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Formulation and Development of Vancomycin hydrochloride for injection

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ABSTRACT

The aim of present study is to formulate an intravenous injection of Vancomycin Hydrochloride. It was belongs to the category of antibiotic and it was widely used in staphylococcal endocarditis. The main objective of the study was to develop rapid reconstituted formulation of Vancomycin Hydrochloride by using lyophilization technology. The lyophilization was carried in different batches of the Vancomycin Hydrochloride were prepared with varying the excipients and its concentration while keeping the lyophilization cycle constant. An optimized lyophilization cycle of 36 hours was achieved. The optimized lyophilized product was subjected to in vitro parameters such as cake appearance, reconstitution time, PH, assay, RS, particulate matter, water content, IR, color value, % light transmittance, DSC. After considering all product characteristics batch-6 was considered as an optimized formulation. All the in-vitro evaluation parameters compiled the limits as per the specification of USP. Scale up batch was done by increasing the batch size. Accelerated stability studies were also conducted and from the result it was concluded that the scale up formulation was found to be stable. Finally, it was concluded that the lyophilization is a suitable technique to increase reconstitution the Vancomycin Hydrochloride of injection (500 mg/vial).

Key Words: Staphylococcal endocarditis, Lyophilization technology.

1. INTRODUCTION

1.1 Parenteral Dosage Form

Parenteral derived from two words: Para-other than; enteron-intestine meaning to avoid intestine1. "Parenteral are Injectable preparations, sterile products intended for administration by injection, infusion or implantation in to the body."

Parenteral should be free of physical, chemical and biological contamination. Parenteral route is the best, when oral route is not suitable.

1.1.1. Advantages of Parenteral Dosage forms

- Rapid and reliable systemic effects.
- Long term drug delivery.
- Targeted Drug Delivery.

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1.2.2. Disadvantages of Parenteral Dosage forms

- Potential for infection at the site of injection and thrombosis.
- Tissue damage and/or pain upon injection.
- Use of specific requirements for specific equipment, devices and techniques.

1.3.3. Routes of administration

- Intravenous
- Intramuscular
- Subcutaneous
- Intradermal
- Epidural
- Intra-articular
- Intrathecal

1.4.4. Types

Based on the volume they are classified as:

Small Volume Parenterals (Injections): These are supplied in single or multiple doses. The volume is generally less than or equal to 100 ml.

Large Volume Parenterals: These are supplied for single dose having more than 100 ml. These are delivered through IV route. These generally provide electrolytes, nutrition to the body.

1.5.5. Classification

- Drug Injection: It is a liquid preparation consisting of drug.
- Drug for Injection: The drug is present in the form of powder and solvent is added to form solution that has the properties of injection.
- Drug Injectable Suspensions: As the name suggests, the drug particles are suspended in suitable vehicle

1.6.6. Ideal Properties of Parenterals

- Every ingredient must be free from microorganisms and pyrogenic materials.
- Almost all the parenterals should be isotonic with body fluids, and other parenterals should be near to isotonicity.

1.2 LYOPHILIZATION

Lyophilization, also known as Freeze-drying or cryodesiccation. The word is derived from Greek, and means "made solvent-loving". Freeze drying as a practical commercial process was introduced around the time of Second World War, and found its first application in preservation of blood plasma. Soon after World War II, the pharmaceutical industry began considering the process for the preparation of the sterile injectable dosage forms, which could not be formulated into stable solutions [2].

Lyophilization is a way of drying something that minimizes damage to its internal structure. The objective in a freeze-drying process is to convert most of the water into ice in the freezing stage, remove the ice by direct sublimation in the primary drying stage, and finally remove most of the unfrozen water in the secondary drying stage by desorption. The water removed from the product is reconverted into ice by the condenser.

Lyophilization gives unstable chemical solutions a long shelf life when they are stored at room temperature. This process gives product excellent solubility characteristics, allowing for rapid reconstitution. Heat and moisture-sensitive compounds retain their viability. Lyophilization ensures maximum retention of biological and chemical purity [3].

1.2.1 Applications of freeze drying

- It is applicable in preservation of blood plasma, and manufacture of penicillin and other antibiotics.
- It is applicable to manufacture of pharmaceuticals, vaccines, steroids, vitamins, Enzymes, biological, serums and hormones.
- It is applicable to wide range of diagnostic products which are thermo labile or otherwise unstable on aqueous solutions for prolonged storage periods, but which are stable in dry state.

1.2.2 Desired characteristics of Freeze-Dried Products

- Intact cake
- Sufficient cake strength
- Uniform color
- Sufficiently dry cake
- Sufficiently porous cake
- Sterile
- Chemically stable

1.2.3 Key benefits

- Rapid, aseptic dispensing into vials, tubes, or bulk trays
- Sealing under vacuum
- Functional preservation
- Longer shelf lifes

2. Materials

The API Vancomycin Hydrochloride is used for the study. The excipients used are Ethyl Alcohol, Mannitol, Polyethylene Glycol 400 and Cyclodextrin.

2.1. Material and sources

Refer Table 1 in page 291

2.2. List of Instruments used

Refer Table 2 in page 291

2.3. Preformulation Study on Vancomycin hydrochloride

Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing optimum drug delivery system.

Prior to the development of dosage form with drug candidate, it is essential to determine certain fundamental physical and chemical properties of drug molecule and other derived properties of the powder. This information will be useful in developing a formulation and this learning phase is known as preformulation.

The main aim of pre formulation study is to ascertain that the drug substance complies with the pharmacopoeias standards.

The following studies were conducted on the API during the Preformulation stage-

- Description
- Solubility
- Identification
- Appearance of solution
- pH

- Water content
- Chromatographic purity
- Related substances
- Heavy metals
- Bacterial endotoxins
- Assay

Description
The powder was visually assessed.

Solubility:
The solubility study was carried out for API in distilled water and other solvents by adding excess amount of the drug to the flask containing solvents and subjected for stirring.

Identification:
Transfer about small quantity of KBr into agate mortar, triturate evenly and perform as the blank disc. Triturate about 3 mg of Vancomycin Hydrochloride into powdered 300 mg of KBr evenly to again a blank disc. Scan blank flake and sample flake and record the Chromatogram.
Identification was done by Jasco FTIR spectrometer. The Vancomycin Hydrochloride sample spectrum of was compared with standard spectrum of Vancomycin Hydrochloride.

pH:
500 mg of sample was weighed and dissolve in 10 ml of water. The pH of the solution of Vancomycin Hydrochloride is measured and recorded.

Water content (by Karl Fisher):
Karl Fisher titration is used as an analytical method for quantifying water content in the drug; methanol was used as a solvent.

$$\text{Formula} = \frac{\text{Volume Consumed} \times \text{KF} \times 100}{\text{Weight taken (mg)}}$$

Assay:
Vancomycin Hydrochloride assay was done by using microbiology.

Related Substance:
The Related Substance was determined by HPLC. The unknown impurity and total impurity was determined and recorded.

DSC:
Glass transition temperature was determined by DSC.

Procedure:
300mg of sample was weighed and prepared a pellet. Both reference and sample pellets were placed in the Differential scanning calorimeter. In this sample was frozen at a temperature - 40°C at the rate of 2°C/min and increase the temperature upto 25°C at the rate of 1°C/min and the DSC was recorded.

2.4. Drug – Excipients Qualitative Formula:
Refer Table 3 in page 292

2.5. FORMULATION TRIALS OF VANCOMYCIN HYDROCHLORIDE FOR INJECTION:

Potency calculation for API
Conversion factor = Mol. weight of Vancomycin Hydrochloride/ Mol. weight of Vancomycin
= 1485.74/1449.5
= 1.025Quantity required for the batch
= Actual Quantity×1.025
Strength =500mg/vial
Refer Table 4 in page 292

Potency calculation for API
Conversion factor = Mol. weight of Vancomycin Hydrochloride/ Mol. weight of Vancomycin
= 1485.74/1449.5
= 1.025
Quantity required for the batch = Actual Quantity×1.025
Strength =500mg/vial

2.6. METHOD OF PREPARATION:
Entire manufacturing process was carried out under Aseptic conditions which include washing and sterilization of vials, lyostoppers, and Aluminium seals. The vials were filled in class 100 laminar air chamber.

2.6.1. TRAIL NO.1:

Objective:
To check the stability of Vancomycin Hydrochloride in water about 48 hrs.

Method of Preparation:
- 13.325 g of Vancomycin Hydrochloride was accurately weighed.
- 40 ml of WFI was taken in a beaker and it is cooled to 30°C and then API was added slowly and stirred continuously until clear solution was formed by using magnetic stirrer.
- Then finally make up the volume up to 50 ml by using WFI and stability of solution observed for about 48 hrs.

Conclusion:
This formulation was not stable and gel formation is observed after 24 hrs.

2.6.2. TRAIL NO.2:

Objective:
To stabilize the Vancomycin Hydrochloride solution by using Alcohol.

Method of Preparation:
- 13.325 g of Vancomycin Hydrochloride was

accurately weighed.
- 40 ml of WFI was taken in a beaker and it is cooled to 30°C and then to this alcohol was added and stirred for 10 min.
- Then API was added slowly and stirred continuously until clear solution was formed.
- Then finally make up the volume up to 50 ml by using WFI and stability of solution observed for about 48 hrs.

Conclusion:

This formulation was stable and no gel formation was observed after 24 hrs.

2.6.3. TRAIL NO.3:

Objective:

To lyophilize the stabilized formulation of Vancomycin Hydrochloride by incorporating bulking agent Mannitol.

Method of Preparation:

- 13.325 g of Vancomycin Hydrochloride was accurately weighed.
- 90% of WFI of total batch size was taken in a beaker and it is cooled to 30°C and then to this alcohol was added and stirred for 10 min.
- Mannitol was added and stirred until clear solution is formed.
- Then API was added slowly and stirred continuously until clear solution was formed.
- Then make up the volume to 95 % of total batch size with WFI and PH of the solution was checked and it was found to be 3.15.
- Then PH was adjusted to 2.81 with 1N HCl.
- Then finally make up the volume up to 100 ml by using WFI and filtered through sterilized 0.22µ membrane filter.
- That solution is transferred to the USP Type I tubular flint glass vials and the fill volume is 3.75ml i.e. 500 mg/vial.
- Then the filled vials were partially stoppered and loaded into Lyophilizer.
- As the lyophilization cycle is completed, the rubber plugs were stoppered under Nitrogen and sealed with Aluminum seals and stored at suitable temperature.

Conclusion:

Reconstitution time of lyophilized formulation was found to be 2 min.

2.6.4. TRAIL NO.4:

Objective:

To decrease the reconstitution time of formulation by using reconstitution enhancer Cyclodextrin.

Method of Preparation:

- 13.325 g of Vancomycin Hydrochloride was accurately weighed.
- 90% of WFI of total batch size was taken in a beaker and it is cooled to 30°C and then Cyclodextrin was added and stirred for 10 min until clear solution is formed
- Then to this alcohol was added and stirred for 10 min.
- Mannitol was added and stirred until clear solution is formed.
- Then API was added slowly and stirred continuously until clear solution was formed.
- Then make up the volume to 95 % of total batch size with WFI and PH of the solution was checked and it was found to be 3.25.
- Then PH was adjusted to 2.75 with 1N HCl.
- Then finally make up the volume up to 100 ml by using WFI and filtered through sterilized 0.22µ membrane filter.
- That solution is transferred to the USP Type I tubular flint glass vials and the fill volume is 3.75ml i.e. 500 mg/vial.
- Then the filled vials were partially stoppered and loaded into Lyophilizer.
- As the lyophilization cycle is completed, the rubber plugs were stoppered under Nitrogen and sealed with Aluminum seals and stored at suitable temperature.

Conclusion:

Reconstitution time of lyophilized formulation was found to be 45-50 sec.

2.6.5. TRAIL NO. 5:

Objective:

To decrease the reconstitution time of formulation by using reconstitution enhancer PEG-400.

Method of Preparation:

- 13.325 g of Vancomycin Hydrochloride was accurately weighed.
- 90% of WFI of total batch size was taken in a beaker and it is cooled to 30°C and then Poly ethylene glycol (6%) was added and stirred for 10 min until clear solution is formed.
- Then to this alcohol (4%) was added and stirred for 10 min.
- Mannitol was added and stirred until clear solution is formed.
- Then API was added slowly and stirred continuously until clear solution was formed.
- Then make up the volume to 95% of total batch size with WFI and PH of the solution was checked and it was found to be 3.25.
- Then PH was adjusted to 2.75 with 1N HCl.

- Then finally make up the volume up to 100 ml by using WFI and filtered through sterilized 0.22µ membrane filter.
- That solution is transferred to the USP Type I tubular flint glass vials and the fill volume is 3.75ml i.e. 500 mg/vial.
- Then the filled vials were partially stoppered and loaded into Lyophilizer.
- As the lyophilization cycle is completed, the rubber plugs were stoppered under Nitrogen and sealed with Aluminum seals and stored at suitable temperature.

Conclusion:

Reconstitution time of lyophilized formulation was found to be 10-15sec.

2.6.6. Trail No.6 : (Optimization Batch)

Objective:

To decrease reconstitution time of formulation by decreasing the concentration Vancomycin Hydrochloride.

Method of Preparation:

- 12.3gms of Vancomycin Hydrochloride was accurately weighed.
- 90% of WFI of total batch size was taken in a beaker and it is cooled to 30°C.
- Then Poly ethylene glycol (3%) was added and stirred for 10 min until clear solution is formed.
- Then to this alcohol (2%) was added and stirred for 10 min.
- Mannitol was added and stirred until clear solution is formed.
- Then API was added slowly and stirred continuously until clear solution was formed.
- Then make up the volume to 95 % of total batch size with WFI and PH of the solution was checked and it was found to be 3.25.
- Then PH was adjusted to 2.85 with 1N HCl.
- Then finally make up the volume up to 100 ml by using WFI and filtered through sterilized 0.22µ membrane filter.
- That solution is transferred to the USP Type I tubular flint glass vials and the fill volume is 4.06ml i.e. 500 mg/vial.
- Then the filled vials were partially stoppered and loaded into Lyophilizer.
- As the lyophilization cycle is completed, the rubber plugs were stoppered under Nitrogen and sealed with Aluminum seals and stored at suitable temperature.

Conclusion:

Reconstitution time of lyophilized formulation was found to be 5-6sec.

2.7. SCALE UP BATCH:

Objective:

To check the feasibility of the batch in large scale by increasing the batch size.

Batch Size: 5 L

Refer Table 4 in page 292

Method of preparation:

Scale up batch method of preparation is same as that of Trial 6 (optimization batch).

Conclusion:

Vancomycin Hydrochloride rapid reconstituted formulation manufacturing in large scale was found to be feasible.

Lyophilization Cycle 1 for Trials 1-5

Refer table no 5 in page 292

Lyophilization Cycle 2 for Trial 6 & Scale up Batch

Refer table no 6 in page 292

Freezing time: 10.08h (605min)

Primary Drying time: 21.25h (1275 min), Pressure: 0.15mbar

Secondary Drying time: 5.58 h (335min), Pressure: 0.08mbar

Total Duration: 36.91 h

2.8. Stability Study:

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 through out 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.

The International Congress of Harmonization titled 'Stability testing of a new drug substances and product' describes test requirements for the drug registration application in the European Union, Japan and USA.

ICH specific length of study and storage

- Long term stability studies: 25±5 °C /60±5% RH for 12 months.
- Accelerated stability studies: 40°C±2°C/75%±5% RH for 6 months.

Accelerated testing studies were designed to increase the rate of chemical or physical degradation of the drug substance/product by using exaggerated storage conditions as per USFDA, and a rapid detection of deterioration of the drug in different formulations can be known in short time. The stability studies were carried out as per ICH guidelines. The Accelerated study was carried at the temperatures of 40°C±2°C/75%±5% RH and the sample was withdrawn at one month interval

and analyzed for evaluation parameters such as assay, pH and loss on drying, reconstitution time, color value, and particulate matter [32].

2.9. Physiological Compatibility Study:

Objective:

To know the compatibility of reconstituted formulation in different physiological solutions like Ringer Lactate, 0.9%NaCl, 5% Dextrose.

Preparation of Reconstituted Solution:

- All the vials were collected from the batch and reconstituted each vial with 100 ml of different physiological solutions Ringer Lactate 0.9% NaCl and 5% Dextrose respectively.
- Those filled vials were kept for stability study in different condition according to table 6 in page 292

At regular time points the samples are analyzed for the following:

- Description, clarity & color of solution
- PH
- Chromatographic purity
- Limit of Monodechlorovancomycin
- Related substance
- Assay by microbiology

2.9.1. EVALUATION OF LYOPHILIZED PRODUCT:

The lyophilized product was evaluated for the following formulation characteristics-

- Description
- Reconstitution time
- pH
- water content
- Identification by IR
- Particulate matter
- Color value
- Light transmission
- Vancomycin B
- Assay
- Related Substance

2.9.2. ASSAY:

Glassware required:

i. Roux bottle
ii. 250 ml conical flask
iii. Pipettes
iv. Test tubes with test tube stand
v. Petri plates (90 mm)

Reagents

i. Potassium dihydrogen orthophosphate - AR grade
ii. Sodium hydroxide - AR grade

Reagent preparation

Buffer PH 8: 50.0ml of 0.2M potassium dihydrogen orthophosphate was mixed with 46.8ml of 0.2M sodium hydroxide and diluted with freshly prepared distilled water to produce 200ml.

0.2M Potassium dihydrogen orthophosphate: 0.02722g of potassium dihydrogen orthophosphate was dissolved in water to produce 100ml.

0.2M Sodium hydroxide: Dissolve 0.08g of Sodium hydroxide with water to produce 100ml

Culture and assay media:

Peptone	6.0g
Pancreatic digest of casein	4.0g
Yeast extract	3.0g
Beef extract	1.5g
Glucose monohydrate	1.0g
Agar	15.0g
Water	1000ml
pH after sterilization	8.0 ± 0.1

Inoculum required:

Inoculum required was Bacillus subtilis ATCC 6633.

Inoculum preparation:

A slant of the organism on the surface of the antibiotic media No. 11 was sub cultured in a test tube and incubated at 37°C to 39 °C for 18 to 24 hours.

1. A roux bottle with 250 ml of sporulating agar spread on one surface of the bottle to get a large surface area was prepared. Using 3 to 5 ml of saline solution, the organism from the 24 hours agar slant was washed and transferred to the large surface of sporulating agar medium in the roux bottle, using glass beads if required, and spread throughout the surface and incubated for 5 days at 37°C to 39.0 °C
2. After incubation, using sterile water, the growth was washed, which consists mainly of spores, and centrifuge.
3. The sediment was resuspended in sterile saline & stored at 8°C (Stock suspension).

Preparation of plates

1.0 ml of inoculum was added to 125 ml media at 37°C to 39°C and swirled at attain a homogenous suspension. 25 ml of the media was poured to each petridish. The plates were cooled for solidification. Punch 4 cavities were punched in such a way that 4 cm or 4.5 cm away from each other in all the five petridishes.

Sample preparation

i. 25 mg of sample was weighed accurately and transferred in to a 25 ml volumetric flask.
ii. Then the sample was dissolved and diluted with water to volume up to 25 ml.
iii. Further diluted the 5 ml of the solution to 50 ml with buffer pH 8 (TH).
iv. 5 ml of TH was diluted to 25 ml with buffer

pH 8 (TL).

Standard preparation

i. 25 mg of Vancomycin hydrochloride WS was taken in a 25 ml volumetric flask and dissolved and diluted with water to 25 ml.
ii. Further 5 ml of the solution was diluted to 50 ml with buffer pH 8 (SH).
iii. 5 ml of TH was diluted to 25 ml with buffer pH 8 (SL).

Procedure

i. 0.1 ml of the test and standard solutions were applied in the cavities as per the design to obtain each of TH, TL, SH and SL. For this five plates were used.
ii. The solutions were inoculated in all the Petri plates.
iii. The plates were inoculated at room temperature for 2 to 4 hours as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of the different solutions.
iv. Then the plates for incubated for 18 to 24 hours at 37°C to 39°C.The diameters of the zones were measured using vernier calipers and the calculations were using following formula.

Calculation

TH: Sum of the zone diameters of test high from four plates in mm

TL: Sum of the zone diameters of test low from four plates in mm

SH: Sum of the zone diameters of standard high from four plates in mm

SL: Sum of the zone diameters of standard low from four plates in mm

$$a = \frac{(TH + TL) - (SH + SL)}{(TH - TL) + (SH - SL)}$$

i = Dilution factor i, e., 1: 5

Log10 i = 0.6989

$$\% \text{ Potency} = a \times \log i = \frac{(TH + TL) - (SH + SL)}{(TH - TL) + (SH - SL)} \times 0.6989$$

Content of Vancomycin in IU per mg

Note: Fudicial limit: Not less than 95% and not more than 105%.

3. RESULTS

3.1. Preformulation Studies

Preformulation Studies Results: Refer table 9 in page 292

FTIR Spectrum of Vancomycin Hydrochloride: Refer figure 1 in page 296

DSC of Vancomycin Hydrochloride: Refer figure 2 in page 296

3.2 ANALYTICAL RESULTS OF FORMULATIONS

3.2.1. Analytical Results for Trial Batches of 1& 2: Refer table no 7 in page 293

3.2.2. Analytical Results for Trial Batches After Lyophilization: Refer table no 11 in page 293

3.2.3. FTIR spectrum of Trail-6 formulation: Refer figure 3 in page 297

3.2.4. Interpretation: Refer figure 4 in page 297

3.3. ANALYTICAL RESULTS FOR SCALE UP BATCH:

Refer table no 12 in page 293

3.4. Analytical Results for Stability Batch:

3.5. Physiological Compatibility Study (PSC):

3.5.1. PSC-1 (Dilution with RL): Refer table no 14 in page 293

3.5.2. PSC-2 (Dilution with NaCl):

Refer table no 15 iin page 293

3.5.3. PSC-3(Dilution with 5% Dextrose):

Refer table no 16 in page 294

4. DISCUSSION OF RESULTS

The objective of this research work was to increase the stability Vancomycin Hydrochloride solution and to reduce the reconstitution time of Vancomycin Hydrochloride. Vancomycin Hydrochloride is an antibiotic used in severe gram positive staphylococcal infections especially infective staphylococcal endocarditis.

4.1. Physicochemical Properties of Vancomycin Hydrochloride:

As a primary step the drug was subjected to preformulation studies as per USP specification. The description of the drug was found to be white crystalline powder, which complies with the standard. The solubility was determined and it was found that the drug was soluble in water, methanol and ethanol. The drug was identified by IR spectrum and which complies with standard drug spectrum. The assay was carried out by HPLC method which was found to be 100% and relative substances gunine, unknown impurity were found to be complies with the specification as per Pharmacopoeia. The percentage of water content of the drug was analyzed by Karl Fischer titration method and was found to be 1.68%w/w, pH of the solution was found to be 3.35.These results were found to be with in the USP limits and glass transition temperarature was found to be -18˚C.

4.2. Formulation of Parenteral Dosage form:

The present study was carried out to develop a stable Vancomycin Hydrochloride solution and its lyophilization to get rapid reconstitution of the formulation i.e. Vancomycin Hydrochloride for injection.

The development of this dosage form was based on different trials by varying or incorporating different

excipients and by varying the percentage of excipients to optimize the formula. After optimizing the formula it was subjected to scale up to optimize the process.

4.2.1. Process involved in optimizing the formulation:

The main aim of the study was to produce a rapid reconstitution of the formulation Vancomycin Hydrochloride by lyophilization technique. For this purpose a literature review was made on lyophilization technique, antibiotic i.e. Vancomycin Hydrochloride and their dosage forms available. Most of the manufacturing scale lyophilizations are desirably carried out as small volume as possible to reduce lyophilization time. Hence the lyophilization time of Vancomycin Hydrochloride is reduced by increasing the concentration of Vancomycin Hydrochloride solution. Hence my approach was made to increase the concentration of Vancomycin Hydrochloride so as to reduce the fill volume and to get rapid reconstitution of the formulation by incorporating different types of excipients. By keeping all this in viewF-1 formulation was selected. According to BCS classification Vancomycin Hydrochloride Class I having highly soluble and high solubility and permeability drug.

Trail No.1 (F-1):

The formulation F-1 was made to check the stability of Vancomycin hydrochloride in water with increase of its concentration. But formulation was failed due to gel formation after 24 hours due to high concentration. This gel formation create a many handling problems and increase the lyophilization related problems .i.e. it can form a dry plug which may dissolve slowly rather than as an easily soluble, flowable powder. So, I was gone for second approach to stabilize high concentrated Vancomycin Hydrochloride solution.

Trail No.2 (F-2):

The second approach was made to stabilize the high concentration of Vancomycin Hydrochloride i.e. 13% by using 4% gel inhibitory agent Ethanol. With the result showed in table it was found that formulation was stable after 24 hours. In this approach I was achieved the desired stability of formulation by using Ethanol. Then I was gone for third approach to do the lyophilization to get rapid reconstituted formulation.

Trail No.3 (F-3):

In the third approach, the stabilized Vancomycin Hydrochloride solution using Ethanol and bulking agent was subjected to lyophilization so as to get rapid reconstitution of the formulation of the formulation but in this approach reconstitution was found to be more than 2 min. I wanted to reduce the reconstitution time I was gone for fourth approach.

Trail No.4 (F-4):

In the fourth approach, I was incorporated the bulking agent Mannitol of 5% and Cyclodextrin as reconstitution enhancer to the above formulation and subjected for lyophilization. The reconstituted time of that formulation was found to be 45- 50 sec. Hence there is a need to decrease that reconstitution time therefore I was taken for fourth trial.

Trail No.5 (F-5):

This fifth approach wad made to increase reconstitution by using PEG-400 in place of Cyclodextrin I a concentration of 6% and this formulation was subjected to lyophilization. After, lyophilization the reconstitution time was found to be 10-15 sec. But the residual solvent crosses the IIG limit so as to encounter that problem and to still reduce the reconstitution time as low as possible I was gone for 6th approach.

Trail No.6 (F-6):

This approach was made to reduce the concentration of Vancomycin Hydrochloride from 13% to 12% so as to reduce reconstitution time and I also reduce decrease the concentration of both Ethanol and PEG-400 so as to meet IIG residual solvent limit. Then the formulation was subjected to lyophilization. Then the reconstitution time of this formulation was found to be 5-6 sec and residual solvent was found to be within IIG limit i.e. 5000ppm.

All the parameters were found to be satisfactory hence this formulation and lyophilization cycle both are optimized.

In the lyophilization cycle pressure was increased in primary drying step to increase the efficiency of lyophilizer to remove the vapours above the product in the chamber. Also pressure was also reduced to 0.08 mbar to increase the rate of drying.

During the formulation all parameters were measured.

- PH -2.758
- Water content-0.45%
- Antibiotic assay-102.9%
- Chromatographic purity-95.38%
- Reconstitution Time-5-6 sec
- Residual solvents limit-1690ppm

Once the formula and lyophilization cycle optimized scale up

(F-7) was proposed in order to optimize the process by increasing the batch size to 5 lit. In the scale up batch no problem was observed. All the parameters were found to be satisfactory and compared with F-6. During the formulation all parameters were measured.

Analytical parameters (results) for scale up batch (F-7) were as follows:

- PH -2.83

- Water content-2.62%
- Antibiotic assay-102.3
- Chromatographic purity-96.4%
- Reconstitution Time-6-7 sec
- Residual solvents limit-1466ppm

4.3. Stability studies:

Scale up batch samples (vials) were subjected to stability studies as per ICH guidelines i.e. accelerated stability conditions i.e. 40°C/75% RH for two months. Stability samples were analyzed for description, PH, water content, assay and reconstitution time.

Description:

It was found that there was no significant change in the color, physical appearance in all the vials during first and second month stability study.

Purity:

There was no significant change in purity in the stability study samples.

Water content:

There was no drastic or significant change in the water content during 1st and 2nd month stability study.

Assay:

Assay result was found to be within the limits of pharmacopeia during 1st and 2nd month stability study.

Reconstitution time:

In reconstitution time, there was no significant change between initial and stability samples.

4.4. Physiological compatability study:

Physiological compatatability study was carried out by reconstituting freeze dried vials with different physiological solutions like Ringer Lactate, 0.9% Sodium Chloride, 5% Dextrose solutions to get concentration of 5 mg/ml respectively and kept at 2-8˚C. After regular intervals at 2nd, 4th and 6th day of time the diluted or reconstituted sample was withdrawn from each bag respectively. They were evaluated for moisture content, RS, PH, RT, color value, % of light transmittance. All evaluated parameters are found to be in the limits of pharmacopeia. Hence the formulation was compatible with 3 physiological solutions i.e. Ringer Lactate, 0.9% Sodium Chloride, 5% Dextrose solutions.

5. Summary and Conclusion

5.1. SUMMARY

- The ultimate object of this project work is to increase the stability of Vancomycin Hydrochloride which is widely used in treatment of staphylococcal gram positive infections mainly staphylococcal endocarditis.
- The Vancomycin Hydrochloride in aqueous solution not stable beyond 48 hours and slowly gel formation takes place. Hence the formulation was stabilized by using gel inhibitory agent and that formulation was subjected to lyophilization to improve reconstitution time.
- The objective of the study was to perform Preformulation studies on the drug, formulation of injectable dosage form, lyophilization of the formulation and finally perform short term stability studies.
- Lyophilization technique was adopted with various excipients, to formulate Parenteral Vancomycin Hydrochloride for injection. The lyophilization was carried out in different batches by varying the excipients, their concentration and the concentration of API by keeping lyophilization cycle constant.
- The lyophilized product was evaluated for moisture content, PH, cake formation, reconstitution time and drug content (assay).The reconstitution time of lyophilized product in the F-6 was found to be 5-6 sec and residual solvent limit was found to be within IIG limit and establishment of the specifications are important for both product release and stability. In F-6, there was no problem of sticking of the product with vials and no melt back was observed.
- Reconstitution time was determined by reconstituting freeze dried cake with WFI, the time was found to be 5-6 sec in F-6 less compared to other batches which signifies no partial loss of potency as the drug is completely dissolved. The PH of formulation was found to be 2.83. Assay of lyophilized product was found to be 102.3% which was within the pharmacopeial limits.
- After successfully optimizing the formulation, the lab scale batch was transferred to production scale by taking a scale up batch i.e. F-7 in order to optimize the process. All the evaluation parameters of freeze dried product were found satisfactory for this scale up batch.
- The accelerated stability studies were carried out as per ICH guide lines at condition such as 40˚C ± 75% RH for 2 months. After each one month interval the product evaluated for moisture content, RS, PH,RT, color value, % of light transmittance. From the above result it was incurred that all the evaluated parameters were found to be within the pharmacopeial limits, even after 2 months of stability studies. Finally it was concluded that the formulation F-6 was the best formulation.
- Physiological compatatability study was performed by reconstituting them with different

physiological solutions like Ringer Lactate, 0.9% Sodium Chloride, 5% Dextrose solutions at 2-8˚C. After regular intervals of time reconstituted vials were evaluated for moisture content, RS, PH,RT, color value, % of light transmittance. The evaluated parameters were found to be within the pharmacopeial limits. From that result it was concluded that formulation F-6 was compatible with all 3 physiological solutions hence Vancomycin Hydrochloride for injection was infused with either of the above diluents.

5.2. CONCLUSION

In present study, an attempt was made to formulate a rapid reconstituted formulation (lyophilized formulation) containing antibiotic drug. The antibiotic drug is Vancomycin Hydrochloride. From the study carried out, following conclusions were made below:

Preformulation studies were performed which showed that the API met the required pharmacopeial limits.

- First one of two approaches, second one was made to establish good stability and in that approach I got good result i.e. the formulation was stable after 48 hrs without any gel formation.
- Then various approaches were done by incorporating different types of excipients and varying their concentration followed by lyophilization.
- The desired RT was achieved by varying the concentration of API and replacing the Cyclodextrin by PEG-400 and decreasing the concentration in the formulation followed by lyophilization in the 6th approach. The RT of F-6 formulation was found to be within 10 sec i.e. 5-6 sec. In this F-6 trial only I got desired RT when compared to all other trials.
- The dosage form was optimized with respect to formulation and processing parameters i.e. lyophilization parameters which indicated that formulation F-6 had the desired optimum characteristics for taking up scale up batch.
- After successfully optimizing the formulation, scale up batch F-7 was taken by increasing the batch size.
- During the manufacturing in large scale no processing problems were observed and all the analytical results were found satisfactory.
- Stability studies were carried out as per ICH guide lines. After 2 months of accelerated stability studies, it was found that F-7 was sufficiently stable.
- Physiological compatatability study was performed with different physiological solutions. From that study result, I concluded that my formulation was physicochemically compatible with those physiological solutions.

Scope for Further Study:

- The work can be extended to tubing compatibility study, rubber stopper compatibility study and reconstitution stability study.
- The work can be extended to take an exhibit batch by increasing the batch quantity in order to check the feasibility of the product on large scale.
- The work can be extended to bioavailability studies for formulated product.
- The work can be extended to long term stability studies.

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Table 1: List of Materials and Suppliers

Materials Used	Source
Vancomycin Hydrochloride	Strides Arco lab Ltd, Bangalore
Ethyl Alcohol	Hayman Ltd, Mumbai
Mannitol	DMV Fonterra Ltd, Nagpur
Polyethylene Glycol 400	Rockette Ltd, Ahmadabad,
Cyclodextrin	Amoli Organic Pvt ltd, Mumbai
Hydrochloric Acid	Nitica chemicals, Jaipur

Table 2: List of Instruments used

S.No	Instrument	Company	Model
1	Lyophilizer	Tofflon, shangai	0.54 cipsip
2	Weighing balance	Sartorius, Japan	Bp2215
3	PH meter	Eutech, Singapore	Ph 510
4	Vial filling machine	Wheatson	Omnispence plus 230vac
5	HPLC	Agilent, USA	1100 series

6	Magnetic stirrer	Remi equipments pvt. ltd, Mumbai	1mlh
7	FT-IR	Jasco-ft-ir, Japan	4100
8	Melting point apparatus	Campbell electronics, Mumbai	Thermonik
9	Auto kf titrator	Mayura analytical Pvt ltd.	703 metro ohm
10	DSC	Thermo-Japan	Q-100 series

Table 3: Drug - Excipient Qualitative Formula

S.No	Ingredients	Functions
1	Vancomycin Hydrochloride	Active Pharmaceutical Ingredient
2	Alcohol	Gel Inhibitory Compound
3	Mannitol	Bulking Agent
4	Cyclodextrin	Reconstitution Enhancer
5	Polyethylene Glycol 400	Co-solvent

Table 4: Formulation Trials of Vancomycin Hydrochloride for Injection

S.No	Ingredients	Formulation Trial Batches					
		1	2	3	4	5	6
1	Vancomycin Hydrochloride	13%	13%	13%	13%	13%	12%
2	Alcohol	-	4%	4%	4%	4%	2%
3	Mannitol	-	-	5%	5%	5%	5%
4	Cyclodextrin	-	-	-	6%	-	-
5	Polyethylene Glycol 400	-	-	-	-	6%	3%
6	Hydrochloric Acid	-	-	qs	qs	qs	Qs
7	Water for Injection	qs to 100ml	qs to 100ml	qs to 100ml	qs to100ml	qs to 100ml	qs to 100ml

Table 5: Ingredients quantitative formula

Ingredients	Quantity/ml
Vancomycin Hydrochloride	0.123gm
Mannitol	5 mg
Alcohol	0.02ml
Polyethylene Glycol 400	0.03ml
Hydrochloric Acid	qs
Water For Injection	qs

Table 6: Lyophilization Cycle 1 for Trials 1-5

Lyocycle steps	Temperature (°C)	Time		Pressure (mbar)
		Ramp (min)	Hold (min)	
Freezing	-40	60	180	-
	-15	35	120	-
	-40	30	180	-
Primary Drying	15	75	1200	0.1
Secondary Drying	40	35	300	0.1

Table 7: Lyophilization Cycle 2 for Trial 6 & Scale up Batch

Lyocycle steps	Temperature (^{0}C)	Time		Pressure (mbar)
		Ramp (min)	Hold (min)	
Freezing	-40°C	60	180	-
	-15 °C	35	120	-
	-40 °C	30	180	-
Primary Drying	15 °C	75	1200	0.15
Secondary Drying	40 °C	35	300	0.08

Table 8: For time intervals for analysis

Sample Details	Sample storage condition	PSC-1 Diluted with RL	PSC-2 Diluted with 0.9% NaCl	PSC-3 Diluted with 5% Dextrose
Initial	NA	1 Bag	1 Bag	1 Bag
2nd Day	2-8°C	1 Bag	1 Bag	1 Bag
4th Day	2-8°C	1 Bag	1 Bag	1 Bag
6thDay	2-8°C	1 Bag	1 Bag	1 Bag

Table 9: Results of Preformulation Studies

Test	Specification as Pharmacopeia (USP)	Result
Description	White or almost white powder	White powder
Solubility	Freely soluble in water, insoluble in ether and Chloroform	Freely soluble in water, insoluble in ether and Chloroform
Identification A (IR)	The sample exhibited the maxima at wavelengths as same as that of the standard.	The sample exhibited the maxima at wavelengths as same as that of the standard.
Identification B (HPLC)	The retention time of the principle peak in the chromatogram of the test should be similar to that of standard.	The retention time of the principle peak in the chromatogram of the test is similar to that of standard.
Appearance of solution	10%w/v solution in water should be clear and absorbance at 450nm is 0.0038.	10%w/v solution in water was clear and absorbance at 450nm is 0.0038.
PH	2.5 - 4.5	3.35
Water	≤5%w/w	1.68%w/w
Chromatographic Purity	≤93.0%	97.78%
Limit of Monodechlorovancomycin	≤4.7%	Complies
Assay by microbiology	≥900µg/mg	100%
Related substances	≤4.0%	Complies
Glass transition temperature	-	-18˚C

Table 10: Analytical Results for Trial Batches of 1 & 2

Trial	Appearance of solution after 24 H	Appearance of solution after 48 H
1	Viscous	Gel
2	Clear	Not viscous

Table 11: Analytical Results for Trial Batches after Lyophilization

Test	Specification as per Pharmacopeia (USP)	Results			
		Trial 3	Trial 4	Trial 5	Trial 6
Description	A white masses or white powder	A white mass	A white mass	A white mass	A white mass
Identification(1)	By UV: maxima between 279 and 283nm	Complies	Complies	Complies	Complies

pH	Between 2.5-4.5	2.78	2.56	2.62	2.758
Purity(1)	Absorbance at 465 nm is NMT 0.05	0.0338	0.0238	0.0238	0.0289
Purity(2) Related Substances	NMT 12.0%	Complies	Complies	Complies	Complies
Water	NMT 5.0% on 0.1gm	3.23%	2.38%	2.10%	0.45%
Foreign Insoluble matter	The sample should be free visible particles	Complies	Complies	Complies	Complies
Insoluble particulate matter	≥10µm:max 6000/ vial,≥25µm:max 600/vial	Complies	Complies	Complies	Complies
Assay	95-105%	101.5%	102.1%	102.3%	102.9%
Chromatographic Purity	-	98.12%	96.12 %	96.06%	95.38%
Reconstituted Time	-	2 min	45-50 sec	10-15sec	5-6sec
Limit of Monodechloro Vancomycin	NMT 4.7%	Complies	Complies	Complies	Complies
Residual solvents Ethanol	5000ppm	Not done	Not done	Not Complies	Complies (1690 ppm)

Table 12: Analytical Results for Scale up Batch

Test	RESULTS	Specification as per Pharmacopeia (USP)
Description	A white mass	A white masses or white powder
Identification (1)	280nm	By UV: The sample should show maxima between 279 and 283nm
Identification (2)	Complies	A white turbidity should produced
PH	2.83	Between 2.5-4.5
Purity (1) Clarity and color of solution at 465 nm	The solution is clear to pale yellow. Absorbance at 465n is 0.0188	The solution is clear to pale yellow. Absorbance at 465 nm is NMT 0.05
Purity (2) Related Substances By HPLC	Complies	NMT 4.0%
Water	2.62%	NMT 5.0% on 0.1gm
Bacterial Endotoxins	Complies	Less than 0.25EU/mg
Uniformity of Dosage unit by mass variation	L1:1.2	L1:15
Foreign Insoluble matter	Complies	The sample should be free from undissolved matter or visible particles

Table 13: Analytical Results for Stability Batch

Test	RESULTS	
	Stability 40°C/75%RH, 1st Month	Stability 40°C/75%RH, 2nd Month
Description	A white mass	A white mass
Identification	Complies	Complies
PH	2.609	2.73
Purity (1) Clarity and color of solution at 465 nm	The solution is clear to pale yellow. Absorbance at 465nm is 0.0292	The solution is clear to pale yellow. Absorbance at 465nm is 0.0295
Related Substances By HPLC	Complies	Complies
Water	0.67%	0.63%

Antibiotic Microbial assay (vancomycin in %/vial)	102.8%	102.0%
Chromatographic Purity	91.84%	90.13%
Reconstituted Time	7sec	6sec
Limit of Monodechloro vancomycin	2.4%	2.4%

Table 14: PSC-1 (Dilution with RL)

S.No	Test	Initial	Day2	Day4	Day6
1	Description	Complies	Complies	Complies	Complies
2	Clarity and color of solution	0.0112	0.0234	0.0405	0.0046
3	pH	4.32	4.31	4.29	4.24
4	Chromatographic Purity Vancomycin B	94.59	95.10	94.26	93.61
5	Limit of Monodechlorovancomycin	1.6	2.2	2.2	2.2
6	RS	Complies	Complies	Complies	Complies
7	Particulate Matter	Complies	Complies	Complies	Complies
8	Assay by Microbiology	103.0	102.8	102.2	101.6

Table 15: PSC-2 (Dilution with NaCl)

S.No.	Test	Initial	Day2	Day4	Day6
1	Description	Complies	Complies	Complies	Complies
2	Clarity and color of solution	0.0125	0.025	0.0432	0.0077
3	PH	4.02	4.14	4.51	4.99
4	Chromatographic Purity Vancomycin B	94.95	95.21	94.33	93.08
5	Limit of Monodechlorovancomycin	1.9	2.1	2.3	2.6
6	RS	Complies	Complies	Complies	Complies
7	Particulate Matter	Complies	Complies	Complies	Complies
8	Assay by Microbiology	103.2	103	102	101.8

Table 16: PSC-3(Dilution with 5% Dextrose)

S.No.	Test	Initial	Day2	Day4	Day6
1	Description	Complies	Complies	Complies	Complies
2	Clarity and color of solution	0.0186	0.0194	0.0053	0.0005
3	PH	3.89	3.93	3.97	3.98
4	Chromatographic Purity Vancomycin B	94.37	95.22	95.23	93.25
5	Limit of Monodechlorovancomycin	1.7	2.2	2.5	2.5
6	RS	Complies	Complies	Complies	Complies
7	Particulate Matter	Complies	Complies	Complies	Complies
8	Assay by Microbiology	103.8	103.6	102.2	101.8

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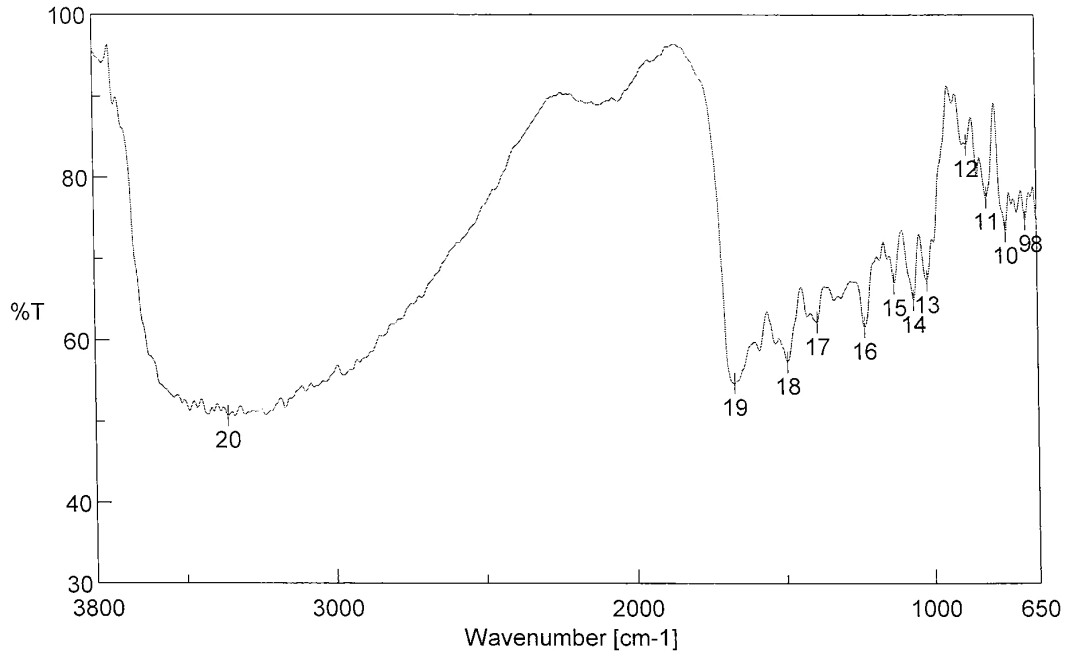
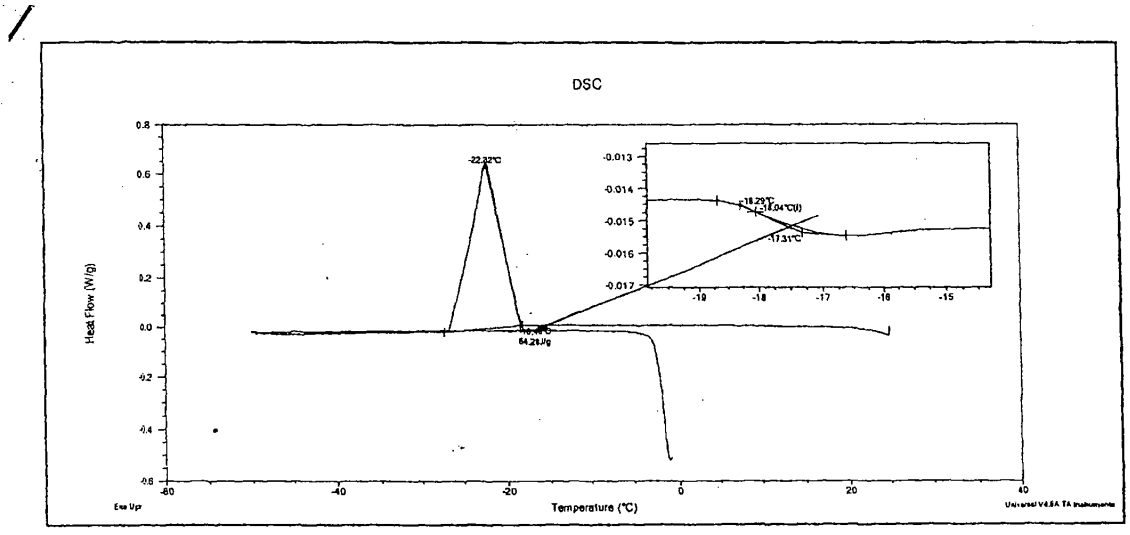


Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity
1	426.191	17.9148	2	447.404	22.1889
3	468.617	44.2804	4	505.258	49.5185
5	520.686	49.6138	6	563.112	66.1515
7	619.038	71.7192	8	649.893	74.7302
9	688.463	74.8189	10	754.031	73.3567
11	817.67	77.4704	12	883.238	83.9788
13	1018.23	67.2398	14	1064.51	64.8822
15	1130.08	66.907	16	1232.29	61.5351
17	1392.35	62.0778	18	1492.63	57.2383
19	1670.05	54.6115	20	3359.39	50.6032
21	3835.72	94.6786			

Fig. 1: FTIR Spectrum of Vancomycin Hydrochloride



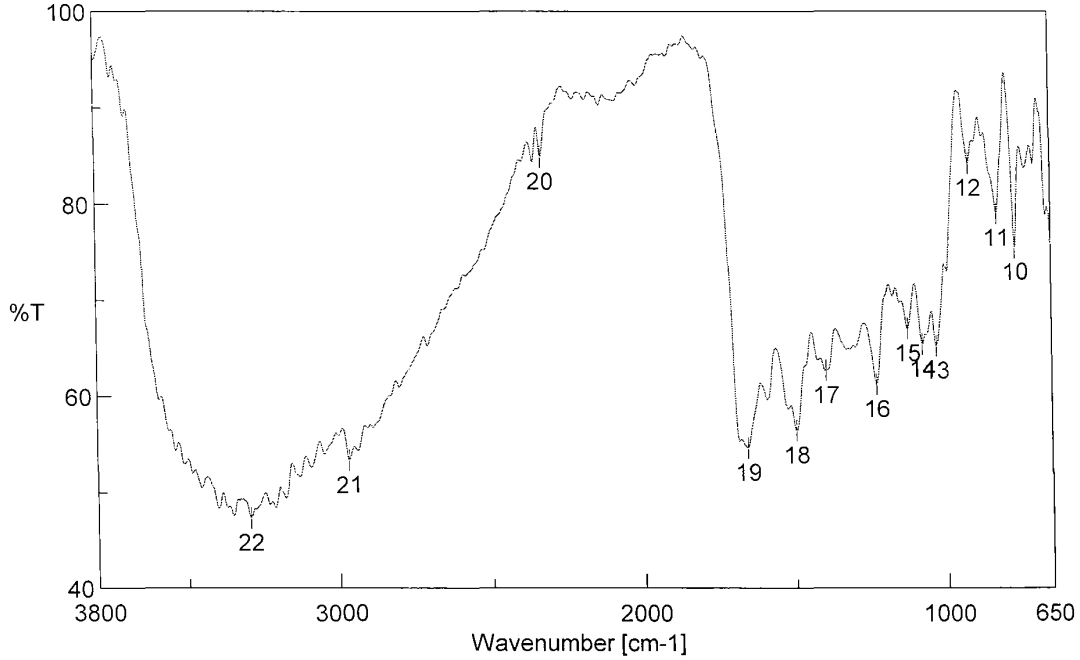
Peak Integration

Start	Onset	Maximum	Stop	Area
°C	°C	°C	°C	J/g
-18.22	-18.46	-22.32	-27.24	64.28

Glass Transition

Onset	Midpoint (I)	End	Height	Delta Cp
°C	°C	°C	W/g	J/(g·°C)
-18.29	-18.04	-17.31	0.0008539	0.05122

Fig.2: DSC of Vancomycin Hydrochloride



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity
1	430.048	19.494	2	449.333	28.9833
3	474.403	12.0616	4	499.473	8.45072
5	518.758	29.5661	6	541.899	56.5471
7	584.325	73.4595	8	622.895	79.0383
9	647.965	74.9751	10	767.53	75.403
11	827.312	78.9302	12	919.879	84.1461
13	1031.73	65.1346	14	1078.01	65.3337
15	1126.22	66.9642	16	1230.36	61.1429
17	1398.14	62.592	18	1496.49	56.3328
19	1654.62	54.4918	20	2333.45	84.8258
21	2967.91	53.2406	22	3291.89	47.2812
23	3916.72	92.3216			

Fig. 3: FTIR spectrum of Trail-6 formulation

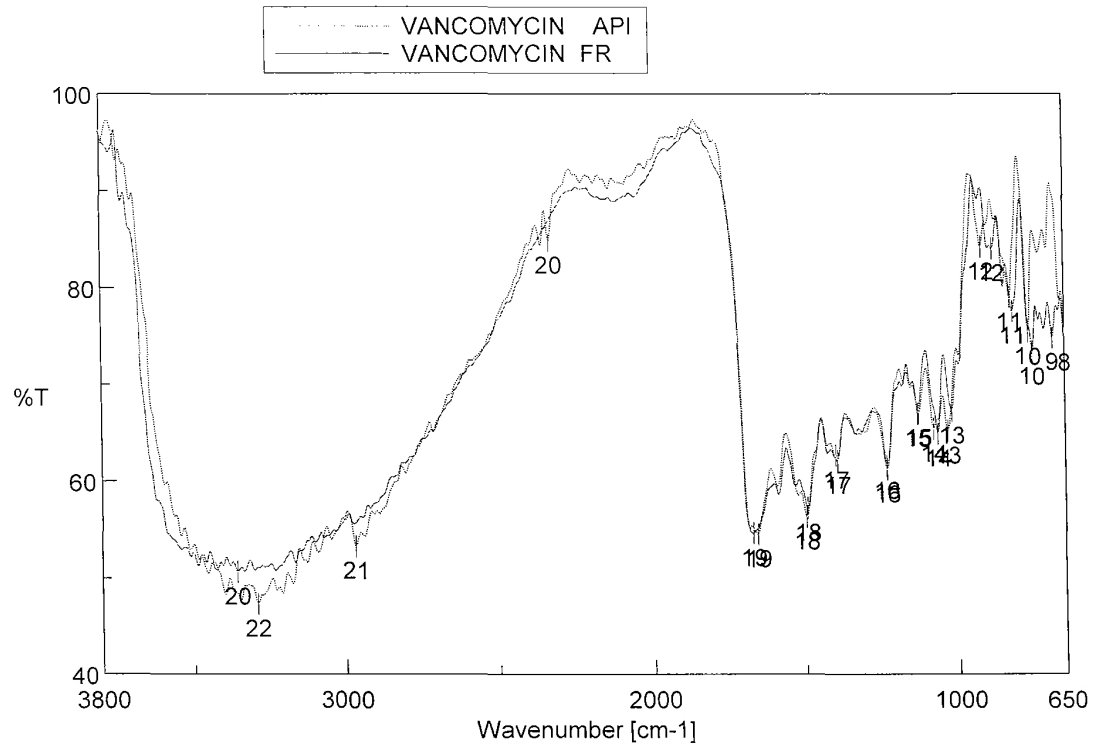


Fig. 4: Interpretation of formulation with Vancomycin HCl (API)