International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.4, pp 1581-1589, Oct-Dec 2013

Formulation and Evaluation of Lyophilized Antibacterial Agent

D.R.Kumar [1], Vasanth PM*[1], Ramesh T[2], Ramesh M[2].

[1]Dept of Pharmacy, UCEV-JNTUK, Vizianagaram, A.P, India.
[2]Dept of Biotechnology, UCEV-JNTUK, Vizianagaram, A.P, India.

***Corres.Author: vasanthpharma@gmail.com**
Mob No – 9247886185, Fax: 08922-277488

Abstract: Lyophilization of pharmaceutical solutions to produce a stable and elegant product has been a practice employed to manufacture many marketed injectable formulations, since a very long time. The present work is designed to formulate a combination of lyophilized β-lactam antibiotic and β-lactamase inhibitor powder for injection, with decreased lyophilization time, increased stability and thus making the product more economical. To improve the stability and cake characteristics of the lyophilized formulation, three bulking agents (mannitol, lactose and dextrose) were selected in different concentrations. Preformulation studies of the drugs were performed and compatibility of the drugs with excipients was determined. Results were found to be within the limits. A total of six formulations with different concentrations of bulking agents were prepared and were lyophilized using three different lyophilization cycles. Lyophilization cycle 3 and formulation F2 with 5% mannitol were evaluated to produce good results. Formulation F2 with 5% mannitol was loaded for accelerated stability studies for three months. The product was evaluated for pH, moisture content, reconstitution time, assay and other evaluation parameters at the end of each month. All the results were found to be within the USP limits. Thus from the results we can conclude that formulation F2 with 5% mannitol was found to be the best formulation to produce stable injectable dosage form of combination of β-lactam antibiotic and β-lactamase inhibitor with improved cake structure and decreased lyophilization time.
Keywords: β-lactam antibiotic, β-lactamase inhibitor, Lyophilization.

Introduction:

Antimicrobial drugs are the greatest contribution of the 20th century to therapeutics. Drugs in this class differ from all others in that they are designed to inhibit/ kill the infecting organism and to have no/minimal effect on the recipient. This type of therapy is generally called chemotherapy. It is defined as treatment of systemic infections with specific drugs that selectively suppress the infecting microorganism without significantly affecting the host. The basis of selective microbial toxicity is the action of the drug on a component of the microbe (cell wall) or metabolic process (folate synthesis) that is not found in the host.The term antibiotic was introduced by Waksman in 1942; before that time they were called toxins, lysins, bacteriostatic or bacteriolytic agents. An antibiotic can be defined as substances produced by microorganisms, which suppress the growth or even kill other microorganisms at very low concentrations.[1-6]

Parenteral is derived from two words *"para"* and *"enteron"* meaning to avoid the intestine. Parenterals according to USP are defined as preparations intended for injection through the skin (or) other external boundary tissue, rather than through the alimentary canal, so that the active substances they contain are

administered, using gravity or force, directly into a blood vessel, organ, tissue, or lesion. Parenterals are prepared by methods designed to ensure that they meet Pharmacopeial requirements for sterility, pyrogens, particulate matter, and other contaminants, and, where appropriate, contain inhibitors of the growth of microorganisms.[7,8]

Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. It is based on the principle of sublimation of ice, without entering the liquid phase. In this process, moisture content of the product is reduced to such a low level that does not support biological growth or chemical reactions. The technique therefore, finds special use in formulation development of drugs which are thermolabile and/or unstable in aqueous medium. Lyophilization is often used to stabilize various pharmaceutical products. The process consists of three steps or processes: Freezing, primary drying (sublimation), and secondary drying (desorption). Since freeze drying is a change in state from solid phase to gaseous phase, material to be freeze dried must be adequately frozen. The method of freezing and the final temperature of frozen product can affect the ability to successfully freeze dry the material. During primary drying, 98-99% of water is removed or sublimated. During this phase, pressure is lowered and heat is supplied to the material for water to sublime. This phase is generally slow, taking few hours to several days. During secondary drying , traces of remaining water is removed. During this stage, the temperature is increased to promote adequate desorption rates and achieve the desired residual moisture. The temperature of the solid is raised to as high as 50^0C to 60^0C. Because the cost of specialized equipment required for freeze drying can be substantial, process may appear to be an expensive undertaking. Stabilizing an unstable product at ambient temperatures, without using refrigerator, will compensate more than that when compared to that invested in freeze dry equipment.[9-14]

Materials And Methods

Materials

Active pharmaceutical ingredients, Mannitol, Lactose and Dextrose were obtained from Aurobindo Pharma Limited, Hyderabad.

Method

Required quantities of drugs and excipients were collected and weighed accurately. Water for injection was freshly collected and to this β-lactam antibiotic was added slowly followed by Sodium Citrate with constant stirring (A). In another beaker Water for injection was taken and to this β-lactamase inhibitor was added slowly followed by Sodium Citrate with constant stirring (B). Solution B was added to solution A, followed by addition of required quantity of selected bulking agent. Final volume was made with WFI and mixed properly until completely dissolved using a sonicator. pH was checked and adjusted with Sodium Citrate to 5.5 – 7.0, if required. 0.22µ Millipore syringe filter was used to filter this solution. The solution was filled into vials. The vials were partially stoppered and loaded into the lyophilizer. The lyophilization cycle was started and after the cycle was completed the vials were removed from the lyophilizer and were stored at suitable temperature for further analysis. 6 formulations were prepared (F1 – F6). Composition of each formulation was tabulated in table 1. Lyophilization cycles were tabulated in tables 2 - 4.

Table 1 Formulation trials

INGREDIENTS	F1	F2	F3	F4	F5	F6
Mannitol	3%	5%	-	-	-	-
Lactose	-	-	3%	5%	-	-
Dextrose	-	-	-	-	3%	5%
Water for injection	Qs	Qs	Qs	Qs	Qs	Qs

Table 2 Lyophilization cycle 1 (Trail 1)→ 26 hours 10 minutes

TEMPERATURE (^{0}C)	RAMP (minutes)	SOAK (minutes)	VACCUM (µbar)
FREEZING			
10 to -10	5	5	-
-10 to -25	20	50	-
-25 to -40	40	120	-
PRIMARY DRYING			
-40 to -20	160	120	300
-20 to -5	80	100	350
-5 to 10	65	150	375
10 to 25	55	120	400
25 to 40	50	150	400
SECONDARY DRYING			
40 to 50	10	220	125
THERMAL TREATMENT			
50 to 25	30	20	125

Table 3 Lyophilization cycle 2 (Trail 2)→ 30 hours 05 minutes

TEMPERATURE (^{0}C)	RAMP (minutes)	SOAK (minutes)	VACCUM (µbar)
FREEZING			
10 to -10	5	5	-
-10 to -25	20	50	-
-25 to -40	40	120	-
PRIMARY DRYING			
-40 to -20	200	140	300
-20 to -5	100	130	350
-5 to 10	80	175	375
10 to 25	60	150	400
25 to 40	60	190	400
SECONDARY DRYING			
40 to 50	10	220	125
THERMAL TREATMENT			
50 to 25	30	20	125

Table 4 Lyophilization cycle 3 (Trail 3)→ 32 hours 35 minutes

TEMPERATURE (^{0}C)	RAMP (minutes)	SOAK (minutes)	VACCUM (µbar)
FREEZING			
10 to -10	5	5	-
-10 to -25	20	50	-
-25 to -40	40	120	-
PRIMARY DRYING			
-40 to -20	200	140	300
-20 to -5	100	130	350
-5 to 10	80	175	375
10 to 25	60	150	400
25 to 40	60	190	400
SECONDARY DRYING			
40 to 50	20	300	125
THERMAL TREATMENT			
50 to 25	50	60	125

Evaluation parameters

Reconstitution time: The lyophilized vials of the formulations were reconstituted with water for injection. Time required for the formation of clear solution was reported.

Water content: The water content was checked by auto Karl Fischer titrator and the results were reported.

pH: The lyophilized formulations were reconstituted with water for injection and the pH of the reconstituted solution was checked.

Drug content: Assay was carried out using HPLC according to USP and the results were reported.

Particulate matter: Light Obscuration Particle Count Test was conducted for this purpose. It is based on the principle of light blockade. It gives automatic evaluation of particulate matter. In this a stream of sample is allowed to pass between a light source and a sensor. If the sample contains any particulate matter, it blocks the path of the light. The instrument measures the cross-sectional area of the particle, and from this the size of the particle can be easily determined.

USP Limits: According to USP general chapter (788)-Particulate matter in injections, allows up to 6000 and 600 average number of particles, of 10µm and 25µm size respectively.

Accelerated stability studies: The stability studies were carried out as per ICH guidelines Q1A (R2). The accelerated study was carried out at temperature of 40 ± 2^0C/75% ± 5% RH for a period of 3 months. Samples were analyzed for drug content and other evaluation parameters, and the results were reported.[15]

Results And Discussion

Lyophilization or freeze drying is a process of removal of water by, conversion of ice directly from solid to vapor without passing through a liquid phase. It is based on the principle of sublimation of ice. The process consists of three processes: freezing, primary drying (sublimation) and secondary drying (desorption).

Initially the drugs were subjected to preformulation studies as per USP specifications. The drugs were identified by HPLC, and the sample retention time corresponds with the standard retention time. Lyophilization was carried out by selecting appropriate excipients. Drug-excipients compatibility studies were carried out for 1 month at accelerated conditions. Based on the results it was concluded that the excipients were compatible with the drugs.

6 different formulations (F1-F6) were formulated; 3 different lyophilization cycles were developed and subjected to lyophilization. The formulations include 3% w/w and 5% w/w each of mannitol, lactose and dextrose.

Lyophilization cycle 1 was carried out for 26 hours 10 minutes, which resulted in damp, collapsed cake in all 6 formulations (F1-F6). Moisture content was found to be 9.1-10.1% and reconstitution time was found to be 21-28 minutes. Lyophilization cycle 2 was carried out for 30 hours 05 minutes. In this cycle, primary drying time was increased approximately by 3 hours 55 minutes. In cycle 2, moisture content and reconstitution time were decreased when compared to cycle 1, but the cake appears to be shrunk or collapsed. Moisture content was found to be 4.8-5.9% and reconstitution time was found to be 12-15 minutes. Lyophilization cycle 3 was carried out for 32 hours 35 minutes. In this cycle, secondary drying and thermal treatment time was increased approximately by 2 hours 30 minutes. In F2 (5% mannitol) formulation, cake appeared to be uniform, distinct and intact without any shrinking and collapse. In other formulations (except F2) cake appears to be shrunk or collapsed. Individual lyophilization cycle results were tabulated in tables 5 - 7.

Cake Analysis For Individual Lyo Cycles

Table 5 Cycle 01

SNO	FORMULATION	DESCRIPTION	WATER CONTENT (%)	RECONSTITUTION TIME (minutes)
1	F1	Damp cake	9.8	24.31
2	F2	Damp cake	9.5	23.28
3	F3	Damp cake	9.1	21.54
4	F4	Damp cake	9.6	22.43
5	F5	Damp cake	10.1	25.46
6	F6	Damp cake	9.9	27.32

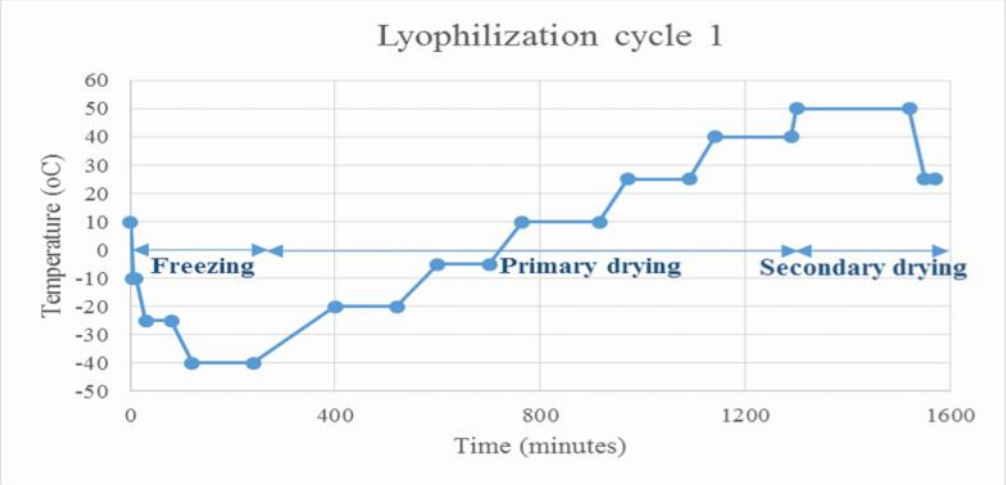


Table 6 Cycle 02

SNO	FORMULATION	DESCRIPTION	WATER CONTENT (%)	RECONSTITUTION TIME (minutes)
1	F1	Damp cake	5.6	12.25
2	F2	Damp cake	5.2	13.45
3	F3	Damp cake	4.8	13.34
4	F4	Damp cake	5.3	13.58
5	F5	Damp cake	5.9	14.56
6	F6	Damp cake	6.1	15.43

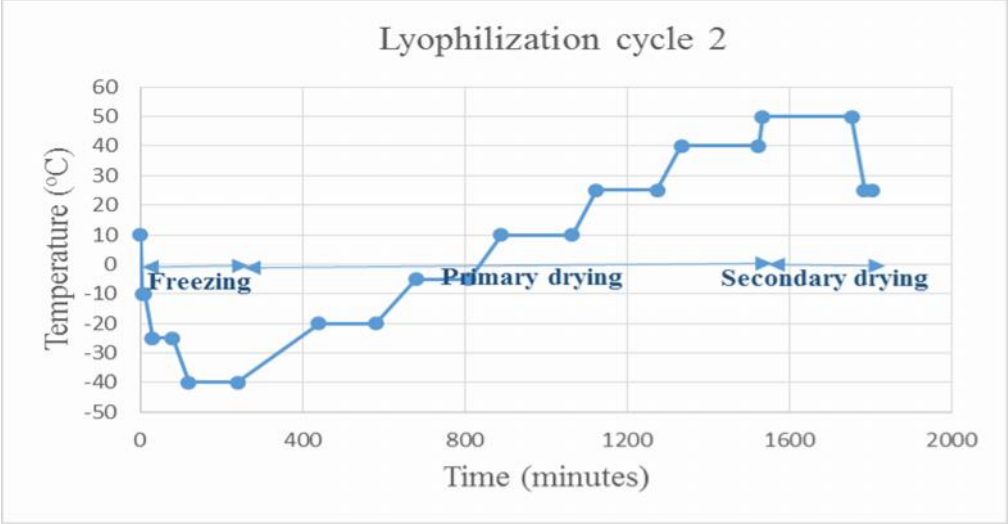
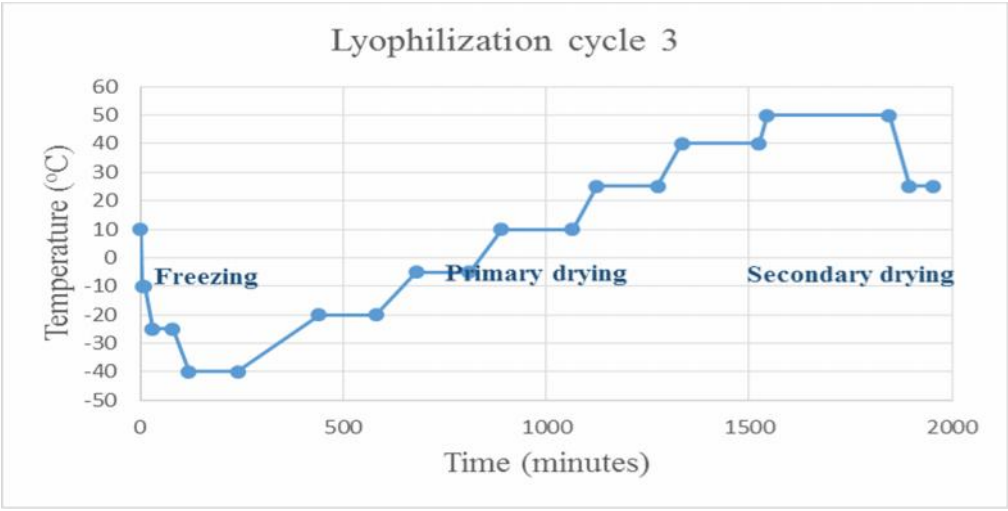


Table7 Cycle 03

SNO	FORMULATION	DESCRIPTION	WATER CONTENT (%)	RECONSTITUTION TIME (minutes)
1	F1	Collapsed	1.5	4.56
2	F2	White lyophilized cake	1.1	4.22
3	F3	Collapsed	1.5	5.21
4	F4	Collapsed	1.3	4.56
5	F5	Collapsed	2.1	5.54
6	F6	Collapsed	2.2	6.01



In F2 cake appears to be intact and elegant. Moisture content of F2 was found to be 1.1% and reconstitution time was found to be 4.22 minutes. pH of the reconstituted solution of F2 was 6.6 and the assay was 99.92% for β-lactam antibiotic and 98.59% for β-lactamase inhibitor. Lyophilization cycle 3 gave best results when compared to the other 2 cycles, and the F2 evaluation results were found to be within the limits. Evaluation results of F2 for dry powder were reported in tables 8 and for reconstituted solution were reported in table 9.

F2 formulation was loaded for stability studies. Stability studies were conducted as per ICH guidelines at 40 ± 2^0C/75% RH ± 5% for 3 months. After three months of stability studies moisture content of F2 was found to be

1.21% and reconstitution time was found to be 4.30 minutes. pH of the reconstituted solution of F2 was 6.85 and the assay was 99.79% for β-lactam antibiotic and 98.49% for Tazobactam. All the evaluated parameters were found to be within the USP limits even after three months of stability studies. Stability study reports were tabulated in table 10.

Therefore the lyophilization cycle 3 was found to be suitable for developing lyophilized β-lactam antibiotic and β-lactamase inhibitor powder for injection, which is more economical and produces a stable product.

Analysis Report Of Formulation F2 (After Lyophilisation)

(F2 is selected as best formulation)

Table 8 For dry powder

SNO	TEST	RESULT	SPECIFICATION
1	Description	White cryodessicated powder	White cryodessicated powder
2	Identification by HPLC	Retention time of major peaks in chromatogram of sample solution corresponds to that of principal peaks in chromatogram of standard solution as obtained in assay	Retention time of major peaks in chromatogram of sample solution should corresponds to that of principal peaks in chromatogram of standard solution as obtained in assay
4	Sterility	Sterile	Must be sterile
5	pH[4%(w/v) of solution]	6.6	5.5 – 7.0
6	Water	1.1%	NMT 2.5%
7	Particulate matter		
	>= 10 microns	126 particles/vial	NMT 6000 particles/vial
	>= 25 microns	15 particles/vial	NMT 600 particles/vial

Table 9 For reconstituted solution

SNO	TEST	RESULT	SPECIFICATION
1	Appearance	Light yellow colored clear solution	White to light yellow colored clear solution
2	Completeness and clarity of solution	Solution dissolved completely, leaving no visible residue or undissolved material The constituted solution is not less clear than the equal volume of diluent contained in a similar vial	Solution should dissolve completely, leaving no visible residue or undissolved material The constituted solution should not be less clear than the equal volume of diluent contained in a similar vial
3	Particulate matter	Solution is free from particles of foreign matter that can be observed on visual inspection	Solution must be free from particles of foreign matter that can be observed on visual inspection

Table 10 Accelerated stability studies: (40 ± 2^0C/75% ± 5%)

Month	Description	Water content	Reconstitution time	pH of reconstituted solution	Assay	
					A	B
1	White lyophilized cake	1.12%	4 minutes 15 seconds	6.8	99.89	98.54
2	White lyophilized cake	1.15%	4 minutes 26 seconds	6.75	99.81	98.51
3	White lyophilized cake	1.21%	4 minutes 30 seconds	6.85	99.79	98.49

A: β-lactam antibiotic
B: β-lactamase inhibitor

Conclusion

Extended spectrum β–lactam antibiotic is useful in treating many gram positive and gram negative pathogens. β –lactamase inhibitor inhibits the action of bacterial β-lactamases. It is combined with β–lactam antibiotic, to prevent it from destruction by bacteria.

The present research work was to formulate a combination of lyophilized β-lactam antibiotic and β-lactamase inhibitor powder for injection, with decreased duration of lyophilization, increased stability of the cake and thus making the product more economical.

The drugs were formulated by using various excipients such as mannitol, lactose and dextrose in different concentrations and were lyophilized. A total of six formulations with different concentrations of excipients were prepared and were lyophilized using three different lyophilization cycles. Lyophilization cycle 3 and formulation F2 with 5% mannitol were evaluated to produce good results. Formulation F2 with 5% mannitol was loaded for accelerated stability studies for three months. The product was evaluated. All the results were found to be within the limits. Thus from the results we can conclude that formulation F2 with 5% mannitol was found to be the best formulation. Finally it was concluded that lyophilization cycle 3 was the best process, with F2 – 5% mannitol being the best formulation passing all the tests.Hence, F2 with 5% mannitol was selected as the best formulation.

References

1) Ashutosh Kar. Medicinal Chemistry - 4th ed (2007). New Age International (p) Ltd. 736-754.
2) Burger's. Medicinal Chemistry and Drug Discovery. Vol 5 – Chemotherapeutic agents – 6th ed. 607-654.
3) Foye's. Principles of Medicinal Chemistry – 6th ed. (2008). Lippincott Williams and Wilkins. 1028-1056.
4) Goodman & Gilman's. The pharmacological basis of therapeutics – 11th ed. (2006). Chapters 42 and 44
5) H.P.Rang, M.M.Dale, J.M.Ritter, R.J.Flower. Pharmacology - 6th ed. Churchil Livingstone. 647-668.
6) Wilson and Gisvold's. Text Book of Organic Medicinal and Pharmaceutical Chemistry – 12th ed (2011). Lippincott Williams and Wilkins. 258-278.
7) James Swarbrick. Encyclopedia of Pharmaceutical Technology – 3rd ed. Vol 2 – Dosage forms - Parenterals (2007). Informa Healthcare, New York. 1001-1012.
8) USP–NF, 32nd revision of the United States Pharmacopeia (USP 32) and the 27th edition of the National Formulary (NF 27), General Chapter 1, Injections.
9) Guide to inspections of lyophilization of parenterals (FDA). Available at: http://www.fda.gov/ICECI/Inspections/InspectionGuides/ucm074909.htm
10) James Swarbrick. Encyclopedia of Pharmaceutical Technology – 3rd ed. Vol 3 – Freeze drying (2007). Informa Healthcare, New York. 1807-1834.
11) L. Rey and J. May. Freeze drying/ Lyophilization of Pharmaceutical and Biological Products. (2004) Marcel Decker, Inc, New York.

12) Fakes, Mandar V, Thomas A, R. Morris, Sailesh A and Serajuddin studied Moisture Sorption Behavior of Selected Bulking Agents Used in Lyophilized Products. PDA journal of pharmaceutical science and technology. March/April 2000 54:144-149.
13) Soham Shukla done a review on freeze drying process: a review International Journal of Pharmaceutical Sciences and Research, Vol 2, Issue 12, 2011, Pages 3061-3068.
14) S.C Tsinontides, P Rajniak, D Pham, W.A Hunke, J Placek, S.D Reynolds done a research on Freeze drying—principles and practice for successful scale-up to manufacturing. International Journal of Pharmaceutics, Vol 280, Issues 1–2, 6 August 2004, Pages 1–16.
15) ICH Guidelines. Stability testing of new drug substances and products Q1A(R2). Available at http://www.ich.org/.

*****