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Research Article

**DESIGN AND DEVELOPMENT OF LYOPHILIZATION CYCLE
FOR PARECOXIB POWDER FOR SOLUTION FOR INJECTION
40 MG*** ¹Kabirdas B. Ghorpade, ²Rohit Bhalke, ³Sharda M. Shinde, ⁴Ujwal Hawelkar*^{1,2} Hetero Biopharma Ltd. Jedchelra.(Telangana) India.²School of Pharmacy SRTMU, Nanded. (Maharashtra) India.⁴Institute of Pharmaceutical Education and Research, Wardha (Maharashtra) India.**Abstract:**

Solution stability of the drug is the major challenge of the pharmaceutical industries. Lyophilization is also known as freeze drying is the subject of current research for most of the scientists to overcome these challenges. Lyophilization consists of three steps freezing, primary drying and secondary drying. Freezing is the pre requisite step for the primary drying where all the unbound water turns into freeze concentrate and water molecules removed by sublimation. Primary drying is the longest step of the lyophilization. In secondary drying the bound water which is associated with the drug molecules can be removed by the desorption by the application of heat and vacuum. The objective of study is Parecoxib different formulations used to Optimization of Lyophilization cycles and the main focus is to minimize consistently drying times, while maintaining constant Parecoxib Powder for Solution for Injection product quality.

Methods: The various formulations were studied for thermal events by DSC technique and the determination of the critical events by FDM analysis. By collaborated observations from these studies the lyophilization cycles were developed and studied.

Results: The data obtained from DSC and FDM analysis of drug solution was used to determine set temperature to be used during freezing, primary drying and secondary drying set temperatures. The set vacuum for the chamber during the primary drying and secondary drying was determined from the operation manual of Lyodryer LSI Freeze dryer. The optimized cycle was about 66 hrs used for better productivity. Evaluated the lyophilized product for water content and assay and x ray diffraction. Pharmaceutical equivalence study was conducted for Parecoxib powder for solution for injection with innovator product (Dynastar®).

Conclusion: Finally concluded that the present developed lyo cycle was used to execute the Parecoxib Powder for Solution for Injection product.

Keywords: Parecoxib, Lyophilization cycle, Impedance, FDM analysis, DSC Analysis, Poor thermal stability, Freezing, Primary drying, Secondary drying, X-Ray diffraction.

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INTRODUCTION:

Parecoxib Sodium is a water-soluble, injectable sodium salt form of parecoxib, an amide prodrug of the cyclooxygenase II (COX-2) selective, non-steroidal anti-inflammatory drug (NSAID) valdecoxib, with anti-inflammatory, analgesic, and antipyretic activities. Upon intravenous or intramuscular administration, parecoxib is hydrolyzed by hepatic carboxylesterases to its active form, valdecoxib. Valdecoxib selectively binds to and inhibits COX-2. This prevents the conversion of arachidonic acid into prostaglandins, which are involved in the regulation of pain, inflammation, and fever. This NSAID does not inhibit COX-1 at therapeutic concentrations and, therefore, does not interfere with blood coagulation. (1) Due to the huge capital investment and operational expenses, the optimization of the lyophilization cycle is the most important on-going challenge for the pharmaceutical industries. Optimization of the drying time with the constant product quality is the main task. (2). Obviously, the primary drying step should be carried out at the highest temperature possible, which is limited by the so called "maximum allowable temperature". This temperature indicates the eutectic temperature for a solute that crystallizes during freezing or the "collapse temperature" for a system that remains amorphous. Therefore, process control means control of the product temperature vs. time profile during lyophilization [2]. To reach this goal the balance between heat and mass transfer which determines the product temperature must be more or less equal Heat and mass transfer are also key issues during scale-up of lyophilization processes. Differences in (1) the degree of super cooling between laboratory, pilot and manufacturing plants, (2) heat transfer owing to differences in dryer design, and (3) the efficiency in the condenser or refrigerator system can result in substantial heterogeneity in sublimation rates and/or desorption rates and hence variation in drying time.

For optimum process control an indicator for the end of primary drying is needed, because an increase in shelf temperature before all vials have completed

primary drying carries a high risk of collapse of the product. Some well-established methods for distinguishing between primary and secondary drying are available, such as commonly-used thermocouples, the pressure rise test, manometric temperature measurements or comparative pressure measurement. New investigations in modern process control have also been made. Near-infrared spectroscopy, mass spectrometry and a remote electrode system are some examples representing a growing research area.

MATERIAL AND METHODS:**Materials:**

Parecoxib Sodium, drug substance was supplied as a gift sample by M/s MSN Organics Pvt. Ltd. Hyderabad (India). Disodium hydrogen Phosphate anhydrous, Phosphoric acid (Ortho-Phosphoric acid 85%) and Sodium hydroxide was supplied by merck. All the other chemicals used were of analytical grade.

Methods:**Glass Transition Temperature (T_g) Determination by DSC (10,11, 12)**

The Glass Transition Temperature (T_g) value of formulation was determined by differential scanning calorimetry (DSC). The principal information obtained from DSC analysis is the glass transition temperature in the frozen state (T_g) is the point at which an amorphous material shows signs of softening indicating a possible loss of structure may occur.

Collapse Temperature (T_c) Determination by FDM (Lyostat Analysis) (15,16)

Formulation was analyzed using the freeze-drying microscope (FDM) equipped with Linksys32 image and data capture software in accordance with BTL SOP AN 301. A 2 µL sample of each formulation was pipetted between a quartz slide and glass cover slip to create a 70 µm thick 'sandwich' of sample during FDM analysis ensures that the sublimation front can be observed so that the critical temperatures can be established. The principal information obtained from FDM analysis is the collapse (T_c) or Eutectic (T_{eu}) point of each formulation.

Table 1 : Temperature Profiles used in Determination of Collapse Temperature

Formulation	Procedure
Parecoxib 40 mg Lyophilized Powder for Solution for Injection	The sample was cooled to -40°C at a rate of 20°C/min and held at this temperature for 5 minutes before a vacuum was applied. The sample was dried at -40°C to allow a sublimation front to develop, at which point the sample temperature was raised until total collapse of the drying structure was observed.
Parecoxib 40 mg Lyophilized Powder for Solution for Injection (annealed)	The sample was cooled to -40°C at a rate of 20°C/min and held for 5 minutes. The sample was then ramped at 20°C/min to -10°C and held for a further 10 minutes to anneal before cooling back down to -40°C at 20°C/min to complete the annealing step. The temperature A second analysis was carried out that included an annealing step. An annealing step may be employed in order to encourage the crystallisation of one or more sample components that may exhibit a crystalline form in the frozen state but may be reluctant to do so upon initial cooling, as well as to increase the size of the ice crystals and to expand the ice crystal network. was held for 5 minutes before a vacuum was applied. The sample was dried at -40°C to allow a sublimation front to develop, at which point the temperature was raised until total collapse of the drying structure was observed.

Table 2: Composition of the Parecoxib Powder for Solution for Injection

Pharmaceutical Ingredient	Quantity/mL	Quantity/vial
Parecoxib sodium*	21.18 mg	42.36 mg
Disodium hydrogen Phosphate anhydrous	1.42 mg	2.84 mg
Phosphoric acid (Ortho-Phosphoric acid 85%)	Qs to pH adjustment	Qs
Sodium hydroxide	Qs to pH adjustment	Qs
Water for Injection ^Δ	Qs to 1.0 mL	Qs to 2.0 mL

* Quantity of Parecoxib sodium is based on 100% m/m Assay on anhydrous and solvent-free basis and nil water content.

Δ does not remain in lyophilized finished product except traces.

Manufacturing Procedure of Preparation of Bulk solution of Parecoxib 40 mg Powder for Solution for Injection

- Water for Injection was collected in a suitable container and cooled to 20° – 25°C.
- About 80% batch size of Water for Injection (WFI) from step 1 was transferred to a stainless-steel container.
- Disodium hydrogen phosphate anhydrous was added to the solution of step 2 under stirring and continued the stirring till to get clear solution.
- pH of step 3 solution was adjusted to 8.1 using 0.1M phosphoric acid solution.
- Parecoxib Sodium was added to the solution of step 4 under stirring and continued the stirring till to get clear solution.
- pH of step 5 solution was adjusted to 8.1 using 0.1M phosphoric acid solution.
- Volume was made up to the target batch size using cooled WFI of step 1 and stirring was continued to get clear solution.

Lyophilization Optimization Cycle Design

Based on previous lyophilization experience and knowledge about solute concentrations in formulation and formulation critical temperatures i.e. glass transition temperature (T_g) and collapse temperature (T_c), A cycle was designed and run.

- Freezing
Freezing is the most important and pre requisite step of the sublimation (Primary drying).
- Primary Drying:
Primary drying is the step where water is removed from the freeze concentrate when applied vacuum by sublimation. For the sublimation the solution should be full frozen. We had performed freeze drying microscopy and observed that the “collapse temperature (T_c)” of the bulk solution of Parecoxib with concentration of 20 mg/ mL of approximately -17.7°C (Lower collapse zone) and recommended Lyophilization drying temperature was -19.7°C to -24.7°C.

The shelf temperature in primary drying shall be ramped up from -25°C to $+30^{\circ}\text{C}$ in four steps. The heating rates during early steps of this ramp up would be kept below 3°C per hour. This ensures a gradual heating of the product vial the vacuum would be maintained 150 m Torr such that melt back during the process does not happen.

- Secondary drying:
It is the final step of the Lyophilization cycle where bound water molecules which are associated with the drug molecule can be removed by the application of the heat. Temperature of the secondary drying is depended on the stability of the molecule at particular temperature.

Table 3: Lyophilization Recipes for cycle optimization

Lyo recipe	RD0051-001				RD0051-002				RD0051-003			
	Temp (°C)	RAMP (mins)	HOLD (mins)	Vacuum (mT)	Temp (°C)	RAMP (mins)	HOLD (mins)	Vacuum (mT)	Temp (°C)	RAMP (mins)	HOLD (mins)	Vacuum (mT)
Loading	5	--	--	--	5	--	--	--	5	--	--	--
Freezing	-5	100	60	--	-5	40	180	--	-25	60	120	--
	-40	350	240	--	-40	360	240	--	-50	180	300	--
Primary Drying	-25	300	500	150	-30	300	400	200	-35	120	240	50
	0	200	840	150	0	300	420	150	-20	60	300	100
	25	300	300	150	25	420	660	150	-10	60	300	100
	35	20	300	150	35	20	300	150	0	300	420	100
	--	--	--	--	--	--	--	--	25	600	500	100
	--	--	--	--	--	--	--	--	35	20	300	50
Secondary Drying	55	40	360	50	55	40	300	50	55	40	300	50
Total Time	3910 minutes (65:10 hrs)				3980 minutes (66:20 hrs)				4220 minutes (70:20 hrs)			

RESULTS:

Glass Transition Temperature (T_g) Determination by DSC

Bulk solution of Parecoxib 40 mg Powder for Solution for Injection was exhibited glass transition temperature (T_g) of around -26.91°C . See figure 1.

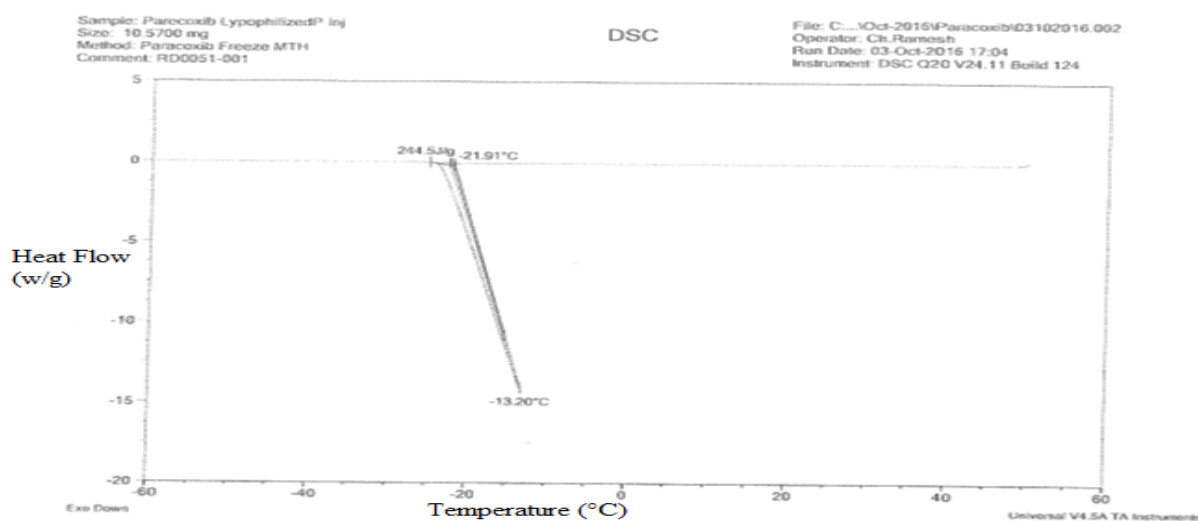


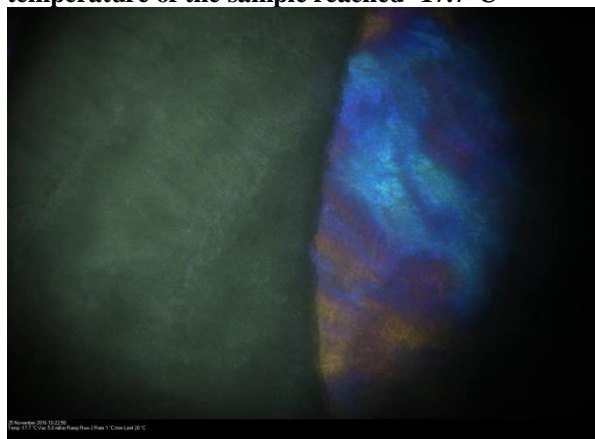
Figure 1 : Glass Transition Temperature (T_g) by DSC

Collapse Temperature (Tc) Determination by FDM (Lyostat Analysis)**Table 4 : Summary of observations made in Lyostat analysis**

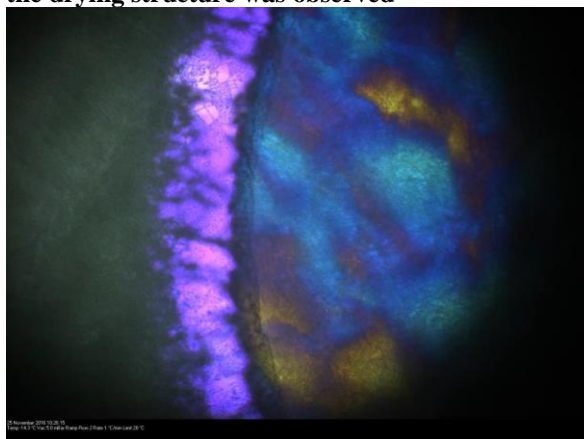
Formulation	Temperature at which the drying structure began to collapse	Temperature at which total collapse of the drying structure was observed
Parecoxib 40 mg Lyophilized Powder for Solution for Injection	-17.7°C	-14.3°C
Parecoxib 40 mg Lyophilized Powder for Solution for Injection (annealed)	-21.5°C	-14.4°C

Figure 2: Without Annealing Run Lyostat Images

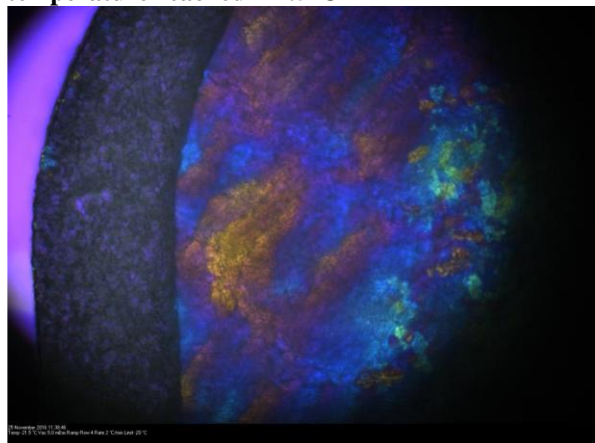
First signs of collapse onset observed as the temperature of the sample reached -17.7°C



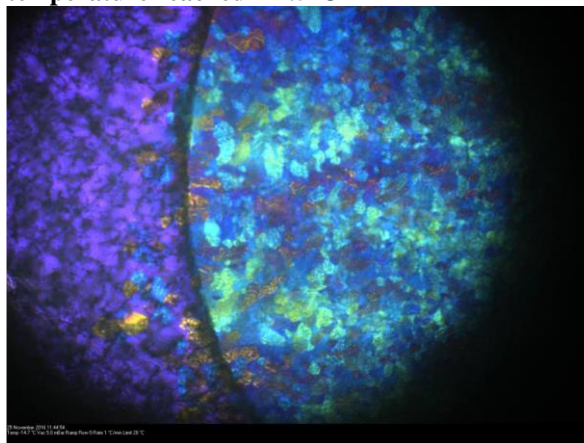
As the temperature reached -14.3°C, total collapse of the drying structure was observed

**Figure 3 : Annealing run Lyostat Images**

First signs of collapse onset observed as the sample temperature reached -21.5°C



First signs of collapse onset observed as the sample temperature reached -21.5°C



Physico chemical Analysis of the Lyophilized Finished Product :

- Cake Appearance
- The lyophilization cake was found uniform and elegant. See figure 4. The cake description was White to off-white lyophilized cake or powder.

Table 5 : Physico chemical Analysis of the Lyophilized Finished Product :

Batch No.	Specification	RD0051-001	RD0051-002	RD0051-003
Tests performed on lyophilized cake / powder				
Description	White to off-white lyophilized cake or powder	Off-white cake	Off-white cake	Off-white cake
Water content (% w/w)	NMT 3.5	1.06	0.65	1.17
Tests performed on reconstituted solution				
Description	Clear, colorless solution	Clear, colorless solution	Clear, colorless solution	Clear, colorless solution
Reconstitution time	NLT 60 seconds	30 seconds	21 seconds	35 seconds
pH	Between 7.5 and 8.5	7.95	8.01	7.98
Assay of Parecoxib (% w/w)	NLT 95.0 and NMT 105.0	101.4	100.7	100.4



Figure 4 : Description of the Finished Product

- Water content and Assay Analysis:

Water content of the Parecoxib Powder for solution for injection 40 mg vials varied from 0.65 % w/w to 1/17% w/w which is within the limit of NMT 3.5% w/w. Based on observations it can be concluded that drying of the product is uniform & elegant. Hence the Lyophilization parameters can be use for further batches. The results were shown in table 5.

The recommended method for assay of Parecoxib was optimized. The method was found suitable for assay analysis. The results are found satisfactory. Hence the recommended test method is suitable for analysis of assay of finished product. The results were shown in table 5.

Polymorphic identity determination: (20)

X -ray diffraction is the important analytical technique used to understand the freezing behavior of the freeze concentrate in frozen state and the crystallized component in freeze dried components. Figure 5.

Water content results are obtained in the range of 0.65% to 1.17% which is within the specified release limit of NMT 3.5%. The optical inspection of the batch showed a uniform product with no signs of shrinkage or melt back at the end of the lyophilization cycle. Parecoxib sodium exists Crystalline Polymorphic Form A. During the process of lyophilization, drug substance was converted into amorphous form. Based on the results obtained, no changes are required in lyophilization cycle recipe and it was concluded that the lyophilization process provides adequate process controls to lyophilize the drug product meeting the established release specifications.

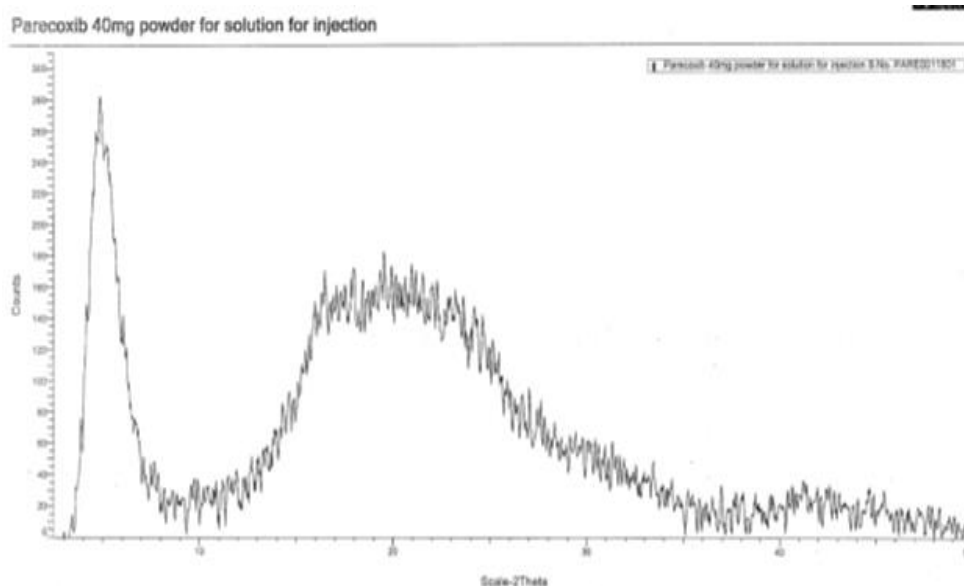


Figure 5 : X -ray diffraction (Polymorphic identity determination)

CONCLUSION:

DSC & FDM analysis were conducted on bulk solution. The data obtained from DSC & FDM analysis of drug solution was used to determine set temperature to be used during freezing, primary drying and secondary drying set temperatures. The set vacuum for the chamber during the primary drying and secondary drying was determined from the ice vapour pressure data. The proposed cycle was executed in batch. The operational ranges at different stages of the process were optimized. Results revealed that all the parameters are satisfactory. Pharmaceutical equivalence study was conducted for Gemcitabine for injection USP with innovator product (Dynastat). The data shows that both the products are pharmaceutically equivalent

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