Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (5):200-205
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Formulation of carbetocin injection by lyophilization technique

***Patel Mitesh N.[1] and Patel Amit A.[2]**

[1]*Department of Pharmaceutical Science, JJT University, Jhunjhunu, Rajasthan*
[2]*B M Shah College of Pharmaceutical Education and Research, Modasa, Gujarat*

ABSTRACT

The objective of this experiment is to formulate the Carbetocin injection by Lyophilization technique for betterstability by reducing Oxidative degradation, Hydrolytic degradation and alkaline degradation and for long term storage. Mannitol as bulking as well as isotonic agent, Glacial acetic acid as buffer and L-Methionine as Antioxidant were used with water for injection into 2 ml tubular vials with pre and post purging nitrogen. The filled vials were loaded into Lyophilizer and lyophilized them as per cycle. Different composition of additives was used and the different pH concentrations of 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 were adjusted with 1% Glacial Acetic acid were tried to formulate the formulation. The formulation F (3) which has been optimized, theresults of the cycle was observed to be optimized. Carbetocin was developed as lyophilized formulation for betterstability. The obtained results suggested that a stable formulation for drug Carbetocin was developed which wasComparable to reference listed product.

Keywords: Parenteral, Lyophilization, Carbetocin

INTRODUCTION

Lyophilization cycle development typically focuses on optimizing the primary drying step. That is the most time consuming of the three steps, and the primary drying parameters are easily adjustable. They can affect both the time involved and the quality of the resulting cake. Extensive investigation of primary drying has demonstrated that two important parameters are chamber pressure and shelf temperature [1-10]. They are usually adjusted to maximize the rate of heat transfer to each vial (speeding ice sublimation) without causing cake collapse. Less attention has been paid to the freezing conditions and their potential effect on the primary and secondary drying processes and on the characteristics of the final product. Kochs et al. reported the effects of freezing conditions on primary drying in a specially designed aluminum and plastic sample cell [9]. They observed variations in vapor diffusion coefficients (a measure of the ease of water-vapor flow) as a function of position and cooling rate. The variations appeared to be largely due to variations in sample morphology. Searles et al. reported some effects of freezing on the rate of primary drying in vials [10]

Carbetocin is a long-acting synthetic analogue of oxytocin [11-12] that can be administered as a single-dose injection, either intravenously or intramuscularly [15]. Intravenously administered carbetocin has a half-life of approximately 40 min [26], around 4– 10 times longer than that reported for oxytocin [13-14]. Following intramuscular injection, carbetocin reaches peak plasma concentrations in less than 30 min and has 80% bioavailability [15].

Postpartum haemorrhage occurs in up to 15% of vaginal deliveries [16] and represents the most important cause of maternal morbidity and mortality worldwide [17–23]. The risk of postpartum haemorrhage is much higher for women undergoing caesarean section [24], particularly in developing countries where the majority of operations are carried out as an emergency procedure [25]. In most cases, uterine atony is responsible for the occurrence of excessive bleeding during or following childbirth [20-21]. Current strategies for preventing postpartum haemorrhage include the prophylactic use of uterotonic agents to enhance natural uterine contraction and retraction following caesarean section and in the third stage of labour for vaginal delivery [16, 20-22]. Oxytocin is the most widely used uterotonic agent [16, 21–23], but only has a half-life of 4–10 min [25–27] so must be administered as a continuous intravenous infusion to achieve sustained uterotonic activity.

Another uterotonic drug frequently used in vaginal deliveries is syntometrine, which contains 5 IU/ml oxytocin and 0.5 mg/ml ergometrine [30, 31]. Syntometrine combines the rapid onset of action of oxytocin and the prolonged uterotonic effects of an ergot alkaloid. Intramuscular syntometrine use in active management of the third stage of labour is associated with a significant reduction in the risk of non-severe postpartum haemorrhage (<1000 ml of blood loss) compared with intramuscular oxytocin [22,28,29].Although intramuscular syntometrine is equally effective as intravenous oxytocin [22,32], gastrointestinal and cardiovascular side effects such as maternal nausea, vomiting and raised blood pressure [22,30–32] are more frequent due to stimulation of smooth muscle contraction and vasoconstriction by ergometrine [31,32]. Oral and rectal administration of misoprostol, a synthetic analogue of prostaglandin E1, have demonstrated lower efficacy thaninjectable uterotonic agents in preventing excessive bleeding following vaginal delivery [33,34] and are associated with a high incidence of shivering, fever and a possible risk of severe hyperthermia [34–36]. These factors deem misoprostol unsuitablefor routine prevention of excessive postpartum bleeding indeveloped countries, despite low cost and ease of use [22, 33-34].Although injectable prostaglandins such as prostaglandin 15-methyl F2a or sulprostone can prevent excessive bleedingfollowing vaginal delivery, safety concerns and cost limit theirsuitability for routine use in active management of the third stageof labour. However, they remain useful therapeutic options forpostpartum haemorrhage treatment when other interventionsprove ineffective [22, 33]. Recent interest has focused on theprophylactic use of the oxytocin receptor agonist carbetocin(DURATOCIN, PABAL, LONACTENE, Ferring Pharmaceuticals SA, St.Prex, Switzerland) [35–37].

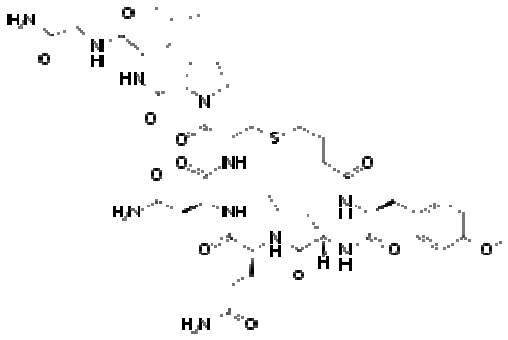

Fig.1: Structure of Carbetocin

Carbetocin is (2S)-1-[(3S,6S,9S,12S,15S)-12-[(2S)-butan-2-yl]-9-(2-carbamoylethyl)-6-(carbamoylmethyl)-15-[(4-hydroxyphenyl)methyl]-16-methyl-5,8,11,14,17-pentaoxo-1-thia-4,7,10,13,16-pentazacycloicosane-3-carbonyl]-N-[(1S)-1-(carbamoylmethylcarbamoyl)-3methyl-butyl]pyrrolidine-2-carboxamide. The chemical structure of Carbetocin has presented in Fig. 1, and the molecular formula of Carbetocin is $C_{45}H_{69}N_{11}O_{12}S$, molecular mass 988.162 gm/mol. This is a white powder. The pH of a 0.5% aqueous solution of Carbetocin is 4.0 – 5.0. The reconstituted solution in water is clear colorless, essentially free of particulate matter. [38-41]

Carbetocin is most susceptible for degradation while stability studies in conventional injectable formulation, So we have developed & stabilized the Carbetocin injection by Lyophilization technique for better stability by reducing Oxidative degradation, Hydrolytic degradation and alkaline degradation and for long term storage.

MATERIALS AND METHODS

Materials: Carbetocin is an active ingredient, Mannitol as bulking as well as isotonic agent, Glacial acetic acid as buffer and L-Methionine as Antioxidant andwater for injection as a vehicle for solubility were used for formulation. Activeingredient was procured from Gufic Biosciences Ltd. and all other ingredients used were AR grade.

Methods:

Freeze drying procedure: For optimization of lyophilized cycle, dummy trials were taken as per batch F0. Until the moisture content of the lyophilized vial was within the acceptance limit (Max. 2.0%) and the formation of good cake was observed in the vials. Based on this parameters, freezing temperature, primary drying time, secondary drying time and vacuum during drying was decided.

Manufacturing Procedure:

Formulation of Carbetocin injection by Lyophilization:Different compositions of Mannitol, L-Methionine and Glacial acetic acid has been used for the experiment which was placed in Table. 1. Water for injection (WFI) was collected in a beaker and purged with nitrogen continuously to reduce the dissolved oxygen until the oxygen level less than 2ppm under 2-8°C condition. (Except Batch F13) Nitrogen Purging is necessary throughout manufacturing process. 250 ml WFI of was collected in a beaker, weighed quantity of Mannitol was added and dissolved by stirring until a clear solution was formed. Then L-Methionine was weighed and transferred to the above solution and dissolved by stirring for minimum duration of 5 min under 2-8°C condition. The pH of the solution was checked and adjusted the pH with 1% Glacial acetic acid slowly under 2-8°C condition. Carbetocin was weighed and transferred to the above solution and dissolved by stirring until clear solution was formed. The solution was diluted and made upto 300ml, by WFI under 2-8°C. pH was checked. The final solution was filtered by using 0.22µm membrane filter. The solution was filled into 2ml tubular vials (13mm neck) with pre and post purging nitrogen, and half stopped the vials with 13mm grey bromobutyl full slotted rubber stopper. The filled vials were loaded into lyophilizer and lyophilized them as per standard cycle. After completion of Lyophilization cycle, Stopering the vials without breakage of vacuum. Using hydraulic system. Now break the vacuum of the plant with help of the sterile nitrogen.Unload the vials for sealing and seal the vials.Temperature of room should be below 25^{o}C and humidity below 40% .

Table 1 : Formulation of Carbetocin injection by Lyophilization of Various Batches

Batch No .	Carbetocin with (10% Overages)		Mannitol		L-Methionine		1% Glacial Acetic Acid		Water for injection	
	Mg/Vial	Qty./300ml	Mg/Vial	Qty./300ml	Mg/Vial	Qty./300ml	Mg/Vial	Qty./300ml	ml/Vial	Qty./300ml
F0	-	-	50	15 gm	1	300 mg	-	-	q.s. to 1	q.s. to 300
F1	0.11	33 mg	50	15 gm	1	300 mg	q.s. to pH 4.0	q.s. to pH 4.0	q.s. to 1	q.s. to 300
F2	0.11	33 mg	50	15 gm	1	300 mg	q.s. to pH 4.5	q.s. to pH 4.5	q.s. to 1	q.s. to 300
F3	0.11	33 mg	50	15 gm	1	300 mg	q.s. to pH 5.0	q.s. to pH 5.0	q.s. to 1	q.s. to 300
F4	0.11	33 mg	50	15 gm	1	300 mg	q.s. to pH 5.5	q.s. to pH 5.5	q.s. to 1	q.s. to 300
F5	0.11	33 mg	50	15 gm	1	300 mg	q.s. to pH 6.0	q.s. to pH 6.0	q.s. to 1	q.s. to 300
F6	0.11	33 mg	50	15 gm	1	300 mg	q.s. to pH 6.5	q.s. to pH 6.5	q.s. to 1	q.s. to 300
F7	0.11	33 mg	50	15 gm	2	600 mg	q.s. to pH 4.0	q.s. to pH 4.0	q.s. to 1	q.s. to 300
F8	0.11	33 mg	50	15 gm	2	600 mg	q.s. to pH 4.5	q.s. to pH 4.5	q.s. to 1	q.s. to 300
F9	0.11	33 mg	50	15 gm	2	600 mg	q.s. to pH 5.0	q.s. to pH 5.0	q.s. to 1	q.s. to 300
F10	0.11	33 mg	50	15 gm	2	600 mg	q.s. to pH 5.5	q.s. to pH 5.5	q.s. to 1	q.s. to 300
F11	0.11	33 mg	50	15 gm	2	600 mg	q.s. to pH 6.0	q.s. to pH 6.0	q.s. to 1	q.s. to 300
F12	0.11	33 mg	50	15 gm	2	600 mg	q.s. to pH 6.5	q.s. to pH 6.5	q.s. to 1	q.s. to 300
F13	0.11	33 mg	50	15 gm	1	300 mg	q.s. to pH 5.0	q.s. to pH 5.0	q.s. to 1	q.s. to 300

Analytical Procedure:

UPLC analysis was carried out with a column Acquity UPLC BEH C18 (2.1mm x 10 mm) 1.7µ with column oventemperature maintained at 50°C, flow rate was 0.3 ml/min, detector was UV detector at 220 nm and injection volumewas 1.5 µl, with the runtime of 2 min.

Preparation of Buffer**:** Take 0.68 gm Potassium dihydrogen phosphate (KH_2PO_4) dilute it up to 500 ml with water and adjust pH 6.5 with Dilute Sodium hydroxide Solution (Dilute NaOH)

Mobile phase: Mix buffer and Acetonitrile in ratio of 50: 50. Degas and filter through 0.22 µ filter paper.

Diluent: Water.

Standard preparation: Weigh 10 mg Carbetocin WS and transfer it in to 100 ml volumetric flask. And make it up to the mark with water (100 mcg/ ml)

Assay preparation (Test solution): Take ten Lyophilized vials and each vial reconstituted with 1 ml water for Injection. Mix all the reconstituted solution from each vial in Test tube and Transfer in to injector vial for Assay.

Procedure: Separately inject the equal volumes (about 1.5µl) of the standard preparation (replicate) and the test preparation (duplicate) into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of Carbetocin in percentage from the mean peak area of standard, sample peak and percentage potency of working standard used. The assay percentage should be within 90.0% to 115.0%.

Stability studies:
Accelerated stability study was conducted for the optimised batch under various temperature and humidityconditions. The water content, assay and pH were determined and compared with standard conditions.

RESULTS AND DISCUSSION

Optimization of Freeze drying procedure:
Freeze drying cycle was optimized as, freezing of the product under the lyophilizer at -25°C for two hours. Then Primary drying at -5°C for seven hours along with vacuum 100 to 150 mtor & then at 0°C for 6 hours along with vacuum 100 to 150 mtor, then +15°C for 7 hours along with vacuum 100 to 150 mtor. Secondary drying was carried out at +30°C for 2 hours along with vacuum 10 to 50 mtor.

Optimization of Carbetocin injection by Lyophilization
All batches (F1-F13) as presented in Table no 1 were planned to observe the effect of different pH by adjusting with 1% glacial acetic acidand different concentration of antioxidant, results obtained after completion of lyophilization process were summarized in below table-2.

Table 2: Observation of Carbetocin injection by Lyophilization

Batch No	Description of cake	pH	Water content	Assay
F1	White lyophilized cake	3.82	0.89%	102.17%
F2	White lyophilized cake	4.36	0.96%	103.57%
F3	White lyophilized cake	4.94	0.63%	109.62%
F4	White lyophilized cake	5.47	0.73%	103.29%
F5	White lyophilized cake	5.91	0.88%	102.52%
F6	White lyophilized cake	6.43	0.95%	101.33%
F7	White lyophilized cake	3.86	0.72%	103.28%
F8	White lyophilized cake	4.42	0.96%	102.46%
F9	White lyophilized cake	4.87	0.58%	108.27%
F10	White lyophilized cake	5.39	0.82%	103.18%
F11	White lyophilized cake	5.88	0.89%	101.41%
F12	White lyophilized cake	6.39	0.76%	103.74%
F13	White lyophilized cake	4.86	0.93%	105.11%

After completion of Manufacturing process & its initial analysis, Batch No F3, F9, F 13 has shown minimum degradation of Carbetocin & were selected for the stability studies.

While analytical process, retention time of Carbetocin was found at 0.83 min, the columnefficiency is less than 1500 theoretical plates, the tailing factor was not more than 1.5 and the relative standarddeviation for five replicate injections was not more than 1.0%.

The accelerated stability study was conducted for theoptimized batch for 6 months at 40°C± 2°C/75% RH ± 5% RH. The results obtained from the stability studies were summarized in Table-3.

Table 3: Evaluation of Carbetocin for injection by lyophilization technique of optimized batches (Accelerated study)

Batch No	Description of cake		pH		Water content		Assay		Total Impurities	
	3 Months	6 Months	3 Months	6 Months	3 Months	6 Months	3 Months	6 Months	3 Months	6 Months
F3	White lyophilized cake	White lyophilized cake	4.92	4.94	0.71%	0.89%	108.11%	107.27%	1.02%	2.65%
F9	White lyophilized cake	White lyophilized cake	4.86	4.89	0.75%	0.92%	107.01%	106.16%	1.28%	2.89%
F13	White lyophilized cake	White lyophilized cake	4.83	4.86	1.04%	1.16%	104.43%	102.90%	2.53%	3.64%

Total impurities were found to be 2.65%, 2.89% and 3.64% in F3, F9 & F13 respectively and the limit is NMT 3.0%. The assay of the respective optimized batches were 107.27%, 106.16% and 102.90% in F3, F9 & F13. Out of these three batch F3 has shown minimum Degradation & so it was considered as optimized batch.

CONCLUSION

Total number of impurities in carbetocin in liquid Injection stored at room temperature (The actual storage condition is 2-8°C) was found to be more than 10% which was out of limits. Therefore, it was developed as lyophilized formulation forbetter stability and lower amount of degradation products. The lyophilized cycle was optimized with direct four step freezing at -25°C and changing vacuum with post heat up to 30°C. Carbetocin for Injection 0.1mg was compatible with 2 ml clear glass USP Type I vial,Greybromobutyl rubber closure, nitrogen purging. The formulation of Batch F3 descried in Table no 1 was stable for 6 months on accelerated stabilitystudies. In conclusion a stable formulation of lyophilized Carbetocininjection was developed& optimized, which was comparable to ReferenceListed Drug Product (RLD) of Carbetocin Injection.

Acknowledgement
We thank Gufic Biosciences Ltd for material donation. We thank Dr. Amit for valuable guidance and suggestions on the manuscript.

REFERENCES

[1] S Nail, *PDA J. Pharm. Sci. Technol.*,**1980**,34, 358–368.
[2] M Pikal et al., *J. Pharm. Sci.*, **1983**,72, 635–650.
[3]M Pikal, M Roy, and S. Shah, *J. Pharm. Sci.*, **1984**,73, 1224–1237.
[4] M Pikal, *PDA J. Parenteral Sci. Technol.*, **1985**,39, 115–139.
[5] R Livesey, T Rowe, *J. Parenteral Sci. Technol.*, **1987**,41, 169–171.
[6]M Pikal and S. Shah, *Int. J. Pharm.* **1990**,62, 165–186.
[7]M Pikal, *Pharm. Res.*, **1995**,12, 831–837.
[8] D Overcashier, T Patapoff, C Hsu, *J.Pharm. Sci.*, **1999**, 88, 688–695.
[9]Naveen Krishna. Y, Anton Smith. A, Gudimetta Karteek, Parimalakrishnan.S, Srikanth. J *Der Pharmacia Lettre*, **2012**, 4 (5):1426-1430
[10] J Searles, JCarpenter, T Randolph, *J. Pharm. Sci.*, **2001**, 90(7), 860–871.
[11] A Atke, H Vilhardt, *Acta Endocrinol*, **1987**, 19, 971–6.
[12] M Norstro, A Andersson, H Vilhardt, *Acta Endocrinol*,**1990**,122, 566–8.
[13] GRyden, JSjoholm, *Acta Endocrinol (Copenh)*, **1969,** 61, 425–31
[14] TChard, N Boyd, MForsling, *J Endocrinol*, **1970**,48, 223–34.
[15] GSweeney, AHolbrook, M Levine, *Curr Ther Res*, **1990**,47, 520–40.
[16] WPrendiville, D Elbourne, I Chalmers, *Obstet Gynecol*, **1988,** 95, 3–16.
[17] RRochat, LKoonin, HAtrash, JJewett, *Obstet Gynecol*, **1988,** 72, 91–7.
[18] Li XF, J Fortney, MKotelchuck, L Glover, *Int J Gynecol Obstet*, **1996**,54, 1–10.
[19] World Health Organization,*Geneva: World Health Organization*; 2001.
[20] FBouwmeester, A Boltc , V Geijn, *Curr Pharm Des*, **2005**, 11,759–73.
[21] YChong, L Su , S Arulkumaran, *Curr Opin ObstetGynecol*, **2004**, 16, 143–50.
[22] LWedisinghe, MMacleod, D Murphy, *Eur J ObstetGynecol Reprod Biol*, **2008,** 137, 27–30.
[23] B Ozumba, H Ezegwui., *Obstet Gynecol*, **2006,** 26, 746–8.
[24] GRyden, JSjoholm, *Acta Endocrinol (Copenh),***1969,** 61, 425–31.
[25] MFabian, MForsling, JJones, *J Physiol* 1969, 204:653–8.
[26] T Chard, N Boyd, M Forsling, *J Endocrinol*, **1970**, 48, 223–34.

[27] SMcDonald, Abbott JM, Higgins SP, Cochrane Database Syst Rev, **2004**, IssueI. Art. No: CD000201.
[28] TLiabsuetrakul, TChoobun, KPeeyanajarassri, Q Islam, *Cochrane Database Syst Rev*,**2007**, 2. Art. No: CD005456.
[29] C Choy, W Lau, W Tam, P Yuen, *BJOG*, **2002,** 109, 173–7.
[30] J Dumoulin, *J Obstet Gynecol*, **1981,** 1, 178–81.
[16] MCarey,*J Obstet Gynecol*,**1993**, 100, 865.
[31] A Gulmezoglu, FForna, JVillar, GHofmeyr,*Cochrane Database Syst Rev*.,**2004**, 1, Art. No: CD000494.
[32] JVillar, A Gulmezoglu, GHofmeyr, F Forna, *Obstet Gynecol*, **2002**, 100, 1301–12.
[33] YChong, SChua, SArulkumaran, *Obstet Gynecol*, **1997,** 90, 703–4.
[34] MBoucher, CNimrod, GTawagi, TMeeker, RRennicks White, JVann,*JObstet Gynecol*, **2004**, 26, 481–8.
[35] SLeung, PNg, WWong, TCheung, *BJOG*,**2006**, 113, 1459–64.
[36] LNgan, WKeong, RMartins, *Int J Gynecol Obstet*, **2007,** 97, 152–3.
[37] L Su, YChong, MSamuel, *Cochrane Database Syst Rev*, **2007**, 3, Art. No: CD005457.
[38]NCort, SEinarsson, DSchams, *Am. J.Vet. Res*., **1981,** 42, 1804–1806.
[39] T Barth, VPliska, IRychlik,*Chem. Commun*, **1967**,32, 1058–1063.
[40] TBarth, HHutter, VPliska, FSorm, *Experientia*,**1969**, 25, 646–647.
[41] T Barth, K.Jost, I Rychlik,*Endocrinol. Exp*.,**1975**, 9, 35–42.