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Development and Evaluation of Lyophilized Product of Apo-Acetozolamide

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Abstract: Lyophilization or freeze drying involves the removal of water or other solvent from a frozen product by a process called sublimation followed by desorption.Lyophilization is a multistage operation in which, quite obviously, each step is critical. The main actors of this scenario are all well known and should be under strict to achieve a successful operation.Freeze-dried products for parenteral use are known as Powders for injection or infusion. As the stability of Acetazolamide in aqueous solution form was unstable it was formulated as Lyophilized product.The objective of the present study was to develop a stable lyophilized formulation of drug Apo-Acetazolamide for injection (500mg/vial) which is therapeutically equivalent to the reference listed product, DIAMOX.

OBJECTIVE AND PLAN OF THE WORK:

OBJECTIVE

- As the stability of Acetazolamide in aqueous solution form was unstable it was formulated as Lyophilized product.
- The objective of the present study was to develop a stable lyophilized formulation of drug Apo-Acetazolamide for injection (500mg/vial) which is therapeutically equivalent to the reference listed product, DIAMOX .

PLAN OF THE WORK

To achieve the ultimate goal of formulating lyophilized product of Apo-Acetazolamide, the present work was designed to address the following objectives: -

- Literature survey
- Procurement of API, Excipients and Packaging materials
- Innovator sample procurement and its characterization
- Preformulation studies of the drug
- Formulation of the injectable dosage form
- Development of Lyophilization cycle
- Lyophilization of the injectable dosage form
- Evaluation of lyophilized product
- Stability studies as per ICH guidelines

METHODOLOGY

- The experimental work starts with the literature survey of innovator product followed by the procurement and characterization of innovator product, sourcing of raw materials (API and excipients) and packaging materials.
- Based on the literature review, the proposed generic composition shall contain the following ingredients:

LIST OF RAW MATERIALS AND THEIR SOURCE:

S.No	INGREDIENT	VENDOR
1	Acetozolamide sodium equivalent to Acetozolamide	Aurobindo Pharma Ltd, India
2	Sodium hydroxide	Merck, Germany
3	Water for injection	Aurobindo Pharma Ltd, India

PROCUREMENT & CHARACTERIZATION OF INNOVATOR PRODUCT

Innovator drug product is procured from the respective market of source country and then they are characterized so as to check whether they conform to the product specifications.

Characterization of the innovator product includes:

a) Label Information

Strength, batch number, shelf life, storage instructions, reconstitution(if necessary) etc.

b) Primary Package Details

Vial type, dimensions, volume, fill volume, label size, head space analysis, etc.

c) Product Analysis

Appearance, assay, related substances, impurities concentration, sterility (endotoxin concentration.), pH etc

d) Stability study

PREFORMULATION STUDIES:

API Characterization:

- Preformulation involves the characterization of physical, chemical and microbiological attributes of the drug substance thus providing useful information so as to develop a safe, effective and stable dosage form.
- In the present study, the primary objective of the preformulation study is to ascertain that the drug substance complies with the specifications or pharmacopoeial standards.

The various tests conducted on the API during the preformulation study and the specifications set for the drug substance are tabulated below:

LIST OF PREFORMULATION TESTS AND THEIR SPECIFICATIONS:

TESTS	SPECIFICATIONS
DESCRIPTION	White to faintly yellowish-white, crystalline, odorless powder
SOLUBILITY	Sparingly soluble in practically boiling water, slightly soluble in alcohol, very slightly sluble in water.
IDENTIFICATION a) By IR	The IR spectrum of sample recorded, as KBr should exhibit transmission minima (absorption maxima)mat the same wavelenths to these in the spectrum obtained with the Acetazolamide working standard.

b) By chemical test	A clear, bright yellow solution is produced. No heavy precipitate or dark brown color results after the mixing or heating.
WATER CONTENT (%w/w, by KF, determined on 1g)	Not more than 3.0
pH (0.5g of sample in 25mL of carbon dioxide free water)	Between 9.1 and 9.6
HEAVY METALS (ppm)	Not more than 0.002
ASSAY (By HPLC, %w/w, as $C_4H_6N_4O_3S_2$ on anhydrous basis)	Not less than 98% and not more than 102%

RELATED SUBSTANCES (By HPLC, %w/w)	
5-amino-1,3,4-thiadiazole-2-thiol	Not more than 0.10
5-amino-1,3,4-thiadiazole-2-sulphnamide	Not more than 0.10
N-(5-sulphanyl-1,3,4-thiadiazol-2-yl)acetamide	Not more than 0.10
N-(5-chloro-1,3,4-thiadiazol-2-yl)acetamide	Not more than 0.10
N-(1,3,4-thiadiazol-2-yl) acetaide	Not more than 0.10
5-Acetamido-1,3,4-thiadiazole-2-sulphonic acid	Not more than 0.10
N-[5-[5-acetamido-1,3,4-thiadiazole-2yl]sulphonyl-1,3,4-thiadiazol-2-yl]acetamide	Not more than 0.10
Any unknown	Not more than 0.10
Total	Not more than 0.5
RESIDUAL SOLVENTS: (By GC-HS, µg/g)	
Methanol	Not more than 1000
Acetone	Not more than 1000
N,N-dimethyl formamide	Not more than 880
ADDITIONAL SOLVENT:	
Benzene	Not more than 2

BACTERIAL ENDOTOXINS (USP/mg of Acetazolamide)	Not more than 0.5
ABSORBANCE/ COLOUR OF SOLUTION (Dissolve 0.5g in 25mL of methanol, at 440nm, 1cmcell)	Not more than 0.2

LIST OF APPARATUS AND EQUIPMENTS:

S.No	NAME OF THE EQUIPMENT	MANUFACTURER	USE OF EQUIPMENT
1	Lyophilizer	Tofflon Science and Technology Co Ltd., Shangai, China	To perform the process of Freeze drying
2	Freeze Drying Microscopy	Lyostat3, Biopharma technology limited.	To determine the collapse temperature(T_c).
3	Weighing balance	Sartorius, Japan	To weigh the raw materials and finished product
4	P^H meter	Thermoscientific (Model: Orion 5 star)	To find out the P^H of the product before and after Lyophilization
5	Filtration unit	Millipore	To clarify the drug solution
6	Modulated DSC	TA instruments (Model : Q 2000 series)	To determine the eutectic temperature of the drug
7	HPLC	Shimadzu HPLC LC 2010	To know the assay and related substances of the drug
8	KF titrator	Metrohm	To determine the water content of lyophilized drug
9	Particle size analyzer	Malvern Instruments, UK	To determine the size of particles

BACTERIAL ENDOTOXINS (USP/mg of Acetazolmide)	Not more than 0.5
ABSORBANCE/ COLOUR OF SOLUTION (Dissolve 0.5g in 25mL of methanol, at 440nm, 1cm cell)	Not more than 0.2

FORMULATION DEVELOPMENT:

As the Qualitative & Quantitative composition of Acetazolamide sodium for injection is given in the prescribing information leaflet of DIAMOX, the drug and excipients are selected based on the innovator formula.

PROTOTYPE FORMULA:

Sr. No.	Ingredients	Qty/Vial	Rationale	Present in innovator
1	Acetazalamide Sodium equivalent to Acetazolamide	500 mg	Active	Yes
3	Sodium Hydroxide	qs to pH	For pH Adjustment	Yes
4	Water for Injection	qs to 5mL	Solvent	Yes

MANUFACTURING PROCEDURE:

- Collect required batch size of Water for Injection in SS vessel; and cool the temperature to 4-8°C.
- Transfer 80% of Water for Injection from the above SS vessel to another SS vessel.
- Add weighed quantity of Acetazolamide with continuous stirring to get a clear solution.
- Check the pH and adjust the pH to 9.6 by using 1N NaOH.
- Make up the solution to required batch size with Water for Injection and check the final pH (Adjust if necessary).
- Filter the bulk solution through 0.2µ membrane filter.

- Fill the solution into to 10mL USP Type I clear glass vials and half stopper it with double slotted chlorobutyl rubber stoppers and load for lyophilization as per optimized lyophilization cycle.
- After completion of the lyophilization, stopper the vials under vacuum and seal with aluminium flip-off seals.

EVALUATION OF LYOPHILIZED PRODUCT:

The lyophilized product was evaluated for the following formulation characteristics:

- Description
- Clarity of reconstituted solution
- Reconstitution time
- pH after reconstitution
- Water content
- Assay
- Related substances
- Particulate matter

STABILITY STUDIES:

STABILITY STUDIES AS PER ICH GUIDELINES:

Recommended Stability Condition/Station:

STATION	STORAGE CONDITION		
	40°C/75 % RH	30°C/65% RH	25°C/60% RH
1M	#↓	-	-
2M	#↓	-	-
3M	#*↓@	#↓	#↓
6M	#*↓@	#↓	#↓
9M	-	-	-
12M	-	#*↓	#*↓
24 M	-	-	@↓

- For analysis

* - BET (Bacterial Endotoxin Test)

↓ - Invert

@ - Reconstitution stability study

COMPATIBILITY STUDIES WITH PROCESS COMPONENTS:

Definition: Compatibility is a measurement of how stable a substance when it is in contact with another material. If there is no change in their physical and chemical properties of substance when it contacts with other materials is considered as compatible.

If changes happen in their physical and chemical properties of substances on contact with other material are considered as incompatible.

Types of Compatibility Studies:

- Filter compatibility
- Silicone Tubing compatibility
- SS Vessel compatibility
- 'O' ring compatibility

Study Design: This study can be in two ways.

Static Soak Method: In this study, the test filter shall be soaked in the drug product solution for longer period.

Dynamic Filtratio*n:* In this study, the liquid is continuously circulated through the filter membrane repeatedly.

Of these both methods, static soak method was used for the present study.

Experimental Procedure:

- The test shall be subjected to autoclaving and then use it for the compatibility studies.

- Place material in drug product solution and kept aside.
- At regular time intervals (0, 4, 8, 12, 24 hours) withdraw the required amount of sample and perform analysis of it.

RESULTS AND DISCUSSION

EVALUATION OF INNOVATOR PRODUCT:

Product name : DIAMOX I.V
Drug substance : ACETAZOLAMIDE
Manufactured by : Bedford laboratories
Dosage form : Powder for injection
Storage condition : Store at room temperature
Batch number : AT2008
Expiry date : 15/1/2014

S.No	TEST PARAMETER	OBSERAVATION
1.	**DESCRIPTION**	White freeze dried powder for injection
2.	**ASSAY**	101%
3.	**WATER CONTENT (%w/w)**	4.7
4.	**pH AFTER RECONSTITUTION**	9.6
5.	**RECONSTITUTION TIME**	54s
6.	**CONSTITUTED SOLUTION**	clear and free from visible particles
7.	**RELATED SUBSTANCES** **Total impurities (%w/w)**	0.5%
8.	**Bacterial endotoxins**	Not detected

PREFORMULATION STUDY:

API CHARACTERIZATION:

TESTS	SPECIFICATIONS	OBSERVATIONS
DESCRIPTION	White to faintly yellowish-white, crystalline, odorless powder	A white powder, crysatalline, odorless powder
SOLUBILITY	Sparingly soluble in practically boiling water, slightly soluble in alcohol, very slightly soluble in water	Sparingly soluble in practically boiling water, slightly soluble in alcohol, very slightly soluble in water
IDENTIFICATION a) By IR	The IR spectrum of sample recorded, as KBr should exhibit transmission minima (absorption maxima)mat the same wavelenths to these in the spectrum obtained with the Acetazolamide workingstandard.	Concordant with that of Acetazolamide reference sample
B) By chemical test	A clear, bright yellow solution is produced. No heavy precipitate or dark brown color results after the mixing or heating.	Positive

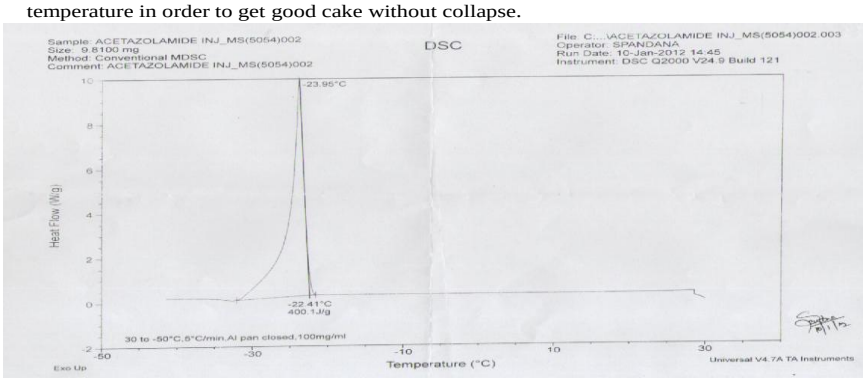
WATER CONTENT	Not more than 0.5	0.5
pH	Between 9.1 and 10	9.5
HEAVY METALS (ppm)	Not more than 0.002	Not more than 0.002
ASSAY	Not less than 98% and not more than 102%	99.8
RELATED SUBSTANCES Total	Not more than 0.5	Not detected
RESIDUAL SOLVENTS: Methanol Acetone N,N-dimethyl formamide **ADDITIONAL SOLVENT:** Benzene	 Not more than 1000 Not more than 1000 Not more than 880 Not more than 2	 Not detected 1002 400 Not detected
BACTERIAL ENDOTOXINS	Not more than 0.5	Not detected
ABSORBANCE OF SOLUTION	Not more than 0.2	0.174

From the above results, the API was found to be within its specifications given by the vendor and thus the drug substance Acetazolamide sodium can be used for the present study.

LYOPHILIZATION CYCLE DEVELOPMENT

DETERMINATION OF EUTECTIC TEMPERATURE

- The Eutectic temperature is determined by using *Modulated Differential Scanning Calorimeter* and was found to be -23.95°C.
- So in the process of lyophilization, the product should be freezed below this temperature in order to get good cake without collapse.



LYOPHILIZATION CYCLES

TRIAL1

PRECOOLING

SL.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180

PRIMARY DRYING

SL.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION(MIN)	PRESSURE (µbar)
1	-32	60	900	150
2	-10	60	480	150
3	0	60	120	150

CYCLE 2

PRECOOLING

SL.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180
3	-30	30	45
4	-45	30	120

PRIMARY DRYING

SL.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION(MIN)	PRESSURE (µbar)
1	-32	120	900	250
2	-10	30	300	100
3	0	120	300	150

SECONDARY DRYING

SL.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION	PRESSURE (µbar)
1	10	60	180	50
2	30	30	180	50
3	40	20	600	50

OBSERVATION: The moisture content observed was 10.1%.

To reduce the water content a new modified cycle was taken in the next trial.

CYCLE 3

PRECOOLING

S.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180

PRIMARY DRYING

S.NO	FINAL T(℃)	RAMP DURATION (MIN)	SOAK DURATION (MIN)	PRESSURE (µbar)
1	-25	120	300	250
2	-10	30	900	100
3	0	120	600	150

SECONDARY DRYING

S.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION	PRESSURE (µbar)
1	10	60	240	50
2	30	30	240	50
3	40	20	240	50

OBSERVATION

- The moisture content was found to be 8.4%

CYCLE 4

PRE-COOLING

S.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180
3	-30	30	45
4	-45	30	120

PRIMARY DRYING

S.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION(MIN)	PRESSURE (µbar)
1	-25	120	300	250
2	-10	30	300	100
3	0	120	300	150

SECONDARY DRYING

S.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION	PRESSURE (µbar)
1	10	60	240	50
2	30	30	240	50
3	40	20	240	50

OBSERVATION

- The moisture content was found to be 6.36%.

CYCLE 5

PRE-COOLING

S.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180

PRIMARY DRYING

Ss.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)	PRESSURE (µbar)
1	-30	120	300	250
2	-25	100	590	150
3	-15	10	600	100
4	0	120	600	150

SECONDARY DRYING

S.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION	PRESSURE (µbar)
1	10	60	240	50
2	30	30	240	50
3	40	20	240	50

OBSERVATION

- The moisture content was found to be 5.7%And time taken by the cycle is 67.80hrs=2.8days.

  To reduce the cycle duration a new modified cycle was taken in the next trial.

CYCLE 6

PRE-COOLING

S.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180

PRIMARY DRYING

S.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)	PRESSURE (µbar)
1	-30	120	300	250
2	-25	120	590	150
3	-15	10	600	100
4	0	120	300	100

SECONDARY DRYING

S.NO	FINAL T(℃)	RAMP DURATION(MIN)	SOAK DURATION	PRESSURE
1	10	60	240	50
2	30	30	240	50
3	40	20	240	50

OBSERVATION

- The moisture content was found to be 4.3% and the cycle time was reduced to 62.3 hrs from 67.80hrs.

EVALUATION OF LYOPHILIZED PRODUCT:

S.No	TEST PARAMETER	SPECIFICATION	OBSERVATION
1.	**DESCRIPTION**	White freeze dried powder for injection	White freeze dried powder for injection
2.	**ASSAY**	98% -102%	101%
3.	**WATER CONTENT (%w/w)**	4.7	4.3
4.	**pH AFTER RECONSTITUTION**	9.6	9.6
5.	**RECONSTITUTION TIME**	54s	50sec
6.	**CONSTITUTED SOLUTION**	clear and free from visible particles	clear and free from visible particles
7.	**RELATED SUBSTANCES Total impurities (%w/w)**	0.5%	0.08%
8.	**Bacterial endotoxins (EU/mg)**	NMT 0.5 USP	Not detected

Based on the results obtained from the characterization product, it can be concluded that the drug product obtained is within its specified limits of the inovator product.

OBSERVATION:

- The manufacturing process adopted for the batch provided a clear colorless solution at the end of the bulk solution compounding. the lyophilized product complied with the tentative specifications for the product with respect to the tested critical parameters like assay, related substances ph and the water content was found to be 4.3%. And time taken by the cycle is 62.30hrs = 2.5days.
- The water content of this batch was reduced and the cycle time was reduced to 62.3 hrs from 67.80hrs.

CONCLUSION: The bulk solution manufacturing process used in this particular trial would be used as standard manufacturing process for all further studies unless started.

- As the water content of this batch was low, the same recipe has been proposed as final cycle for exhibit batch.

RECONSTITUTION STABILITY STUDIES:

RECONSTITUTION WITH 0.9% SODIUM CHLORIDE INJECTION:

S.No	Test parameter	Specification	Reconstitution with 0.9%NaCl			
			0 hrs	6 hrs	12 hrs	24 hrs
1.	**Description**	Clear, colour less solution	Clear, colour less	Clear colour less	Clear colourless	Clear colour less
2.	**Assay**	98-102%	99.7	100.4	100.1	98.9
3.	**pH**	9.6	9.62	9.61	9.62	9.63
4.	**Related substances Total impurities**	NMT 0.5%	0.03	0.2	0.23	0.3
5.	**Particulate matter (per container)**	≥25µ - 600/unit	6	14	213	42
		≥10µ - 6000/unit	131	180	190	430

From the above results, it can be concluded that **0.9% SODIUM CHLORIDE INJECTION** for reconstitution.

COMPATIBILITY STUDIES WITH PROCESS COMPONENTS:

FILTER COMPATIBILITY STUDY:

S.No	Test parameter	Specification	Initial	PVDF Filter compatibility			
				4 hrs	8hrs	12hrs	24hrs
1.	**Description**	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colou r less
2.	**Assay**	90-102%	99.5	99.2	99.4	99.1	98.9
3.	**pH**	9-10	9.62	9.63	9.63	9.62	9.63
4.	**Related substances Total impurities**	NMT 1.0%	0.03	0.04	0.08	0.08	0.08
6.	**Particulate matter (per container)**	≥10µ - 6000	224	310	320	325	957
		≥25µ - 600	6	24	26	32	62

From the above results, it can be concluded that PVDF filter can be used for the purpose of the filtration during the experimental work.

SILICON TUBING COMPATIBILITY STUDY:

1. PHARMA PURE TUBE:

S.No	Test parameter	Specification	Initial	Pharma Pure compatibility (storage at 2-8 °C)			
				4 hrs	8hrs	12hrs	24hrs
1.	**Description**	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Small particles observed	Small particles observed
2.	**Assay**	98-102%	101	99.7	98.2	98.1	97.9
3.	**pH**	9.6	9.62	9.63	9.64	9.63	9.64
5.	**Related substances Total impurities**	NMT 0.5%	0.03	0.01	0.1	0.1	0.1
6.	**Particulate matter (per container)**	≥10µ - 6000	224	367	585	1064	2020
		≥25µ - 600	6	108	120	128	310

2. PHARMA 50 TUBE:

S.No	Test parameter	Specification	Initial	Pharma 50 compatibility (storage at 2-8 °C)			
				4 hrs	8hrs	12hrs	24hrs
1.	Description	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Small particles observed	Small particles observed
2.	Assay	98-102%	101	99.3	99.2	99.2	98.9
3.	pH	9-10	9.62	9.62	9.63	9.63	9.64
4.	Related substances Total impurities	NMT 1.0%	0.03	0.07	0.08	0.08	0.09
5.	Particulate matter (per container)	≥10µ - 6000	224	260	280	630	920
		≥25µ - 600	6	17	22	33	43

From the above results, it can be inferred that the silicon tubings should not be in contact with the drug solution for more than 8hrs because beyond that period particle growth was being observed in the drug solution.

Of both the tubings, Pharma 50 has shown better results than Pharma Pure relating to particulate matter. So Pharma 50 can be used during the experimental work.

3. SS VESSEL COMPATIBILITY STUDY:

S.No	Test parameter	Specification	Initial	SS Vessel compatibility (storage at 2-8 °C)			
				4 hrs	8hrs	12hrs	24hrs
1.	Description	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less
2.	Assay	98-102%	101	99.8	99.5	99.1	98.9
3.	pH	9.6	9.62	9.64	9.64	9.64	9.65
4.	Related substances Total impurities	NMT 0.5%	0.03	0.04	0.04	0.07	0.08
5.	Particulate matter (per container)	≥10µ - 6000	224	180	220	280	1024
		≥25µ - 600	6	4	17	32	81

The above results indicate that SS Vessel was found to be compatible with the drug solution throughout the study period and therefore it can be used for the holding the drug solution before loading in to the lyophilizer.

'O' RING COMPATIBILITY STUDY:

1. SILICON 'O' RING:

S.No	Test parameter	Specification	Initial	Silicon O ring compatibility (storage at 2-8 °C)			
				4 hrs	8hrs	12hrs	24hrs
1.	Description	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less
2.	Assay	98-102%	99.8	99.8	99.4	99.3	99.2
3.	pH	9.6	9.62	9.63	9.63	9.64	9.63
4.	Related substances Total impurities	NMT 0.5%	0.03	0.04	0.03	0.06	0.1
5.	Particulate matter (per container)	≥10µ - 6000	224	223	240	441	820
		≥25µ - 600	6	7	11	22	72

2. PTFE 'O' RING COMPATIBILITY:

S.No	Test parameter	Specification	Initial	PTFE O ring compatibility (storage at 2-8 °C)			
				4 hrs	8hrs	12hrs	24hrs
1.	Description	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less
2.	Assay	98-102%	101	99.8	99.5	99.4	98.7
3.	pH	9.6	9.63	9.62	9.63	9.61	9.62
4.	Related substances Total impurities	NMT 0.5%	0.03	0.07	0.08	0.08	0.03
5.	Particulate matter (per container)	≥10µ - 6000	232	290	312	351	421
		≥25µ - 600	5	7	7	10	13

Even though both the 'O' rings had shown satisfactory results with respect to the assay, pH and related substances, Silicon 'O' ring had favored more particulate growth than PTFE 'O' ring during the analysis period. So, PTFE 'O' ring can be selected for the present study.

Table : Stability data of 1st and 2nd months under accelerated storage condition

S.No	Test parameter	Specifications	Initial	40°C/75 % RH	
				1st Month	2nd Month
1.	Description	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	Assay	98-102%	99.7	98.9	98.7
3.	pH after reconstitution	9.6	9.61	9.63	9.63
4.	Reconstitution time	NMT 60s	10s	11s	13s
5.	Constituted solution	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles
6.	Related substances Total impurities	NMT 0.5%	0.03	0.14	0.15
7.	Particulate matter (per container)	≥10µ - 6000	131	254	326
		≥25µ - 600	6	19	34
		≥50µ	0	2	5

Table : Stability data of 3rd and 6th months under accelerated storage condition

S.No	Test parameter	Specifications	40°C/75 % RH			
			3rd Month		6th Month	
			At the time of reconstitution	12hrs after reconstitution	At the time of reconstitution	12hrs after reconstitution
1.	Description	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	Assay	98-102%	99.7	99.3	99.1	98.9
3.	pH after reconstitution	9.6	9.7	9.6	9.7	9.6
4.	Reconstitution time	NMT 60s	50s	50s	55s	55s
5.	Constituted solution	Clear, color less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, color less solution, free from visible particles	Clear, color less solution, free from visible particles	Clear, color less solution, free from visible particles
6.	Related substance, total impurities	NMT 0 5%	0.01	0.02	0.21	0.3
7.	BET	NMT 0.5 EU/mg	<0.5 EU/mg	<0.5 EU/mg	<0.5 EU/mg	<0.5 EU/mg
8.	Particulate matter (per container)	≥10µ - 6000	315	369	422	511
		≥25µ - 600	56	69	79	96
		≥50µ	11	18	15	32

The accelerated stability study results confirm that all the test parameters were within the specifications.

LONG TERM AND INTERMEDIATE STORAGE CONDITION STABILITY STUDY

S.No	Test parameter	Specifications	25°C/60% RH		30°C/67% RH	
			3rd Month	6th Month	3rd Month	6th Month
1.	**Description**	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	**Assay**	98-102%	99.6	99.1	99.5	98.9
3.	**pH after reconstitution**	9.6	9.62	9.63	9.63	9.64
4.	**Reconstitution time**	NMT 60s	51s	52s	50s	53s
5.	**Constituted solution**	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles
6.	**Related substances, total impurities**	NMT 0.5%	0.01	0.02	0.2	O.3
7.	**Particulate matter test by Light Obscuration test method (per container)**	≥10µ-6000	355	495	411	525
		≥25µ - 600	59	86	66	81
		≥50µ	15	31	20	34

The above results confirm that the developed lyophilized drug was stable at long term storage condition (25°C/60% RH) and intermediate storage condition (30°C/65% RH) for a period of 6 months.

THERMAL CYCLING STUDY

The product was evaluated for the effects of temperature variation on the product when subjected to temperature conditions.

S.No	Test Parameter	Specifications	Initial	Cycle 3
1.	**Description**	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	**Assay**	98-102%	99.6	98.9
3.	**pH after reconstitution**	9.6	9.7	9.6
4.	**Reconstitution time**	NMT 60s	50	51s
5.	**Constituted solution**	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles
6.	**Related substances Total impurities**	NMT 0.5%	0.01	0.2
7.	**BET**	NMT 0.075 EU/mg	<0.075 EU/mg	<0.075 EU/mg
8.	**Particulate matter (per container)**	≥10µ - 6000	158	221
		≥25µ - 600	2	5
		≥50µ	0	1

TEMPERATURE EXCURSION STUDY:

S.No	Test parameter	Specifications	Initial	2 days at -20°C	2 days at 60°C
1.	**Description**	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	**Assay**	98-102%	99.7	98.4	98.2
3.	**Water content by KF**	NMT 7.0%	1.59	4.77	2.10
4.	**pH after reconstitution**	9.2-10	9.7	99.1	98.9
5.	**Reconstitution time**	NMT 60s	50s	52s	50s
6.	**Constituted solution**	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles
7.	**Related substances Total impurities**	NMT 0.5%	0.01	0.04	0.2
9.	**Particulate matter (per container)**	≥10µ - 6000	131	115	320
		≥25µ - 600	6	5	8
		≥50µ	0	0	2

OBSERVATION:

From the results of the Thermal Cycling and Temperature Excursion study we can confirm that the developed lyophilized drug product will be stable when it was subjected to or cycled through temperature conditions that

simulate the short term excursions outside the proposed label storage conditions which are likely to be encountered by the drug product during distribution.

So, it can be concluded that the drug product can be shipped between different climatic conditions without any change in its quality.

SUMMARY AND CONCLUSION

Before the development of the formulation, preformulation studies were conducted. In that solubility studies of the drug product, was determined the suitable conditions for the development of an optimized product.

Eutectic temperature was determined by using DSC and collapse temperature was determined which is the key parameter in the development of lyophilisation cycle recipe. Depending upon the value of eutectic temperature the recipes of the lyophilisation cycles were developed and optimized cycle was decided by trial and error method. Among all the above formulations, cycle 6 produces excellent product which is having desired qualities of the lyophilized product. This product was then compared with the innovator. And product was then subjected to various tests like reconstitution stability, stability studies as per ICH guidelines.

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