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Enhancing Stability of an Anti Ulser Drug through Lyophilization Technique

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Abstract- The aim of present research work was to formulate an intravenous injection of Omeprazole sodium. Omeprazole drug is very slightly soluble in water. Hence *in-situ* conversion of Omeprazole into Omeprazole sodium was opted. But Omeprazole sodium is not stable in solution form. It is stable only for 1-2 days. Hence lyophilization technology was adopted to increase the stability of Omeprazole sodium injection. The lyophilization was carried out in different batches by varying the total cycle time, freezing and holding time, primary drying and secondary drying time while keeping the quantities of all the active pharmaceutical ingredients constant.

Lyophilization was carried out in five different batches with five different lyophilization-cycles of 24.5 hours, 28.5 hours, 30.5 hours, 32.5 hours, and 35 hours respectively. Melt pack was found in Batch 1, Partial melt pack was found in batch 2, cake was sticking to the bottom of vial in batch 3, moisture content was high in batch 4, the optimized good cake was found in batch 5. lyophilization cycle of 35 hours was optimized. The optimized lyophilized product was subjected to evaluation parameters such as cake appearance, reconstitution time, pH, assay, impurities, particulate matter, water content and DSC.

After considering all product characteristics batch-5 was considered as an optimized formulation. All the evaluation parameters complies the limits as per the specification of USP. Accelerated stability studies were also conducted for a period of three months and from the results obtained, it was found that the optimized formulation was found to be stable. Finally, it was concluded that the lyophilization is a suitable technique to enhance the stability of Omeprazole sodium for intravenous injection with a single dose of 40mg/vial.

Index Terms- lyophilization, Freezing, Primary drying, Secondary dying, HPLC, DSC

I. INTRODUCTION

THE FREEZE-DRYING CYCLE:

Lyophilization is the most common method for manufacturing solid pharmaceuticals products and is central to the preservation of materials which must be dried. Thoroughly in order to ensure stability and require a gentle, easily sterilized process. To meet this requirement, a solution's lyophilization occurs in three steps: (1)freezing to convert most of the water into ice, (2) primary drying to sublime the ice, and (3) secondary drying to remove unfrozen water by desorption. To technically realize this manufacturing process, a freeze dryer is commonly constructed with two main parts: a "drying" chamber holding temperature controlled shelves is connected by a valve to a "condenser" chamber, which contains coils capable to achieve very low temperatures between -50°C and -80°C. One or more vacuum pumps in series are connected to the condenser to obtain very low pressures in the entire system. With this, the sublimed water of the primary drying stage is reconverted to ice by the condenser and thus removed from the system. However, the multitude of variables inherent in a large batch of individual vials in a complex chamber setup makes process control difficult. Understanding of the product, the thermodynamic behavior of formulations and principles of the different drying stages are of fundamental Importance to avoid product damage.

The freeze-drying process consists of three stages.
1) Freezing
2) Primary drying
3) Secondary drying

1) Freezing

Freezing is a critical step, since the microstructure established by the freezing process usually represents the microstructure of the dried product. The product must be frozen to a low enough temperature to be completely solidify. Since freeze drying is a change in state from the solid phase to the gaseous phase, material to be freeze dried must first be adequately pre-frozen. The method of prefreezing and the final temperature of the frozen product can affect the ability to successfully freeze dry the material. Rapid cooling results in small ice crystals, useful in preserving structures to be examined microscopically, but resulting in a product that is, more difficult to freeze dry.Slower cooling results in large ice crystals and less restrictive channel in the matrix during the drying process. Products freeze in two ways, the majority of products that are subjected to freeze drying consist primarily of water, the solvent and materials dissolved or suspended in the water, the solute. Most samples that are to be freeze dried are eutectics, which are mixtures of substances that freeze at lower temperature than the surrounding water. Only when all of the eutectic mixture is frozen, then the Solution is said to be properly frozen. This is called the eutectic temperature. It is very important in freeze-drying to freeze the product to below the eutectic temperature before beginning the drying process. The second type of frozen product is a suspension that undergoes glass formation during the freezing process. Instead of forming eutectics, the entire suspension becomes increasingly viscous as the temperature is lowered. Finally the products freeze at the glass transition point forming a vitreous solid. This type of product is extremely difficult to freeze dry.

Typical solutions may cool to produce a partially crystalline / amorphous matrix depending on the solution components, rates of cooling etc.

Ex. 1% NaCl will contain only 1% solids and 99% water. As the solution is cooled, ice will nucleate at approximately 0^0C. The ice crystals continue to grow, pervading the solution until virtually all the freezable water has been converted into ice. Analysis will confirm that the ice crystals are embedded within a solute rich concentrate. Of all pharmaceutical unit operations, drying process contribute the most to the manufacturing cost. Lyophilization is the most expensive of all drying operations both in capital investment and in operating expense. The high cost and commercial value per production batch demands careful attention to process design and process control. The heat input during the lyophilization process must be well controlled to insure that the product temperature does not become too high. The structure of the product deteriorates at too high temperatures and the final quality of the product becomes unacceptable.

Lyophilization stabilizes the formulation by slowing the kinetic clock of the degradation process. It alters the clock by removing the solvent component to levels that never support chemical reactions or biological growth.

2) Primary drying

After pre freezing the product, conditions must be established in which ice can be removed from the frozen product via sublimation, resulting in a dry, structurally intact product.

This requires very carefully control of the two parameters,
1) Temperature and
2) Pressure, involved in freeze-drying system.

The rate of sublimation of ice from a frozen product depends upon the difference in vapor pressure of the product compared to the vapor pressure of the ice collector. Molecules migrate from the high-pressure sample to a lower pressure area. Since vapor pressure is related to temperature, it is necessary that the product temperature is warmer than the cold trap (ice collector) temperature. It is extremely important that the temperature at which a product is freeze dried is balanced between the temperature that maintains the frozen integrity of the product and the temperature that maximizes the vapor pressure of the product. This balance is key to optimum drying.

3) Secondary drying

After primary freeze-drying is complete, and all ice has sublimed, bound moisture is still present in the product. The product appears dry, but the residual moisture content may be as high as 7-8% continued drying is necessary at warmer temperature to reduce the residual moisture content to optimum values. This process is called 'Isothermal Desorption' as the bound water is desorbed from the product. Secondary drying is normally continued at a product temperature higher than ambient but compatible with the sensitivity of the product. In contrast to processing conditions for primary dying which use low shelf temperature and a moderate vacuum, desorption drying is facilitated by raising shelf temperature and reducing chamber pressure to a minimum care should be exercised in raising shelf temperature too highly; since, protein polymerization or biodegradation may result from using high processing temperature during secondary drying.

Secondary drying is usually carried out for approximately 1/3 or 1/2 the time required for primary drying.

The general practice in secondary freeze-drying is to increase the shelf temperature and to decrease chamber pressure to the lowest attainable level. The practice is based on the reason that, the ice is no longer present and there is no concern about "melt-pack" (melt pact is a sticky liquid appearance formed due to improper sublimation of the ice during primary drying) the product can withstand higher heat input. Also, the water remaining during secondary drying is more strongly bound, thus requiring more energy for its removal. Decreasing the chamber pressure to the maximum attainable vacuum has traditionally been thought to favor desorption of water. In successful lyophilization, product should retain the physico-chemical attributes of the starting solution and the structure established during freezing. The dried cake should be uniform in structure, color and texture — ideally, a dense white cake with fine, uniform structure, showing good physical strength and friability

II. INTRODUCTION OF STOMACH DISEASE

A peptic ulcer, also known as PUD or peptic ulcer disease, is the most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. It is defined as mucosal erosions equal to or greater than 0.5 cm. Normally, the lining of the stomach and small intestine is protected against the irritating acids produced in the stomach. If this protective lining is affected it results in the breakdown of lining and hence inflammation (gastritis) or an ulcer. Most ulcers occur in the first layer of the inner lining. A hole that goes all the way through the stomach or duodenum is called a perforation. A perforation is a medical emergency.Gastroesophageal reflux disease (GERD) is a condition in which the stomach contents (food or liquid) leak backwards from the stomach into the esophagus (the tube from the mouth to the stomach). This action can irritate the esophagus, causing heartburn and other symptoms. When refluxed stomach acid touches the lining of the esophagus it may cause a burning sensation in the chest or throat called heartburn or acid indigestion. Erosive Esophagitis is an inflammation and swelling of the esophagus, and is most often caused by acid-containing stomach contents refluxing back up into the esophagus.

Proton pump inhibitors are commonly used to treat the above mentioned diseases. Omeprazole for injection is mostly used among the proton pump inhibitors. But the solubility of Omeprazole is very less and it is very unstable in solution form. So the main objective of the research work is to increase the solubility of Omeprazole by *in-situ* conversion of Omeprazole into Omeprazole sodium and increase the stability by lyophilizing the solution form of Omeprazole sodium. Lyophilization is performed for the substances which are Thermo labile and Unstable in the solution form.

The present work was designed to address the following objectives: -

- Preformulation studies on the drug.

- Selection of the excipients for development of injectable dosage form by lyophilization techniques.
- Formulation of the injectable dosage form.
- Performing Lyophilization and study its parameters.
- Evaluation of the optimized formulation
- Perform stability studies on the optimized best formulation.

Composition per one vial

Composition	Quantity	Rationale
Omeprazole	40mg	Active
Edetate Disodium	0.4mg	Chelating Agent
Sodium Hydroxide	q.s to solubilize Omeprazole Slurry	For conversion of Omeprazole base to its Sodium salt
Water for Injection	q.s to 4ml	Solvent

Precautions taken during manufacturing

- The vehicle used i.e. sterile water for injection was free from oxygen
- Glass or stainless steel apparatus was used
- Entire manufacturing process was carried out under aseptic conditions and includes washing and sterilization of vials, rubber plugs and disinfection aluminium seals. The vials were filled in class 100 laminar air cabinets

Method of Preparation

1. Collect Required water for injection and bring down the temperature below 40°C, by nitrogen bubbling
2. Check and record the pH of WFI (Limit 5.0-7.0)
3. Add and dissolve the weighed quantity of Disodium Edetate in 80% of WFI with continuous stirring
4. To the solution of step-3, Omeprazole was added with stirring to get uniform slurry
5. 1N sodium hydroxide was prepared by using WFI separately
6. To the slurry obtained in step-3, sodium hydroxide solution obtained in step-4 was added slowly with stirring till a clear solution was obtained
7. Volume of the solution is made to 100% with Water for injection. pH was checked (limit 10.3 – 12)
8. The solution of the step-7 was filtered through 0.22µm PVDF membrane and filled into USP type 1 flint glass tubular vials (fill volume 4.0 – 4.1ml), half stoppered with slotted grey bromo butyl rubber plugs and loaded into lyophilizer.

Composition of Special diluent for reconstitution

Sl.No.	Ingredients	Qty/ml
1	Citric acid monohydrate	0.5mg
2	Polyethylene Glycol 400	400mg
3	Water for injection	q.s to 1ml

Trail Batches:

Trail batches were conducted as per below table

Formulation trials of Omeprazole sodium for injection

Constituents	Trail batches				
	I	II	III	IV	V
Omeprazole	40mg/vial	40mg/vial	40mg/vial	40mg/vial	40mg/vial
Disodium edetate	0.4mg	0.4mg	0.4mg	0.4mg	0.4mg
Sodium Hydroxide	q.s to solubilize omeprazole	q.s to solubilize omeprazole	q.s to solubilize omeprazole	q.s to solubilize omeprazole	q.s to solubilize omeprazole

Water for injection	q.s to 4ml	q.s to 4ml	q.s to 4ml	q.s to 4ml	q.s to 4ml
Lyocycle	**LYO 1**	**LYO 2**	**LYO 3**	**LYO 4**	**LYO 5**

Lyophilization Cycle

Lyophilization or Freeze drying fills an important need in pharmaceutical manufacturing technology by allowing drying of heat-sensitive drugs and biologicals at low temperature under conditions that allow removal of water by sublimation or a change of phase from solid to vapor without passing through the liquid phase. Lyophilization occurs in three steps: freezing, primary drying and secondary drying. In freezing processwater is converted into ice, in primary drying to sublime the ice is subjected to sublimation and in secondary drying process unfrozen water is removed by desorption.

During the lyophilization process the material is first frozen and then subjected to drying. To initiate the drying stage, the material in the chamber is subjected to vacuum. Heat is applied carefully to the material, and a condenser is used in the chamber to collect the water. When water is leaving rapidly, its heat of vaporization is taken away from the material and helps to keep it cool and safe.

Before carrying out lyophilization for formulation it was subjected for preliminary DSC studies and based on the DSC results obtained the suitable lyophilization cycles were designed. The glass transition temperature obtained from DSC was used to determine the freezing temperature of the formulation filled into the vials. The cycles thus designed were applied and the product obtained after lyophilization was subjected for physical examination. On the basis of the cake obtained the process variables i.e. temperature and duration of the cycle were applied. Duration taken to attain required temperature was termed as ramp temperature and the duration in which the formulation remained in the attained temperature was termed as soak temperature.

Trail 1:

In this cycle, formulation was subjected for 3.5 hours of freezing, 14 hours of primary drying and 7 hours of secondary drying. Here the freezing temperature was fixed purely based on the glass transition temperature of the formulation. The glass transition temperature of the formulation was found to be -15.78°C. Thus the formulation was frozen to a temperature of -35°C which is -15.78°C lesser than the glass transition temperature. This was carried out in order to ensure complete freezing.

Lyophilization cycle of 24.5 hours

Process & Temperature	**Ramp duration (min)**	**Soak duration (min)**	**Pressure (torr)**
Freezing (-35°C)	120	90	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	120	0.75
10°C	120	150	0.75
Secondary Drying			
20°C	60	90	0.3
35°C	120	150	0.1

Trail 2:

In this trial formulation was subjected for 5.5 hours of freezing, 16 hours of primary drying and 7 hours of secondary drying. Here the freezing temperature was reduced to -40°C. And the duration of the steps like freezing and primary drying was increased. These changes were implemented in order to enhance the freezing of the formulation and to ensure proper drying.

- Lyophilization cycle of 28.5hrs

Process & Temperature	**Ramp duration (min)**	**Soak duration (min)**	**Pressure (torr)**
Freezing (-40°C)	150	180	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	180	0.75
10°C	120	210	0.75
Secondary Drying			
20°C	60	90	0.3
35°C	120	150	0.1

In this trial formulation was subjected for 5.5 hours of freezing, 18 hours of primary drying and 7 hours of secondary drying. Here the freezing temperature was maintained similar to trial 2. But the duration of the steps in primary drying were increased. These changes were implemented in order to enhance proper drying of the formulation.

Lyophilization cycle of 30.5hrs

Process & Temperature	**Ramp duration (min)**	**Soak duration (min)**	**Pressure (torr)**
Freezing (-40°C)	150	180	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	210	0.75
10°C	120	300	0.75
Secondary Drying			
20°C	60	90	0.3
35°C	120	150	0.1

Trail 4:

In this trial formulation was subjected for 5.5 hours of freezing, 19.5 hours of primary drying and 7.5 hours of secondary drying. Here the freezing temperature was maintained similar to trial 2. But the duration of the steps like primary drying and secondary drying were increased. These changes were implemented in order to enhance the proper drying of the formulation.

Lyophilization cycle of 32.5 hours

Process & Temperature	**Ramp duration (min)**	**Soak duration (min)**	**Pressure (torr)**
Freezing (-40°C)	150	180	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	240	0.75
10°C	120	360	0.75
Secondary Drying			
20°C	60	90	0.3
35°C	120	180	0.1

Trail 5

In this trial formulation was subjected for 5.5 hours of freezing, 19.5 hours of primary drying and 10 hours of secondary drying. Here the freezing temperature was maintained similar to trial 2. But the duration of the secondary drying were increased. These changes were implemented in order to enhance the proper drying of the formulation

Lyophilization cycle of 35 hours

Process & Temperature	**Ramp duration (min)**	**Soak duration (min)**	**Pressure (torr)**
Freezing (-40°C)	150	180	NA
Primary Drying			
-20°C	180	60	1.0
-5°C	210	240	0.75
10°C	120	360	0.75
Secondary Drying			
20°C	90	120	0.3
35°C	150	240	0.1

Table 1: Evaluation parameters of all the batches

Sl.No	**Evaluation parameters**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**
1	Appearance	White in Color	White in Color	White in Color	White in color	White in Color
2	Moisture content %	7.45%	6.87%	6.31%	6.22%	4.1%
3	Cake formation	Melt pack	Partial melt pack	Cake sticking to the bottom of vial	Satisfactory cake	Good cake
4	Reconstitution time(sec)	37sec	32sec	29sec	22sec	19sec
5	pH(initial)	8.15	8.34	8.78	8.45	8.31
6	pH(After 24 hours)	8.16	8.33	8.79	8.47	8.33
7	Assay	96.23%	97.34%	97.28%	99.63%	99.78%
8	Particulate matter ≥10µm:Not more than 6000/vial	4356	4567	4768	4598	4362
	≥25µm:Not more than 600/vial	376	389	354	398	341
9	Related substances					

a	4-Desmethyl Omeprazole and Hydroxy Omeprazole	NMT 0.5%	NMT 0.5%	NMT 0.5%	NMT 0.5%	NMT 0.5%
b	Any unknown Impurity	NMT 0.2%	NMT 0.2%	NMT 0.2%	NMT 0.2%	NMT 0.2%
C	Total Impurity	NMT 1.5%	NMT 1.5%	NMT 1.5%	NMT 1.5%	NMT 1.5%

Figure 1: IR Spectra of Disodium Edetate

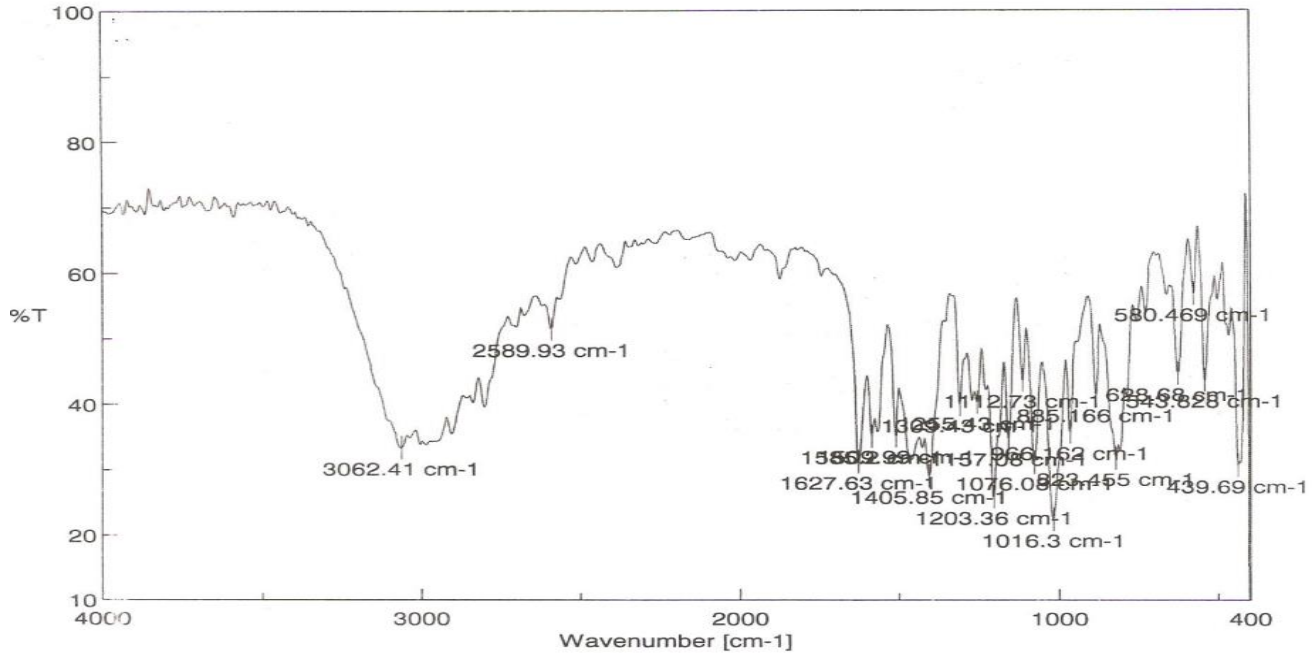


Figure 2: IR Spectra of Omeprazole

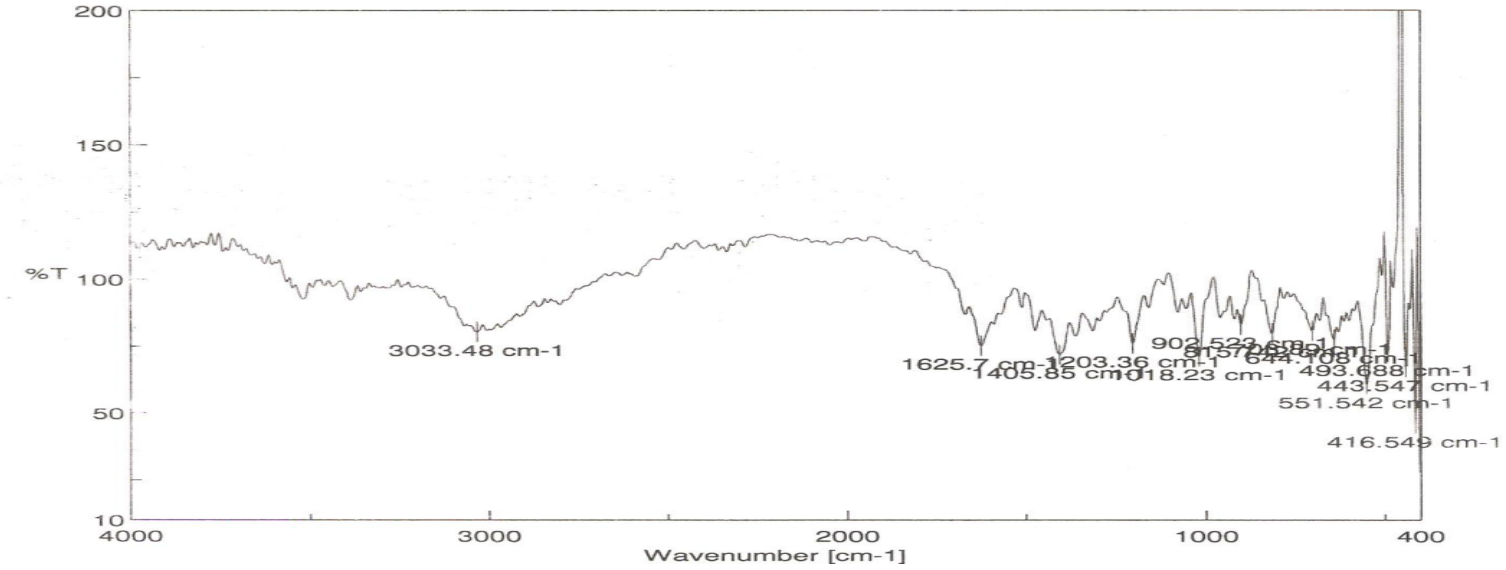


Figure 3: IR of optimized batch V

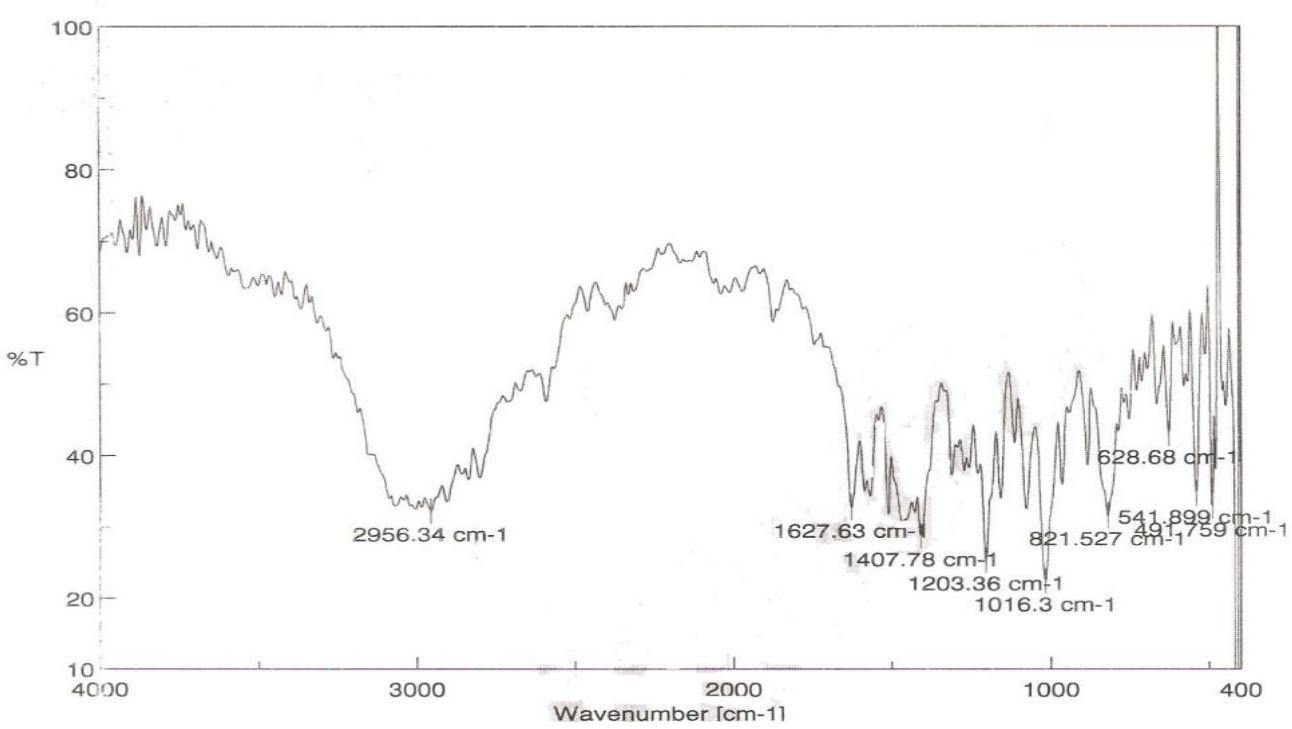


Figure 4: HPLC for Pure drug

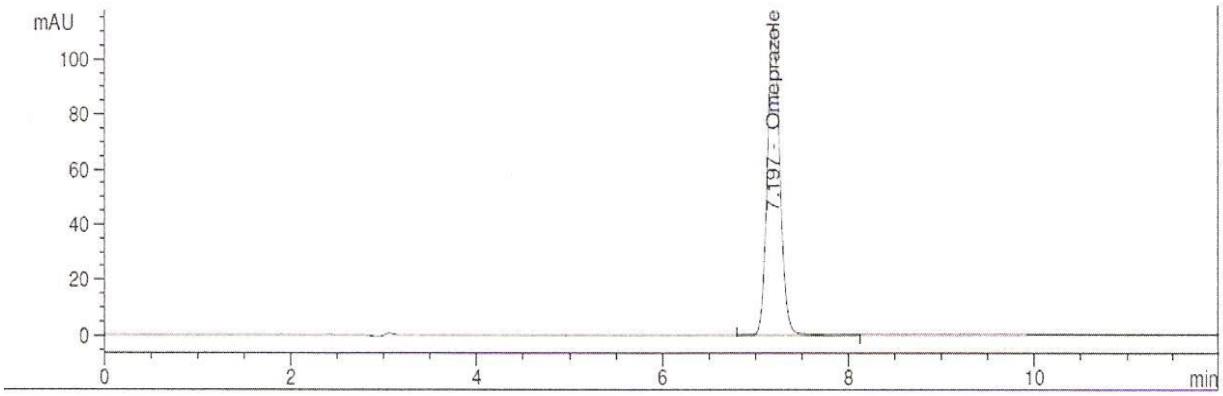


Figure 5: HPLC graph for optimized batch V

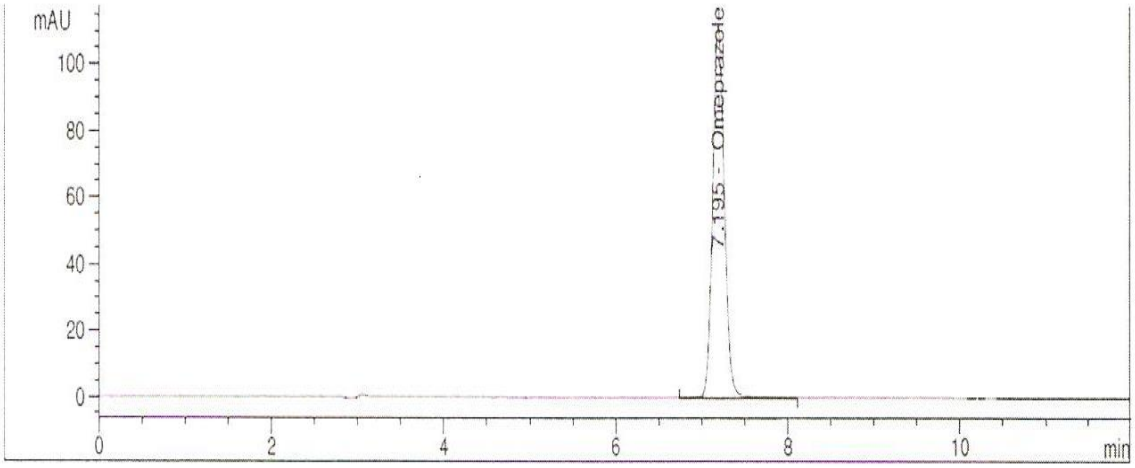


Figure 6: DSC Post-lyophilization

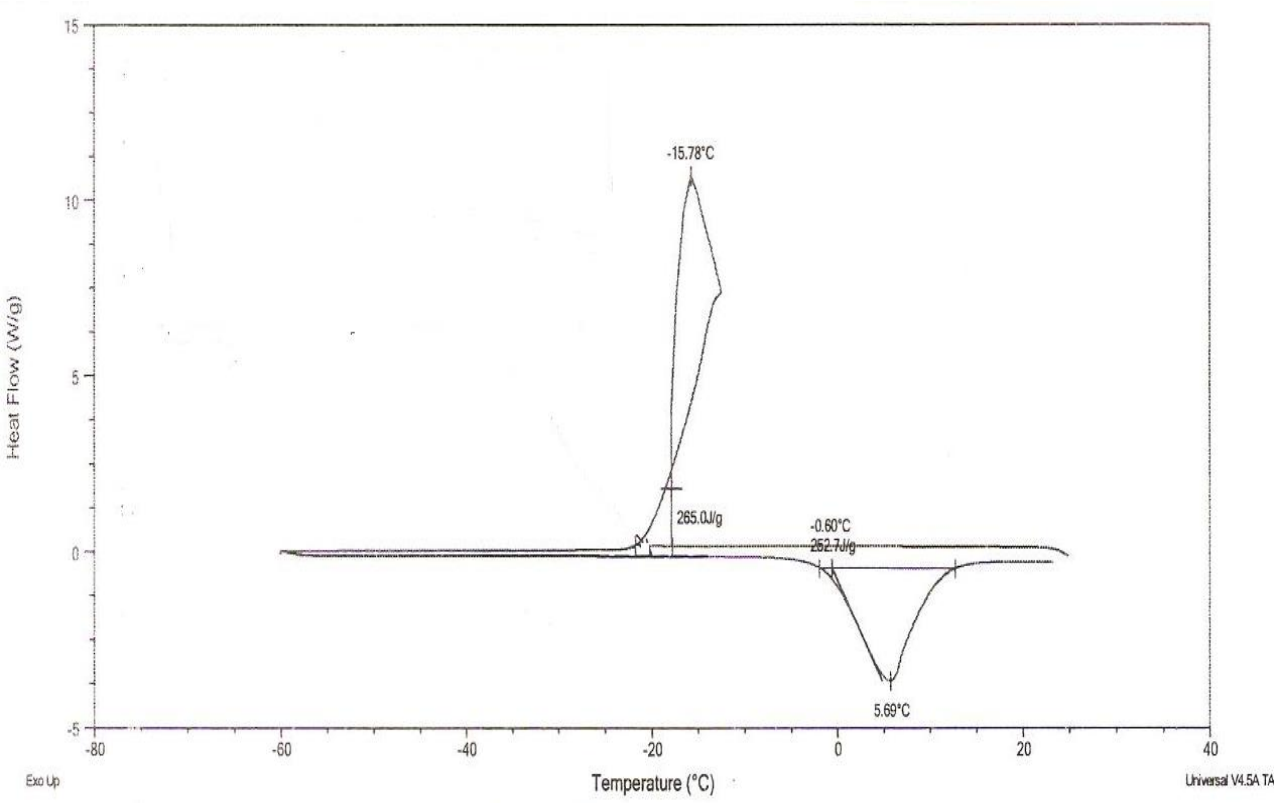


Table 2: Stability conditions: 40°C±2°C, 75%±5%RH

Sl.No	Evaluation parameters	Duration		
		First Month	**Second Month**	**Third Month**
1	Appearance	White in colour	White in colour	White in colour
2	pH	8.34	8.45	8.32
3	Reconstitution time	24sec	26sec	27sec
4	Assay	99.32%	99.84%	98.92%
5	Particulate matter ≥10µm:Not more than 6000/vial ≥25µm:Not more than 600/vial	4376 342	4387 387	4452 329
6	Related substances			
a	4-Desmethyl Omeprazole and Hydroxy Omeprazole	NMT 0.5%	NMT 0.5%	NMT 0.5%
b	Any unknown Impurity	NMT 0.2%	NMT 0.2%	NMT 0.2%
c	Total Impurity	NMT 1.5%	NMT 1.5%	NMT 1.5%

Figure 7: First month stability HPLC peak area at 40^{0}C ±2^{0}C /75% RH for batch-V

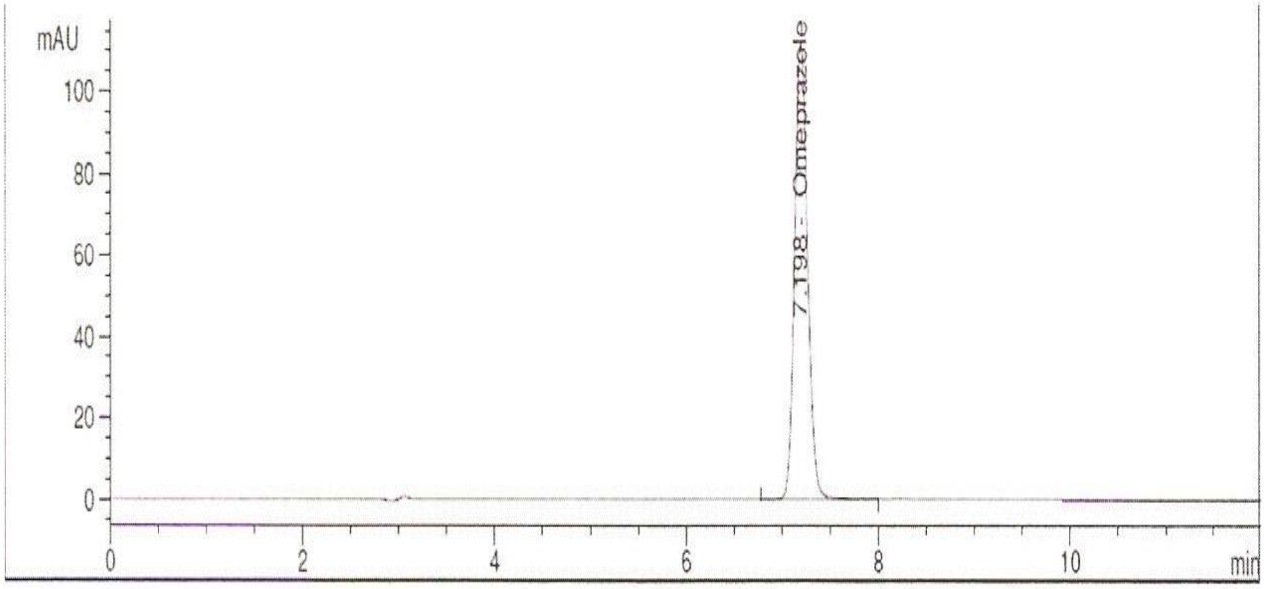


Figure 8: Second month stability HPLC peak area at 40^{0}C ±2^{0}C /75% RH for batch-V

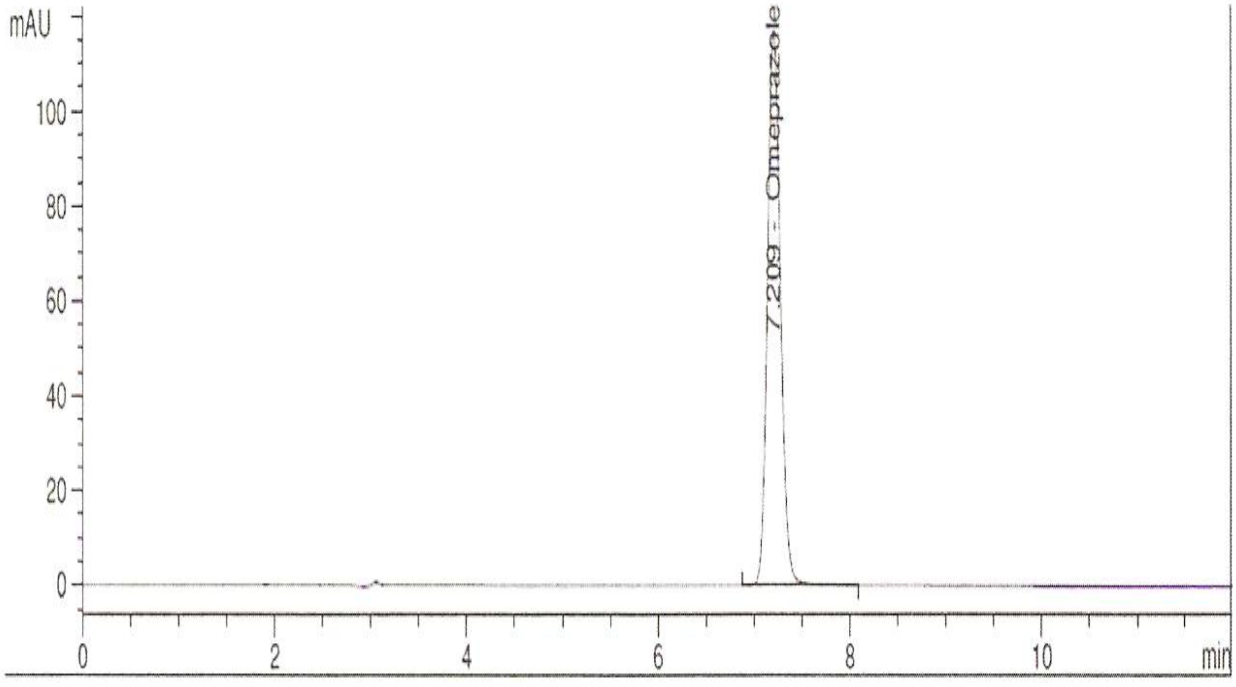
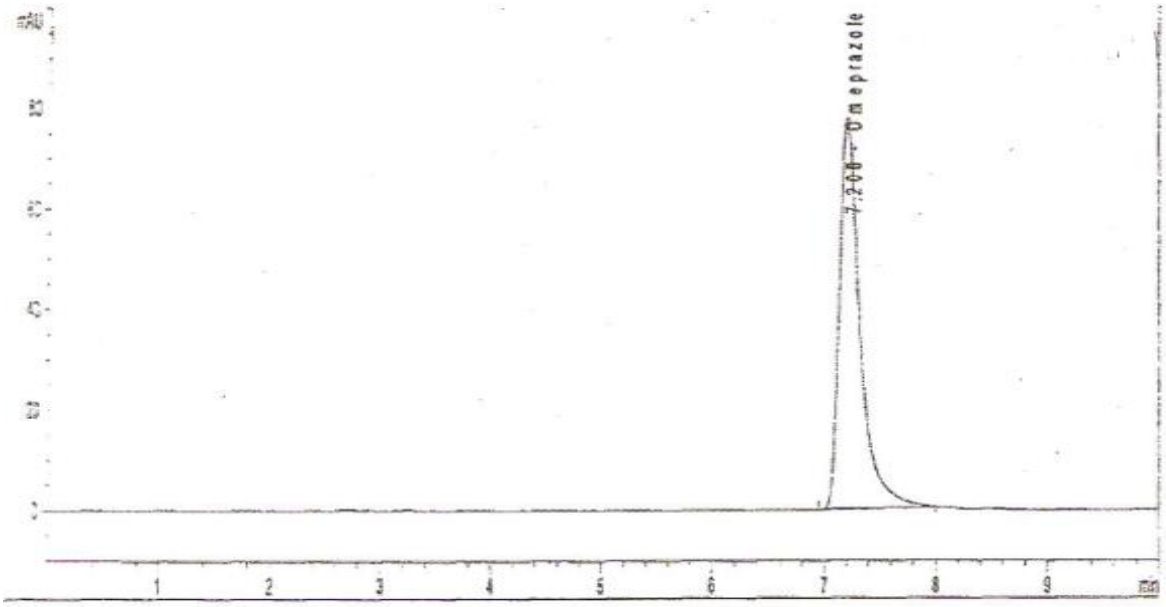


Figure 9: Third month stability HPLC peak area at 40^0C ±2^0C /75% RH for batch-V



III. CONCLUSION

The present research work was designed to develop a lyophilized injectable dosage form of an Anti-Ulcer drug Omeprazole Sodium. The drug is unstable if dispensed as liquid dosage form. Hence the present project was envisaged to overcome the drawbacks associated with Omeprazole sodium and to formulate a stable and therapeutically effective formulation by lyophilization technique which provides extended shelf life.

Based on the physicochemical properties of the drug , disodium edetate (chelating agent) and Sodium Hydroxide (Solubilizing agent), lyophilization technique was adopted to improve the cake characteristics of the lyophilized form of Omeprazole sodium Five different lyo cycle protocols were investigated sequentially to optimize the product characteristics. The batch-V of total duration of 35 hours was considered as the best formulation because it exhibited a good cake formation and the assay, pH, particulate matter and also percentage water content was found to be within the USP limits. Stability studies were conducted for the optimized formulation as per ICH guidelines for a period of three months which revealed that the formulation is stable.

From the above results, it was concluded that the lyophilization technique proves to be an advantage for development of stable injectable dosage form of Omeprazole sodium, hence our objective to develop a stable and therapeutically effective lyophilized injection of Omeprazole sodium was achieved.

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