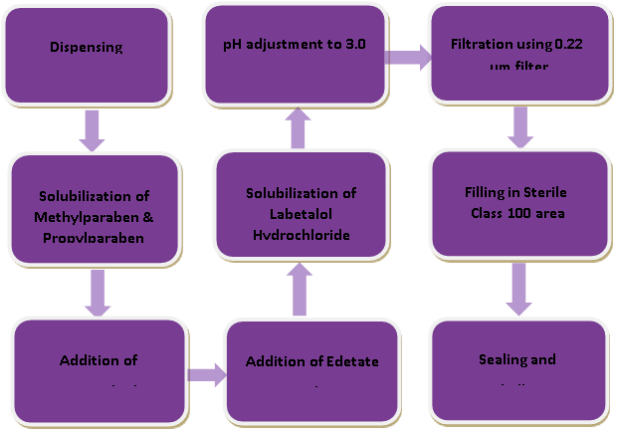

Fig. 1: Manufacturing schematic representation of labetalol hydrochloride.

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**.

Hold time compatibility study for labetalol hydrochloride injection

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.

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.

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 weresoaked 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.

Stress studies

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.

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

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 thedrug 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.

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 sampleserved 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. Thetotal 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.

Quality control study for labetalol hydrochloride injection

Description

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

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

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 setin 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.

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.

Bacterial endotoxin test or LAL test
The LAL (limulus amoebocyte lysate) testing, also known as bacterial endotoxin testing, isan *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

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.

Assay of labetalol hydrochloride injection
About 50 mg of labetalol hydrochloride is mixed with 100 mL of water. The 10 mL of solutionis 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.

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.

RESULTS

Raw material analysis of labetalol hydrochloride
Labetalol hydrochloride drug sample was analysed for various physical and analyticalcharacterizations and was found to comply with USP.

Physiochemical characters of Labetalol hydrochloride
Table 4: Physiochemical characteristics of Labetalol hydrochloride.

S. No.	Test	Results
1	Appearance	White or almost white powder
2	Melting range	About 180^o C
3	Solubility Sparingly solubleInsoluble	in water and in ethanol (96%) in ether and in methylene chloride

DISCUSSION

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 administrationestimated 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.

Raw material analysis of labetalol hydrochloride

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 foundto be 180° C.

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 foundto be 99.2%**.**

Incompatibility study

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**.** 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.

Evaluation study for containers and closure

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 withinthe 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.

Evaluation of closures

Evaluation of closure was done with the rubber closure (bromobutyl rubber). The results showthat 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.

Preformulation study

Determination of RPM

The API (Labetalol hydrochloride) was found to be solubilised easily at 600 rpm. 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.

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 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.

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**.** 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.

Process compatibility study for Labetalol hydrochloride injection

Compatibility with stainless steel

The hold time compatibility with stainless steel vessel was performed with before filtration andafter 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 result data indicates that the Labetalol hydrochloride injection solution was compatible with stainlesssteel vessel (SS316L).

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 inboth the tubings. From the analyticalresults 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.

Filter compatibility study

The filters used for this formulation were analysed for physiochemical tests and both the filtersdid not show any physical changes or discoloration at the end of the study. 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 thanPVDF and hence PES filter was selected for the Labetalol hydrochloride injection.

Stress studies

Oxygen sensitivity study

The oxygen sensitivity of the Labetalol hydrochloride injection was performed and the results of product with nitrogen**.** The physiochemical results of the sample without nitrogen at stability were found to decreasewhen 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.

The Labetalol hydrochloride injection solution was adjusted to pH around 3.0- 4.5. 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.

Freeze thaw study

And the results indicated that the product stability was not affected by the extremetemperature 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 foundto be within the specification limits**.**

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 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.

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. T

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 impurity profile of the drug product complies with the product specification limits. All the critical attributes for the drug products were satisfactory; the drugproduct 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.

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 finallystability 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 tobe 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 allthe test criteria and the results was found to be within specification.

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.

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