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Original research article

Stabilization of the Pharmaceutical Finished Dosages form by Using Various Techniques

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Abstract

The stabilization of the drug product also involves stabilization of the drug product at room temperature which requires refrigerated condition for storage.

One of the major problems faced by the drug product manufacturer is the maintenance of the cold chain during manufacturing, storage, transportation thus increasing the cost of the product to the manufacturer as well as the end user, i.e. patients. The products which requires refrigerated condition for manufacture, storage and transportation are prone to degradation and loss of potency in case when the cold chain is broken inadvertently during any of the stages of the production, storage, transportation or usages.

The lyophilization is one of the technique which can be used for the stabilization of the drug product which are prone to hydrolytic and other types of degradations. The lyophilization process involves three stages (1) Freezing (2) Primary drying and (3) Secondary drying. The lyophilization process removes majority of the water from the frozen drug product during the primary drying and residual water is removed during the secondary drying.

The drug chosen for the stabilization is Posaconazole injection which is an antifungal agent and is being marketed with the brand name of Noxafil by Merck Sharp and Dhome. As per the package insert the Noxafil recommended storage condition is "Store refrigerated at 2-8°C (36-46°F)." thus making it one of the ideal drug product for stabilization at room temperature

Keywords: Stabilization, Posaconazole, Lyophilization, Refrigerated, Room temperature

1. Introduction:

There are many pharmaceutical products which are only stable at refrigerated condition. The refrigerated condition for storage as defined by USP 43 is "A cold place in which the temperature is controlled between 2° and 8° (36° and 46° F)[1].

The requirement to store the drug product at refrigerated condition posses' challenges from the initial manufacturing stage till it reaches the end user. In the developing countries like India or even in the developed countries maintenance of the cold chain[2] is a daunting task during transportation and storage specifically in the remote areas. The lack of availability of the cold chain compromises the safety and efficacy of the drug product and may result in untoward reaction, this leads to the spoilage of the drug product, in addition especially in developing countries the refrigerators are also not available with majority of the population and hence chances of improper storage increases many fold once the drug product is dispensed to the patient. In addition to the unavailability of the refrigerators in the rural areas

of the under developed or developing countries another major problem is availability of the uninterrupted power supply required for proper functioning of the refrigeration equipments.
The lack of availability of the storage facility has lead to the below incidents.
1. The major reason for polio outbreak in South Africa in 1990's was due to an incomplete cold chain of the polio vaccine[3].
2. In Hungary about 38% of the sampled vaccines were damaged due to cold weather[4].
As per the *Pharmaceutical & Medical Packaging News* surveyed supply chain experts in 2015 had arrived at these findings: Of temperature-sensitive products shipped, 51% were ambient, 31% were refrigerated, 17% were frozen, and 32% should not be allowed to freeze[,5].
More types and numbers of drugs are sensitive to temperature or time than ever. That's because drug research and development are evolving past traditional chemical-based, small-molecule therapeutics to more complex and often more effective large-molecule biologics[6,7].
The number of pharmaceutical products harmed by incorrect timing and temperature is difficult to pinpoint
In August 2017, for instance, a shipment from a single lot of Intralipid 20% IV fat emulsion, 100 mL bags (Baxter International, Inc.), was improperly exposed to subfreezing temperatures—those outside the labeled acceptable storage range—on its way to a distribution facility. The company voluntarily recalled this parenteral-nutrition product about two months later, and warned patients to dispose of their supplies. When frozen, the product's emulsion droplets enlarge, forming aggregates that can obstruct pulmonary circulation, leading to serious health problems and possible death[8]. Hence, it would be very beneficial if more and more drugs can be stabilized at room temperature from their present storage condition which require refrigerated or subzero temperature storage for maintaining the quality of the drug throughout the shelf life of the product.
The Posaconazole injection is such drug product wherein the recommended storage of the drug product is 2-8°C[9]. This work will focus on stabilizing the drug product at room temperature by one or more the techniques discussed subsequently in this work.
Hence, in order to stabilize the drug product Posaconazole injection at room temperature various batches were manufactured using complexing agent and surfactant for solubilisation and various combination of antioxidants for stabilization. These batches were further subjected to lyophilization so that hydrolytic degradation can be prevented.
This study was done with the objective of stabilizing the marketed drug product at room temperature which otherwise is stable at 2 - 8°C, this will help in preventing the degradation of the drug product when the drug product is inadvertently exposed to higher temperature i.e. more than the recommended temperature of 2-8°C. The drug product was stabilized by using various stabilizing agents and by removing aqueous media from the formulation by employing the technique called lyophilization.
Thus, by using the appropriate combination of excipients and lyophilizing the formulation the objective of stabilizing the refrigerated product at room temperature was achieved

2. Materials and methods

2.1. Materials

Posaconazole was obtained from Zhejiang Asun Pharmaceutical Company China , Betadex Sulfobutyl Ether Sodium Cyclodextrin (SBECD) was obtained from Zibo, Edetate disodium, Propylene glycol was received as a gift sample from Merck, Dimethyl acetamide was received as a gift sample from Finar, ethanol and sodium metabisulfite, sodium ascorbate were obtained from Pharmonix, Polysorbate 80 was received as a gift sample from Sepic, Kolliphor EL was obtained from Sigma, Arginine, L-cystiene, Butylated hydroxyl anisole,

Butylated hydroxyl tolune, Methionine were obtained from Excimed and Polyethylene glycol was obtained from Sigma

2.2. Methodology for preparation bulk solution.

The solubility of the Posaconazole is a major challenge in the formulation of injection hence, different solvents and solvent systems were tried to solubilise the Posaconazole, in all the trials the drug substance was added after addition of all the excipients as the excipients provides the required solubility and stability to the drug substance. The various trials were executed for solubilisation and stabilization of the drug product at room temperature. The trials are listed in the table below:

The batch/trial manufacturing involves the following steps

1. Collect 80% of water for injection or equivalent water like Milli Q water, purge nitrogen to achieve the dissolve oxygen content of 2 ppm or less. Continue nitrogen purging throughout the batch manufacturing.
2. Check the pH of the Milli Q water
3. Add slowly Betadex Sulfobutyl Ether Sodium (SBECD) to the step 1 under continuous stirring, carry on the stirring till clear solution is obtained.
4. Add Propylene glycol under stirring and stir till it get mixed with the solution of step 3
5. Add sodium metabisulfite and sodium ascorbate, Arginine L cystine, methionine and BHA and BHT sequentially under stirring, after addition of the one ingredient under stirring ensure that it is dissolved before addition of the next excipient's.
6. To the above step, add Polysorbate 80 and Kolliphor stir to dissolve.
7. Adjust the pH of the solution of step 6 to 2.5
8. To the above solution of step 6 add Posaconazole slowly under stirring, continue stirring till the API dissolves completely.
9. Check and adjust the pH to 2.6.
10. Make up the volume to 100% of the batch size
11. Purge nitrogen to achieve the dissolved oxygen content of less than 2 ppm.
12. Filter the solution through 0.2u PES filter membrane
13. Purge nitrogen to maintain the dissolved oxygen content of less than 2 ppm.
14. Fill the solution in glass vials, half stoppered and load into the Lyophilizer.

All the other batches with different composition were manufactured with the similar manufacturing process considering the solubility of the excipients and the function of these excipients.

2.3. Development and Optimization of room temperature stabilized Posaconazole Injection.

Selection of anti-oxidants, solubilizers, other components and optimization of the manufacturing process and the lyophilization cycle were carried out to produce a room temperature stabilized product. The experimental designed to achieve the pre-defined product characteristics are discussed in the below table 1 and in the text sequentially.

Table 1: Optimization of the Formulation Composition

S. No	Composition	innovator (16.7 mL)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10
1	Posaconazole	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0
2	Betadex Sulfobutyl Ether Sodium (SBECD)	400	-	-	-	-	-	-	-	150	200	200
3	Edetate disodium	0.180	-	-	-	-	-	-	-		-	-
4	Propylene glycol	-	200	200	-	-	-	200		-	-	-
5	Dimethyl acetamide	-	-	-	100	150	200	-		-		
6	SMBS	-	4	-	-	-	-	-		25	35	35
7	Sodium ascorbate	-	**5**	-	-	-	-	-		20	20	20
8	Polysorbate 80	-	-	-	-	-	-		20	40	40	40
9	Arginine	-	-				15	-		-	-	-
10	L – cystiene	-	-	-			30	-		-	-	-
11	HCl	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to Ph	Q.S to pH	Q.S to pH	Q.S to pH
12	NaOH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH	Q.S to pH
13	WFI	Q.S to 1 mL	Q.S to 1 mL	Q.S to 1 mL	Q.S to 1 mL	Q.S to 1 mL	Q.S to 1 mL	Q.S to 1 mL	Q.S to 1 mL	Q.S to 1 mL	Q.S to 1 mL	Q.S to 1 mL

SMBS: Sodium Metabisulfite, HCl: Hydrochloric acid, NaOH: Sodium Hydroxide, WFI: Water for Injection.

Trial 1: To the water for injection, sodium ascorbate, sodium metabisulfite, propylene glycol and ethanol were added sequentially followed by addition of Posaconazole under continuous stirring, stirring was continued for 2 hours with intermittent observations to check solubility of the API, however it was observed that there was no significant improvement in the solubilisation during the stirring duration of two hours hence, it was inferred that the API Posaconazole is not soluble in the selected solvent system of water for injection, propylene glycol and ethanol.

Trial 2: In the trial 2 the solution of water for injection, propylene glycol and ethanol was prepared, Posaconazole was added slowly under stirring to the solution of WFI, Propylene glycol and ethanol, stirring was continued for 2 hours with intermittent observation however, there was no appreciable improvement in the solubility of the API hence, trial was aborted and the product was discarded.

Trial 3: In the trial 3 attempt was made to solubilise the API in 10% solution of Dimethyl acetamide, there was slight improvement in the solubility of the API however the desired solubility of 18 mg similar to reference listed drug could not be achieved

Trial 4: In the trial 4 the percentage of Dimethyl acetamide was increased by 5% to 15% in water for injection there was appreciable improvement in the solubility of the Posaconazole, but the API was not dissolved completely

Trial 5: In this trial the percentage of the Dimethyl acetamide was increased to 20% the API solubility was approximately 22 mg/mL, other excipients added were argentine and L-cystine. This composition was not further evaluated due to known harmful effects of Dimethyl acetamide and its difficulty to remove during the lyophilization process.

Trial 6: In trial 6, the Dimethyl acetamide was replaced with equal amount of propylene glycol (20%) and 7.5% of ethyl alcohol to check the solubility of the API, though there was slight increase in the solubility of API as compared to earlier trials with propylene glycol however, the solubility was approximately 12 mg/mL which was way less than the desired solubility of minimum requirement of 18 mg /mL which is in-line with the strength of reference listed drug.

Trial 7: Although in the trial 5 the desired solubility of more than 18mg/mL was achieved however, it was not taken up for further development as Dimethyl acetamide is considered slightly toxic hence, in the trial 7, Polysorbate 80 and ethanol were dissolved followed by addition of Posaconazole 18mg/mL, to the solution of API. The API was not dissolved completely even at the end of 5 hours as the appearance was slightly turbid.

Trial 8: The aim of trial 8 was to check the solubility of the Posaconazole with reduced amount of Sulfobutyl Cyclodextrin with the aid of surface active agent Polysorbate 80. The other ingredients were added for their antioxidant properties. In this trial the reduced quantity of Sulfobutyl Cyclodextrin was taken i.e 150 mg/ mL and same was dissolved in the water for injection, after dissolution of Sulfobutyl Cyclodextrin, 40mg/mL of Polysorbate 80 was added and dissolved under slow stirring to minimize the foaming in the solution. In this solution of Sulfobutyl Cyclodextrin and Polysorbate 80 the active ingredient Posaconazole was added slowly under stirring, the stirring was continued for approximately 3 hours 30 minutes for the dissolution of the API; pH was adjusted using 0.1 N sodium hydroxide or 0.1 N Hydrochloric acid. After dissolution of the Posaconazole sodium metabisulfite and sodium ascorbate was added in the formulation because of their anti-oxidant properties, after dissolution of all the excipients volume was made to 100% of batch size. The solution filtered through 0.2u filter and filled into vials.

Trial 9: This trial was planned to see if the solubilization time can be reduced further, like the other trials the Sulfobutyl Cyclodextrin (200mg/mL) was dissolved in water for injection followed by addition of Polysorbate 80 (40mg/mL) was added and dissolved. To the solution of Sulfobutyl Cyclodextrin and Polysorbate 80, Posaconazole was added slowly under stirring, stirring was continued for 2 hours and 30 minutes and it was observed that the Posaconazole was completely solubilized at the end of the 2 hours 30 minutes. After solubilization of the Posaconazole the anti-oxidants sodium ascorbate followed by sodium metabisulfite were added in the bulk solution under continuous stirring, pH was adjusted using 0.1N Sodium hydroxide or 0.1N Hydrochloric acid, after pH adjustment volume was made up to 100% of the batch size, After volume make up the bulk solution was filtered through 0.2 micron 47 mm filter membrane and filled into vials.

The trial 9 batch was loaded on stability however, at 3 months accelerated stability time point there was decrease in the assay and increase in the impurity profile.

Trial 10: This trial (trial no. 10) was same as the trial no. 09 with respect to composition of the batch and the manufacturing process however, the temperature of the manufacturing process was reduced from room temperature to 2-8°C, the manufacturing temperature was reduced to arrest the degradation during bulk manufacturing. After completion of the compounding of the bulk solution; the bulk solution was filtered through 0.2 micron 47 mm filter membrane and filled into vials. Now to reduce the further degradation over stability the drug product was freeze dried/lyophilized to remove the water from the drug product so as to minimize the degradation due to oxidation and hydrolysis during course of the stability/storage.

After optimization of the manufacturing process of the bulk solution, Optimization of lyophilization cycle was carried out. The optimization of lyophilization cycle involves

1. Determination of collapse/glass transition temperature
2. Optimisation of freezing temperature
3. Optimisation of primary drying
4. Optimisation of vacuum during the drying stage
5. Optimisation of secondary drying

The lyophilization process involves removing the water content from the product at subzero temperature (frozen condition) under vacuum by the process called sublimation. The sublimation of the frozen material initiates at triple point. The triple point is defined as the temperature where the all three phases solid liquid and gases exists[10].

The lyophilization process is divided into the following parts

1. Freezing
2. Annealing
3. Primary drying and
4. Secondary drying

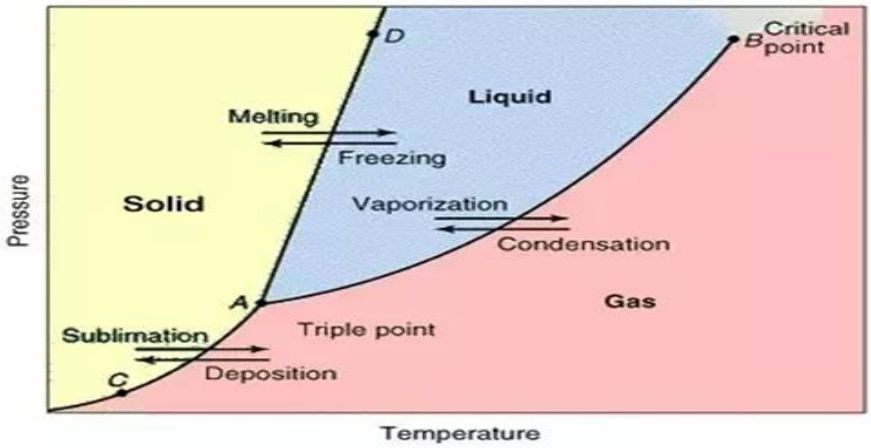


Figure 1: Image demonstrating triple point

Determination of critical /collapse temperature: Tc - Collapse temperature, this is the temperature at which the material softens to the point of not being able to support its own structure. Teu - Eutectic temperature, this is the temperature at which the solute material melts, preventing any structure forming after the solvent has been removed (www.biopharma.co.uk).

The collapse temperature was determined by using freeze drying microscope from BTL. The process involves freezing the small drop of bulk solution in the cryo-stage and then freezing the small drop of the bulk solution with the help of liquid nitrogen to -45°C or lower (depending on the type of product and then heating the frozen solid slowly with the applied vacuum. with the help of onstage camera the events are recorded and are observed on the

screen of the computer. the edges of the frozen solid are dried slowly when the vacuum is applied and the interface between the dried phase is observed for any changes, the temperature at which slight collapse/movement of the frozen material at the interface of dried and frozen phase indicates initiation of collapse, the temperature at which collapse starts is known as collapse or critical temperature. After the determination of the collapse temperature the primary drying temperature was determined and it was ensured that the primary drying is done at or below the determined collapse temperature however, there are literature available that the primary drying can be done above collapse temperature. The products which can be dried above collapse temperature help to reduce the lyophilization cycle time thereby reducing the overall cost of manufacturing, thus making the medicine more affordable[11].

Figure 2: Typical Set up of Freeze Drying Microscope

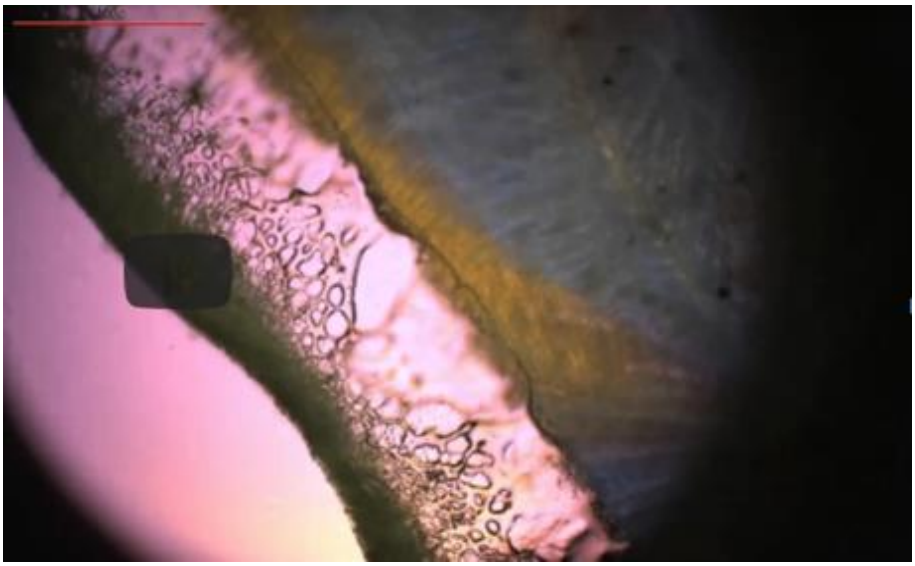


Figure 3: Collapse Temperature Determination

After determination of the collapse temperature, the following lyophilization cycle was executed, the lyophilization cycle was finalised based on the Physical appearance and moisture content. The finalised cycle is given below.

Table 2: Final Lyophilization cycle

Stage	Temperature (°C)	Ramp (min)	Hold time (min)	Vacuum (mtorr)
Pre-Cooling	5	50	60	-
Freezing	-45	133	600	
Primary Drying	0	170	1200	100
Secondary Drying	20	50	600	100
	40	100	1000	
Total Time		503	3460	Minutes
Total Time		3963	66	Hours

Lyo Trial 1: The desired parameters - physical, chemical and reconstitution time were achieved by the above cycle. The trial batch was prepared as per the process given in the trial 10, the bulk solution was filtered and filled in the glass vials, half stoppered loaded into the lyophilzer, the lyophilization cycle was executed as given in the above table, after completion of the cycle the vacuum was partially broken with the nitrogen and the vials were stoppered in the lyophilzer, after stoppering the vials, the vials were sealed and observed for any physical defects. The vials were loaded on stability and samples were analysed at predefined intervals as per ICH requirement.

After the Lyo Trial 1 another repeat trial batch (Lyo Trial 2) was planned to check the repeatability of the process parameters and the batch, the data is presented in the table.

Table 3: Stability Data

			Stability condition				
Test Parameters	**Tentative specification**	**Initial**	**40 ±5°C /75±5% RH**		**25 ±5°C /60±5% RH**		
			3 M (Inv.)	**6 M (Inv.)**	**3 M (Inv.)**	**6 M (Inv.)**	**12 M (Inv.)**
Description	#	#	#	#	#	#	#
pH after reconstitution (15mg/mL)	2.0 to 4.5	2.87	2.60	2.78	2.74	2.81	2.79
Water content (KF)	NMT 4.0 %	2.73	2.67	2.91	2.8	2.79	2.81
Reconstitution time (seconds)	180	100	120	80	100	130	120
Assay (%w/w)	90 to 110	98.2	102.9	100.2	97.5	99.5	98.4
Related substances (%w/w)							
Any unspecified degradation products	NMT 0.2	0.05	0.04	0.04	0.05	0.04	0.05
Total degradation products	NMT 2	0.19	0.16	0.17	0.21	0.17	0.23

#White to off white color cake or powder

The data for both the batches complied with the pre-determined specifications as per ICH Q3B (R2)[12] based on the maximum daily dose of 400 mg. The ICH defines the limits of known and unknown impurities. The table given in the guidance is presented below:

Table 4: Thresholds for Degradation Products in New Drug Products

Reporting Thresholds	
Maximum Daily dose	**Threshold**
≤1 g	0.1%
≥1 g	0.05%
Identification Threshold	
Maximum Daily dose	**Threshold**
<1m g	1.0% or 5 µg TDI whichever is lower
1 mg - 10mg	0.5% or 20 µg TDI whichever is lower
10 mg – 2 g	0.2% or 5 µg TDI whichever is lower
> 2 g	0.10%
Qualification Threshold	
Maximum Daily dose	**Threshold**
<10m g	1.0% or 50 µg TDI whichever is lower
10 mg - 100mg	0.5% or 200 µg TDI whichever is lower
>100 mg – 2 g	0.2% or 3mg TDI whichever is lower
> 2 g	0.15%

The representative chromatograms of the stability batches are presented below

Fig 4: Initial

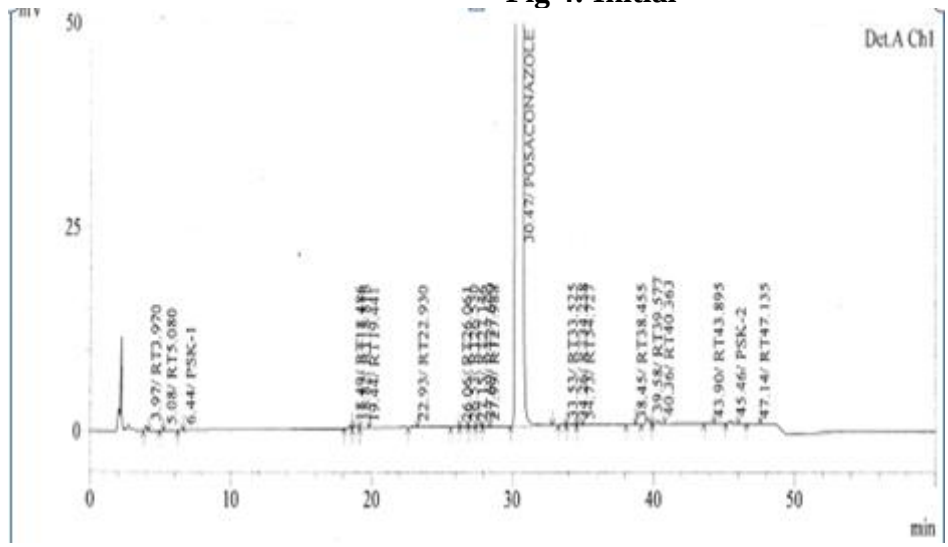


Fig 5: 3 M 25±2°C/60±5%RH

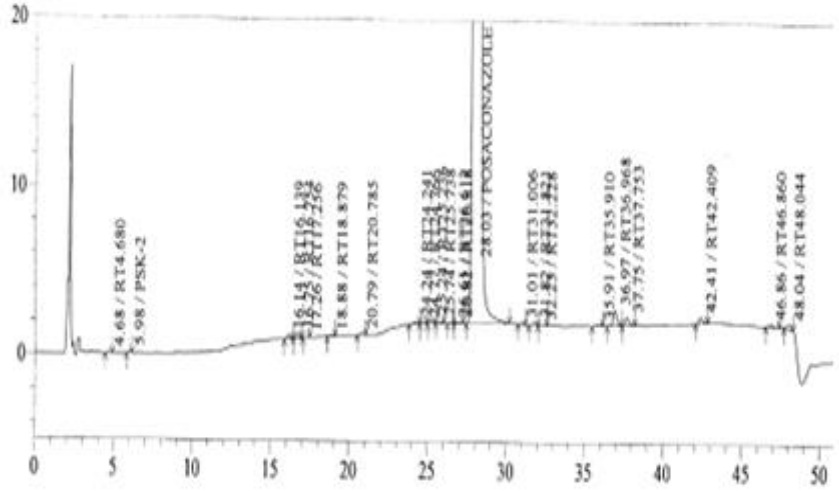


Fig 6: 6M ±2°C/60±5%RH

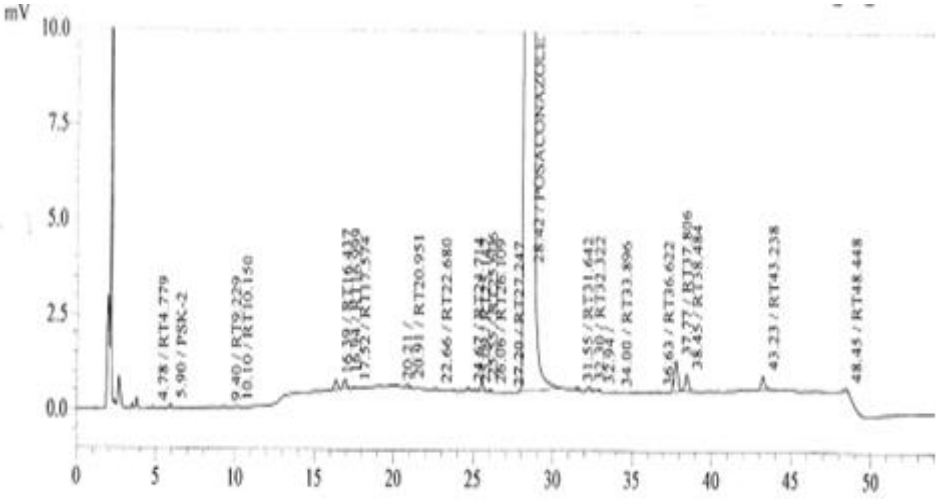


Fig 7: 12 M 25±2°C/60±5%RH

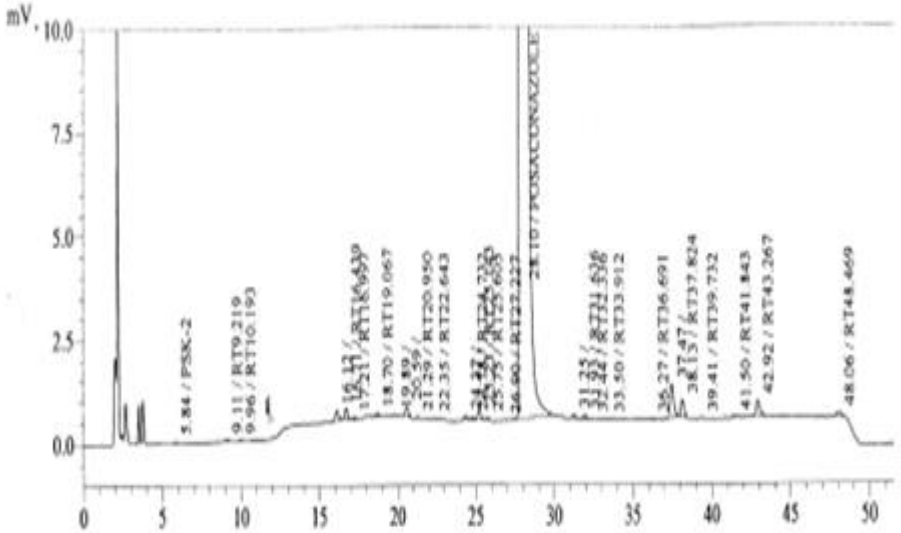


Fig 8: 3 M 40±2°C/75±5%RH

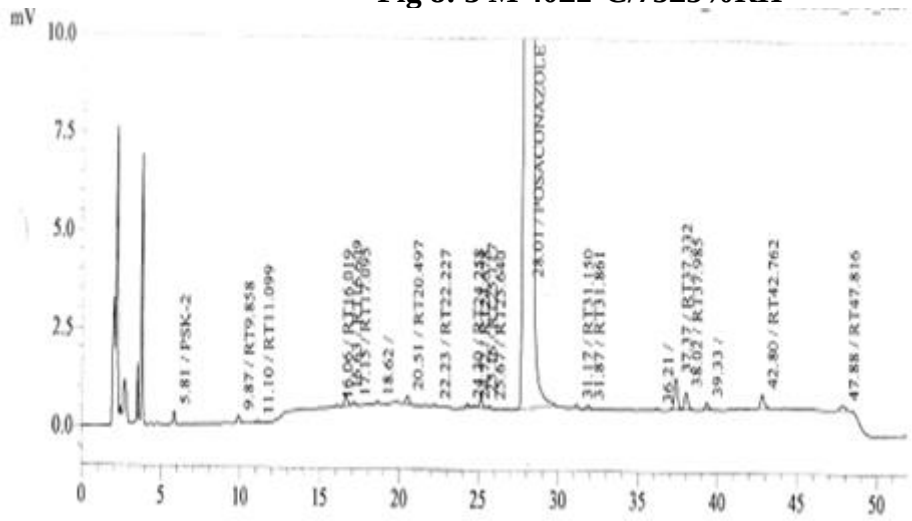
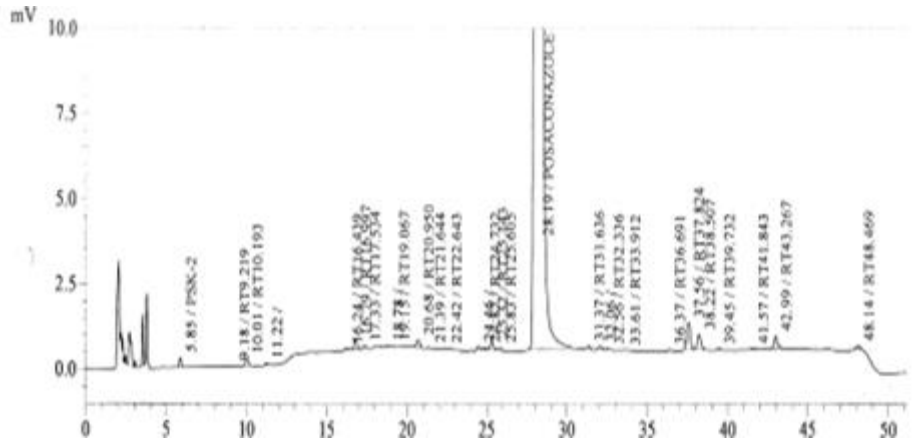


Fig 9: 6M 40±2°C/75±5%RH



Results and Discussion:

The solubility of the Posaconazole was achieved by addition of Sulfobutyl Cyclodextrin alongwith the addition of surface active agent i.e. Polysorbate 80. This lead to decrease in the amount of Sulfobutyl Cyclodextrin required for solubilization of the active pharmaceutical ingredient.

The antioxidant sodium ascorbate and sodium metabisulfite helped to stabilize the formulation against the oxidative degradation. The water from the formulation was removed by the process of lyophilization – this process involves removing the water from the formulation at subzero temperature thus eliminating the chances of degradation due to hydrolysis.

The data given in the table 3 demonstrate drug product stability up to 12 M at real time stability condition and 6 M at accelerated stability condition hence, it can be inferred that by adding suitable excipients and subjecting the drug product to lyophilization has helped to stabilize the drug product at room temperature which was originally stable at refrigerated condition and was not stable at room temperature.

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