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FORMULATION AND *IN-VITRO* EVALUATION OF CLOPIDOGREL BISULFATE IN PARENTERAL DOSAGE FORM USING LYOPHILIZATION TECHNIQUE

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Abstract

The aim of this work was to develop & evaluate in-vitro; a stable clopidogrel bisulfate formulation, in parenteral dosage form using lyophilization technique. Faxon DP et al. have reported use of antiplatelet therapy generally with clopidogrel bisulfate before percutaneous coronary interventions (PCI).[1] Solubility of the drug in different solvents were determined. Solubility study suggested pH dependent solubility. Clopidogrel bisulfate hydrolyzes in aqueous phase into free base. In-order to get a stable parenteral dosage form, lyophilization technique was used. Optimized lyophilized cycle process along with graphical representation is presented. Compatibility study for rubber closures, tubing, and filter membrane were done and absences of any loss of drug due to the adsorption of any of the component were checked. Osmolarity of the lyophilized product was checked. Dilution studies with normal saline, 5% dextrose injection up to 150 mg/ml for 12 Hrs without any degradation and precipitation was observed. Stress studies for thermal cycling, effect in presence of nitrogen and oxygen and photo stability were carried out. Accelerated stability study indicated that the lyophilized product is stable at 40 ^{o}C/75% RH up to 3 months. Polymorphic change during the process was evaluated. Parenteral formulation of clopidogrel bisulfate is of considerable interest in the area of Novel drug dosage form for treating critical cardiovascular diseases.

INTRODUCTION

As per reports and study of Payal Patel et. al. thrombosis superimposed on arteriosclerosis is the principal cause of mortality and morbidity in patients with arteriosclerosis.[2] Use of antiplatelet agents and anticoagulants in the treatment of arteriosclerosis is well established, based on many large randomized trials. Similarly, findings of Jonathan D. Marmur et. al. were reported for high-risk patients such as those with acute coronary syndromes (ACS; unstable angina, myocardial infarction) antiplatelet therapy with clopidogrel is indicated, based on results of the clopidogrel in unstable angina to prevent recurrent events (CURE) trial.[3] Platelet glycoprotein IIb/IIIa agents are powerful inhibitors of platelet function and are indicated for patients undergoing percutaneous coronary intervention. Clopidogrel bisulfate is available as oral antiplatelet agent and belongs to pharmacological category of thienopyridine class. The pharmacological action is to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease.[4,5] Clopidogrel bisulfate removed in oral solid dosage form has a maximum onset time of 1 Hr. Bioavailability of the drug is 50 %. Administering clopidogrel bisulfate through parenteral route will have fast onset of action during critical cardiac emergency condition. For the management of cardiovascular disorder, particularly in view of underperformance of oral antihypertensive therapy in some major interventions trials such as CURE trial in reducing the occurrence of coronary heart disease. All drug delivery through parenteral route seems to be more promising. Lyophilization technique can be one of the methods to prevent conversion of the clopidogrel salt into free base in presence of aqueous phase. In the review report and findings of Jack WC Tan et al. in the CREDO trial (clopidogrel for the reduction of events during observation) in which patients were randomized to 300 mg clopidogrel tablet found that six hours of pre-treatment with 300 mg of clopidogrel has been suggested to achieve the full clinical benefit of this loading dose.[6]

MATERIALS & METHODS

Clopidogrel bisulfate was obtained as gift samples from Aarti pharma (Ahmedabad, India), mannitol and solutol HS were obtained from Signet. Absolute alcohol (99.6% v/v) was obtained from shree chaltan vibhag khand udyog sahakari mandli ltd, Surat. All other chemicals were of analytical reagent grade.

Methods

Calculation of dose for the parenteral formulation: It has been observed in the CREDO trials that 300 mg dose of clopidogrel bisulfate is required through route to achieve the full clinical benefit before Pre-cutaneous Intervention Surgery (PCI). The pharmacokinetic data of clopidogrel bisulfate indicates a bioavailability of 50 % if administered through oral route. Presuming a bioavailability of 100 % through parenteral route, dose of 150 mg / vial can be considered for formulation of clopidogrel bisulfate parenteral formulation.

HPLC analysis of clopidogrel bisulfate estimation: The separation was conducted on column of inertsil ODS 3V.4.6X150 mm. The mobile phase consisted of 20% potassium dihydrogen phosphate pH to 7.5 with triethyl amine and 30% acetonitrile and 50 % methanol. The flow rate was 1.5 ml/min and the injection volume was 10 µL. The retention time was monitored by a UV detector at 220nm as λmax. All analysis was done in triplicate and the mean peak concentration was used to determine the concentration of clopidogrel in the formulation.

Formulation: The drug concentrate was prepared by dissolving clopidogrel bisulfate in ethanol in presence of solutol HS -15. Mannitol was dissolved in water separately. Both the solution were mixed and stirred properly. These solutions were stirred, filtered and lyophilized as per suitable lyophilized cycle. The qualitative and quantitative composition of clopidogrel bisulfate for injection is given in Table 1.

Lyophilization cycle: Freeze drying was executed in a lyophilizer (make: Virtis lyophilizer, USA). Lyophilization or freeze drying is defined as 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.[7] Freezing, primary drying and secondary drying are the three basics steps of freeze drying.[8] Solution of the selected antiplatelet remove drug formulation was prepared in the concentration of 10mg/mL. Initial sample was analyzed for drug content by HPLC. The solution was filled in to vials. After filling, the vials were half stoppered with lyo stoppers and loaded in to lyophilizer. Vials containing clopidogrel bisulfate were frozen to -45 ^{o}C for 19 hrs. Primary drying was carried out in a step wise manner at a rate of 12 ^{o}C / minutes. The drying time was kept at a temperature of – 33 ^{o}C for 1 hr followed by an additional 1 hour at a temperature of – 21 ^{o}C, and finally remove at a temperature of – 9 ^{o}C for 21 Hrs. Secondary drying was carried out at +15^{o}C & +35 ^{o}C for 9 hr & 20 hr respectively. End of the secondary drying was marked by determining the residual moisture content of the product. Secondary drying was continued until the final LOD was below 1.5%.Vaccum during primary and secondary drying was kept at a range from 440 to 530 mTorr respectively. Freeze dried vials were kept in the vials and stored properly at a temperature of 2 to 8 ^{o}C. The optimized freezed drying processes used for lyophilization of clopidogrel bisulfate are presented in Table 2.

Evaluation parameter of critical steps in the formulation:

Solubility studies in different solvents: The solubility of Clopidogrel bisulfate was determined by mixing an excess quantity of the drug with approximately 2 ml of the solvent taken in a screw-capped bottle. The bottles were rotated on a rotary gyratory shaker (Dolphin, Mumbai) at 100 rpm for 24 hrs at room temperature to attain equilibrium condition. The solubility data is presented in Table 3.

Polymorphic study: The Powder X-ray diffraction was done at room temperature with an X-ray diffractometer (Analytical Co., Netherlands) using Ni-filtered Cu K_ radiation (voltage 45 kV, current 40 mA). The scanning rate was 3.35◦/min over a 2ø_ range of 2–40◦ and with an interval of 0.0260◦. The peak intensity measured during initial and accelerated stability conditions are shown in Table 4.

Control Sample: Unfiltered formulation without any of the components such as

rubber stopper, tubing and filter membrane was stored in a SS316L and samples were withdrawn at different intervals. This served as control sample to study the compatibility of the drug with SS316L alone. Unfiltered formulation without any of the aforementioned components was stored in a borosilicate glass vessel and samples were withdrawn at different intervals. This served as counter control sample for compatibility with SS316L.

Compatibility study with rubber Stopper: Unfiltered formulation with different types of rubber stoppers were taken in a ratio of 0.2g of the rubber stopper per 2.0mL of the formulation in separate SS316L vessels.[9] Compatibility study with different types of rubber closures (Fluorotec coated rubber stoppers, Butyl coated rubber stoppers, Teflon coated rubber stoppers and Chlorobutyl coated rubber stoppers), were kept for 96 hrs and analyzed for the drug content by HPLC to determine any loss of drug and related substance along with the control sample. The results of the study are presented in Table 5. The results show that the Chlorobutyl rubber stopper is more compatible to the drug as compared to other stopper.

Compatibility study with tubing: Unfiltered formulations with different types of tubing's were taken in a ratio of 10.0 cm of the tubing per 50 mL of the formulation in separate SS316L vessels.[10] Compatibility study with different types of tubing's (peroxide cured and platinum cured tubing's), were kept for 96 hrs and analyzed for the drug content by HPLC to determine any loss of drug and related substance along with the control sample. The results of the study are presented in Table 6. The result indicates that the platinum cured silicon tubing shows more compatible as compared to peroxide cured silicon tubing.

Compatibility study with filter membranes: Unfiltered formulations with different types of filter membranes (one filter membrane per vessel) per SS316L vessels containing the formulation were studied.[11] Compatibility study with different types of filter membrane (PVDF and nylon membrane), were kept for 96 hrs and analyzed for the drug content by HPLC to determine any loss of drug and related substance along with the control sample. The results of the study are presented in Table 7.The result indicates that nylon

membrane filter shows more compatible as compared to PVDF membrane filter.

Osmolarity: The concentration of osmotically active particles expressed in terms of osmoles of solute per liter of solution.[12] Osmolarity is calculated from experimentally determined osmolality of a solution. Theoretical osmolarity is calculated as per the following formula;

$$Osmolarity = \frac{\text{Concentration}}{Molecular\ weight} * \text{No. of dissociable ions} * 1000$$

The osmolarity of formulations of Clopidogrel bisulfate for Injection was measured using Osmomat–030–D Osmometer. The results is presented in Table 8.

Thermal cycling Study: It is essential to investigate the effect of temperature variation for product suitability. The product was exposed to temperature variations that simulate short-term excursions outside the proposed labeled storage conditions for the product, in order to ensure the quality of the product throughout the shelf life at different temperature conditions. Three cycles, each of 2 days sample exposed at –5°C to –30°C and 2 days sample exposed at 40°C/75%RH were done. The results is presented in Table 9 indicate that, there were no significant changes in the physical and chemical stability of the drug along with the excipients.

Effect of nitrogen or oxygen on the product: To evaluate the effect of oxygen and nitrogen (dissolved or in gaseous state) on the formulation, trials were conducted wherein formulation was processed under oxygen or nitrogen (sparged into the formulation during the manufacturing process and head space back filled after Lyophilization) and then analyzed. [13], [14] Bulk solutions were divided into two parts. One part of the solution was filled in to 5mL vials with nitrogen purging and headspace filled with nitrogen. After filling, the vials were stoppered immediately to avoid loss of nitrogen from headspace and sealed .Another part of the solution was filled into 5mL vials with oxygen purging and headspace filling with oxygen. After filling, the vials were stoppered and sealed. Samples were withdrawn at different time intervals and analyzed for the drug content by HPLC to determine any loss of drug and related substance. The results of the study

are presented in Table 10. The result indicates that bulk solution purged with nitrogen and oxygen shows similar results in both assay and related substances.

Photo-stability Study: The intrinsic photo stability characteristics of new drug substances and products should be evaluated to demonstrate that, as appropriate, light exposure does not result in unacceptable change. Hence the effect of Visible and UV light on the product was evaluated by exposing the product for pre-determined number of days/hours (not less than 126hrs) in order to achieve an overall illumination of not less than 1.2 million lux hours, and an integrated near ultraviolet energy of not less than 200 watt hours/square meter, as required by ICH Q1B in a photo stability chamber.[15] The solution was filled in to vials, stoppered, sealed and initial sample was analyzed for drug content by HPLC. Samples were divided into three parts: first with primary package; vials with label and without carton denoted as sample 1, second with secondary package vials label but inside the carton denoted as sample 2, and finally third as controlled sample vials labeled and wrapped inside the aluminum foils which served as controlled sample denoted as sample 3. Sampled were analyzed for physical and chemical stability and results are presented in Table 11.The results indicates that the formulation was stable after exposed to both visible and UV light.

Optical Control: Flawlessness, intactness and cap fitting are also controlled. Any flaw of vial, stopper, or cap is critical; hence all vials are checked to ensure the integrity of the product.

RESULTS AND DISCUSSION

Optimized lyophilized cycle:

Process parameters (pressures, temperatures, duration) for all phases (freezing, primary and secondary drying) were determined on laboratory equipment. During the lyophilization it is critical for the parameters to stay at set values so as to obtain product that has satisfactory quality. During the lyophilization process, time course is monitored. Different lyophilization cycle were tried to optimize the cycle, which gives stable and uniform cake. Initial chamber temperature was kept at -45 ^{o}C to ensure that the ice formation is properly formed. Primary drying is carried out at a

temperature of -9 ^{o}C in order to prevent crumple of cake and improper drying.[16, 17, 18] The drying time was kept up for 21 Hrs which will prevent any damage of melting of ice ensuring uniform drying over the ice-cake. It was observed drying time below 21 Hrs resulted in melting of ice and improper drying. It has been reported by Lewis LM etal. that desorption of aqueous phase will be faster at a higher secondary temperature and time required will be less.[19] Secondary drying was done in a step wise manner at a temperature of 15 and 35 ^{o}C for 9 and 20 Hrs respectively. The end of the lyophilization process is determined by the residual moisture content of the lyophilized product. After completion of the secondary drying, the residual moisture content was found to be less than 1.5%.Finally the vacuum determines the heat exchange process between the shelf and product. The pressure for creating the vacuum was kept in the range of 440 to 544 mTorr.[20, 21, 22].

Solubility study: Clopidogrel bisulphate shows pH dependent solubility. It has more solubility in acidic media and solubility decreases towards basic media. Clopidogrel bisulfate shows highest solubility of 99.28 mg / mL in a pH of 1.2 whereas in a pH of 7.2 it shows least solubility of 0.051 mg. Acidic based parenteral formulation causes irritation and pain during administration. Hence solubility of clopidogrel bisulfate in ethanol, DMSO and water were investigated. It was observed that the solubility of clopidogrel bisulfate in ethanol, DMSO and water for injection were found to be 182.26 mg / mL, 96.23 mg / mL and 164.47 mg / mL respectively. The required concentration of the drug is 150 mg / ml hence ethanol and water for injection were selected as solvent to formulate clopidogrel bisulphate injection.

Polymorphic study: PXRD studies was carried on Clopidogrel bisulphate Form I formulation. Figure 2 represents initial diffractogram for form I of clopidogrel bisulfate, whereas Figure 3 represents diffractogram of three months accelerated stability condition at 40 ^{o}C /75 % RH. The peak intensity of the Form I 2 θ (theta) values of clopidogrel bisulfate initial and 3 months stability at accelerated condition 40 ^{o}C /75 % RH does not show any significant changes. The result of the study indicates that during the stability the polymorphic integrity of clopidogrel bisulfate form I remain unchanged.

Compatibility study with rubber stopper: Clopidogrel bisulfate shows good stability with all type of rubber stoppers. The assay of Clopidogrel bisulfate and related substances were compared with each other after 96 hrs of study. Finally 20mm Chlorobutyl coated igloo lyo rubber stopper was selected as a primary packaging material based on the least related substance of known and unknown impurity as compared to other rubber stoppers.

Compatibility study with tubing: After 96 hrs of study Clopidogrel bisulfate vials shows good stability with both silicone and peroxide cured tubing. The assay of Clopidogrel bisulfate was compared with that of the control sample. The maximum unknown related substances were found to be higher for peroxide cured silicone tubing as compared to platinum cured silicone tubing. Hence platinum cured silicone tubing was selected as a manufacturing process component during filtration and filling for the product.

Compatibility study with filter membranes: After 96 hrs of study Clopidogrel bisulfate vials showed good stability with both nylon and PVDF based filter membrane. The assay of Clopidogrel bisulfate in both the membrane filters was comparable with that of control sample. The total impurity of nylon membrane filter showed lower total impurity as compared to PVDF membrane filter. Hence nylon membrane filter was selected as a manufacturing process component during filtration and filling for the product.

Osmolarity: The measured osmolality of lyophilized clopidogrel bisulfate concentration was diluted to 0.5mg/mL and 0.05mg/mL with either 0.9% sodium chloride injection or 5% dextrose injection were compared. As the product is meant intravenous administration after dilution with either 0.9% sodium chloride injection or 5% dextrose injection, the osmolality of the product should match with the osmolality of blood. The result indicates osmolarity of 279, 295 and 349 mOsm/kg with 0.9% sodium chloride injection, 5% dextrose injection and WFI. The normal plasma osmolarity is in the range of 280-300 mOsm/kg. Hence 5% dextrose can be the infusion for the clopidogrel bisulfate injection.

Thermal cycling Study: After 3 cycles of freeze-thaw, no significant increase in known and maximum unknown impurities

were observed and no significant drop in assay of Clopidogrel bisulfate was observed. This shows that the formulation is stable to varied temperature conditions. The physical and chemical stability of the drug in the binary mixture of drug, mannitol and solutol was found to be satisfactory. Hence mannitol can be used as excipient (bulking agent) for lyophilization and solutol HS as solubilizer for clopidogrel bisulfate formulation.

Effect of Nitrogen or Oxygen on the product: Initial data of Clopidogrel bisulfate treated with oxygen and nitrogen shows a very slightly higher impurity profile with product process under oxygen. At 3 month accelerated condition study, no significant increase was observed with respect to known and unknown impurity profile compared to that of the initial of either sample. Even though impurity profile of known and unknown impurity was found to slightly higher with product treated with Oxygen, impurities are in controlled limit, which shows that the product is not sensitive to oxidation.

Photo stability Study: The study was divided into three samples. Sample 1 (formulation in primary packaging) i.e. vial with label without carton; sample 2 (formulation in secondary packaging) i.e vials with label in a carton; & sample3 (control sample) i.e vial with label and aluminum wrapped in a carton. After exposing the sample to both UV and Visible light, no significant increase in related substances and no significant drop in assay of Clopidogrel bisulfate were observed. This shows that the formulation is stable after exposure to UV and Visible light for a predetermined time.

CONCLUSION

Development of parenteral dosage of clopidogrel bisulfate by using lyophilization technique involved many studies such as photo stability study, an oxygen sensitivity study, stopper & tubing compatibility study, a thermo cycling study, and an accelerated stability study. The studies result indicated a stable lyophilized product of clopidogrel bisulfate for parenteral dosage form. Freeze drying of clopidogrel bisulfate although is more tedious, time consuming and expensive but resulted in stable formulation with no change in the polymorphic form during stability

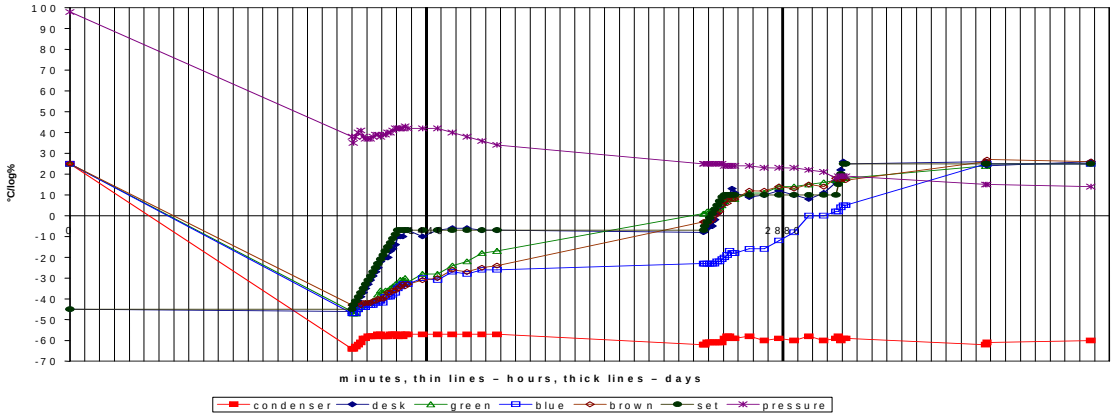

Figure 1:Freeze drying graphical representation of the process: (a) Left y-axis – temperature in °C (b) Right y-axis – pressure in mbar (c) Brown – set pressure (d) Green, blue – measured pressure (e) Cyan – set desk temperature (f) Magenta – measured desk temperature. In the graphs the pressure is expressed in log%; log %=10.log ‰ .Condenser temperature is measured by probe in the wall of condenser. Desk temperature is measured by probe in a desk. Green, Blue, and Brown are probes that are put into vials. Set temperature is set desk temperature.

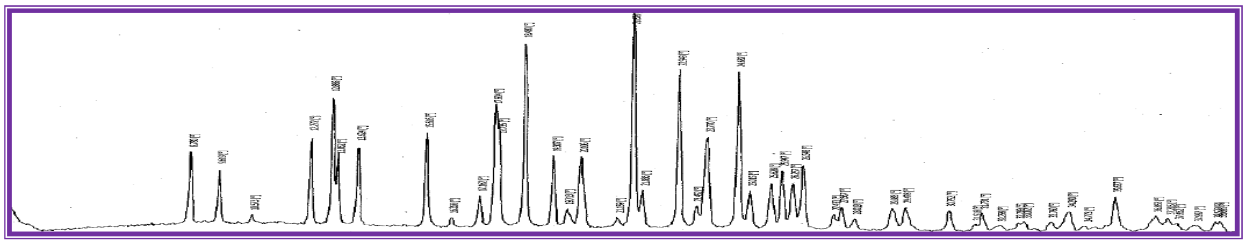

Figure 2 PXRD diffractogram of Clopidogrel bisulfate lyophilized powder (Initial)

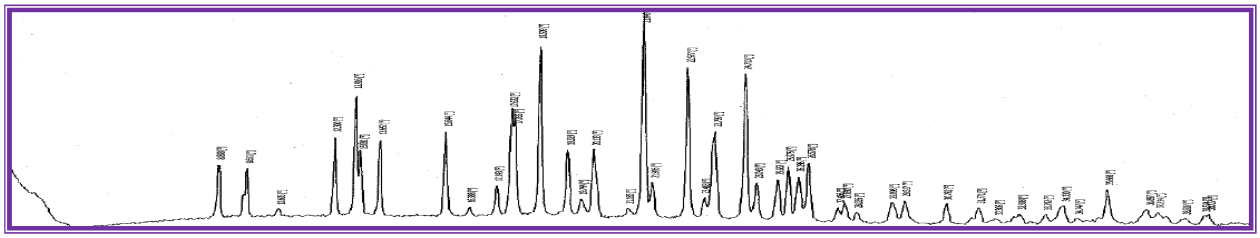

Figure 3 PXRD diffractogram of Clopidogrel bisulfate lyophilized powder (After 3 months accelerated condition of 40 ºC/75 % RH)

Table 1

Qualitative and quantitative composition

Sr. No.	Ingredients	% w/v
1.	Clopidogrel bisulfate	10
2.	Mannitol	58
3.	Solutol HS 15	32
4.	Ethanol	q.s.
5.	Water for Injection	q.s.

Table 2

The Optimized Lyophilization process

Lyophilization steps	Temperature (°C)	Temp increase rate (°C)	Time (Hrs)	Vacuum (m torr)	Ramp® or Hold (H)	Comments
Initial chamber temperature	-45	-	19		H	Vials were ok
Primary drying	-33	12	1	530	R	Vials were ok
	-21	12	1	533	R	Vials were ok
	-9	12	21	544	H	Vials were ok
Secondary drying	+15	24	1	500	R	Vials were Ok
			8	500	H	Vials were ok
	+35	20	1	440	R	Vials were ok
			19	440	H	Vials were ok

The values of log ‰ can be transformed into mbar by following equation:

$$mbar = 0.0034 * e^{0.00965 * \log ‰}$$

Table 3

Solubility study of Clopidogrel bisulfate

Medium	**Solubility (mg/ml)**
0.1 N HCl	99.28
pH 2.0 HCl Buffer	24.50
pH 4.5 Acetate Buffer	4.70
pH 6.8 Phosphate Buffer	15.57
pH 7.2 Phosphate Buffer	0.051
Ethanol	182.26
Water	164.47
DMSO	96.23

Table 4

Peak intensity ratio at 2 θ (theta) values

S. No.	**Initial**	**After 3 months accelerated study**
	2 θ values	2 θ values
1	12.886	12.916
2	18.480	18.509
3	21.620	21.649
4	22.949	22.977
5	24.682	24.713

Table 5

Compatibility analysis data with rubber stopper

Sample description	Duration (Hrs)	Assay (%)	Related Substances				
			Imp-A	Imp-B	Imp-C	Max UK	Total Impurity
API	Initial	-	ND	ND	ND	0.02	0.02
SS control	96 hrs	101.2	ND	ND	ND	0.04	0.04
Glass control	96 hrs	100.5	ND	ND	ND	0.04	0.04
Fluorotec coated rubber stoppers	96 hrs	100.6	ND	0.02	ND	0.06	0.08
Butyl rubber stoppers	96 hrs	101.3	0.01	ND	ND	0.07	0.07
Teflon coated rubber stoppers	96 hrs	100.9	ND	ND	ND	0.06	0.06
Chlorobutyl rubber stoppers	96 hrs	99.6	0.01	ND	ND	0.05	0.05

Table 6

Compatibility analysis data with tubing's

Sample description	Duration (Hrs)	Assay (%)	Related Substances				
			Imp-A	Imp-B	Imp-C	Max UK	Total Impurity
API	Initial	-	ND	ND	ND	0.02	0.02
SS control	96 hrs	101.5	ND	ND	ND	0.04	0.04
Glass control	96 hrs	101.6	ND	ND	ND	0.04	0.04
Peroxide cured silicon tubing	96 hrs	101.5	ND	ND	ND	0.08	0.08
Platinum cured silicon tubing	96 hrs	101.8	ND	ND	ND	0.05	0.05

Table 7

Compatibility analysis data with filter membranes

Sample description	Duration (Hrs)	Assay (%)	Related Substances				
			Imp-A	Imp-B	Imp-C	Max U	Total Impurity
API	Initial	-	ND	ND	ND	0.02	0.02
SS control	96 hrs	101.2	ND	ND	ND	0.04	0.04
Glass control	96 hrs	100.5	ND	ND	ND	0.04	0.04
PVDF membrane filter	96 hrs	100.1	ND	ND	ND	0.07	0.07
Nylon membrane filter	96 hrs	100.3	ND	ND	ND	0.05	0.05

Table 8

Osmolarity data of Clopidogrel bisulfate with different infusion

Osmolality (mOsm/Kg)		
150mg/mL in WFI	150 mg/ml in 0.9%NaCl	150 mg/ml in 5%Dextrose
349	295	289

Table 9

Analysis result of thermal cycling study

Parameters	Specifications	Condition and schedule	
		Initial	Thermal cycling study
Assay	90-110%	102.5	101.6
Imp-A	NMT 0.2%	ND	ND
Imp-B	NMT 0.3%	ND	ND
Imp-C	NMT 1.0%	ND	ND
Max unknown Impurity	NMT 0.1%	0.05	0.06
Total impurity	NMT 1.5 %	0.12	0.32
pH	5.0 to 6.0	5.3	5.5

Table 10

Analysis result of Clopidogrel bisulfate with nitrogen and oxygen

Parameters	Initial		3 months 40°C/75%RH	
	O2	N2	O2	N2
Assay	101.9	101.5	100.6	101.2
Imp-A	0.01	ND	0.02	ND
Imp-B	ND	ND	0.01	ND
Imp-C	ND	ND	ND	ND
Max unknown impurity	0.04	0.03	0.06	0.04
Total impurity	0.05	0.03	0.09	0.04

Table 11

Photo stability data results

Parameters	Specifications	Condition and schedule			
		Initial	*Sample 1	**Sample 2	***Sample 3
Assay	90-110%	102.6	101.8	101.5	101.2
Imp-A	NMT 0.2%	ND	ND	ND	ND
Imp-B	NMT 0.3%	ND	ND	ND	ND
Imp-C	NMT 1.0%	ND	ND	ND	ND
Max unknown impurity	NMT 0.1%	0.05	0.04	0.05	0.03
Total impurity	NMT 1.5 %	0.05	0.04	0.05	0.03

*Sample 1: Vial with label without carton; **Sample 2: Vial with label in a carton;

***Sample 3: Vial with label and aluminum wrapped in a carton (control sample)

REFERENCES

1. Faxon DP: Use of antiplatelet agents and anticoagulants for cardiovascular disease: current standards and best practices Rev. Cardiovasc. Med. 2005; 6 Suppl 4: S3-14.

2. Payal Patel and Lily Cheng: The role of clopidogrel in the emergency department: Can J Emerg Med 2005; 7(1):22-7.

3. Jonathan D. Marmur, Cristina A. Mitre, Elliot Barnathan and Cavusoglu: Benefit of bolus-only platelet glycoprotein IIb/IIIa inhibition during percutaneous coronary intervention: Insights from the very early outcomes in the evaluation of 7E3 for the prevention of ischemic complications (EPIC) trial: American heart journal 2006; 152(5): 876-881.

4. Fontana P, DuPont A, Gandrille S, Bachelot-Loza C, Reny JL, Aiach M and Gaussem P: Adenosine diphosphate induced platelet aggregation is associated with P2Y12 gene sequence variations in

healthy subjects: Circulation 2003; 108:989 –995.

5. Gladding P, Webster M, Zeng I, Farrell H, Stewart J, Ruygrok P, Ormiston J, El-Jack S, Armstrong G, Kay P, Scott D and Gunes A, ML: The antiplatelet effect of higher loading and Maintenance dose regimens of clopidogrel: the PRINC (Plavix response in coronary intervention) trial; J .Am Coll. Cardiol. Intv. 2008; 1:612–619.

6. Jack WC Tan and Kenneth WQ Guo: Antiplatelet therapy during acute percutaneous coronary intervention: New strategies and therapeutics: Annals academy of medicine. 2010; 39 (3):221-229.

7. Stefan C. Schneid, Henning Gieseler, William J. Kessler,Suman A. Luthra, and Michael J. Pikal: Optimization of the secondary drying step in freeze drying using TDLAS technology: AAPS PharmSciTech. 2011; 12(1): 379–387.

8. Aulton ME: Dosage form design and manufacture. Pharmaceutics: The science of dosage form design, 2nd Edition, 2002: (307-339).

9. Kostecka D, Duncan MR and Wagenknecht D.: Formulation of a stable parenteral product; Clonidine Hydrochloride Injection. PDA J Pharm Sci Technol. 1998; 52(6):320-5.

10. Selina Hines and Steve Pleasance.: Compatibility of injectable hydromorphone formulations with typical diluents, components of giving sets and drugs for potential co-administration. EJHP Praticel. 2011, 17(3):47-53.

11. Xiaolin (Charlie) Tang and Michael J. Pikal: Design of freeze-drying processes for pharmaceuticals: practical advice: Pharmaceutical Research.2004, 21(2):191-200.

12. Jain Jain, Clara Fernandes and Vandana Patravale: Formulation development of parenteral phospholipid-based micro emulsion of Etoposide: AAPS PharmSciTech.2010, 11(2): 826-831.

13. Sandhya chaurasia, Sneha Golani, Nishi prakash Jain, Manoj Goyal and Sofiya verma: Comprehensive review on aseptic fill/finish manufacturing as per regulatory guidelines: Journal of current pharmaceutical research 2011; 5 (1): 19-27.

14. ICH Topic Q1B: Photo stability testing of new active substances and medicinal products January 1998: CPMP/ICH/279/95

15. Daoussia R, Vessota S, Andrieua J and Monnierb O: Sublimation kinetics and sublimation end –point times during freezing of pharmaceutical active principle with organic co-solvent formulations.Chem Eng Res Des 2009;87:889-907.

16. Teagarden DL and Baker DS. Practical aspects of freeze-drying of pharmaceutical and biological products using non-aqueous co-solvents systems. Informa Healthcare; 2010; 47: 254-87.

17. Schneid SC and Henning G: Rational approaches and transfer strategies for the scale up of freeze drying cycles. Chimica Oggi 2011: 29: 43-6.

18. Lewis LM, Teagarden DL, Johnson RE and Ahmed SS: Rational design of a freeze dried formulation for a biologic. Am Pharm Rev 2008; 11:91-6.

19. Eyster HC: Effect of temperature on catalase activity. Ohio J Sci 1950; 50: 273-7.

20. Blue J and Yoder HS: Successful lyophilization development of protein therapeutics Am Pharm Rev 2009; 12: 90-6.

21. Tang X and Pikal MJ: Design of freeze-drying processes for pharmaceuticals: Practical advice. Pharm Res 2004; 21:191-200.