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PHYSICO-CHEMICAL STUDIES ON STABILITY OF CLOPIDOGREL TABLET FORMULATIONS

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ABSTRACT

Clopidogrel bisulphate (anti-coagulant drug) is an enantio-selective chrial drug and needs to be monitored for the active form in tablet formulation. Hence, a stable tablet formulation was developed for clopidogrel using two different disintegrants of LHPC (to match as closely as possible to innovator tablet) and PVPP XL. Tablets were evaluated for stability analysis using validated analytical methods. The results showed similar drug release profile and assay on stability. Clopidogrel R-isomer was detected in both LHPC and PVPP XL containing tablet formulations and it was found to be less than 2.5% after six months stability study under accelerated conditions which is simulation of three years storage period

KEYWORDS: Clopidogrel tablets, PVPP XL, LHPC and Stability

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INTRODUCTION

Clopidogrel[Methyl(+)-(S)-alpha-(o-chlorophenyl)-6,7-dihydrothieno[3,2-a]pyridine-5(4*H*)-acetate] (Figure 1) is a routine component of the clinical management of patients after acute coronary syndrome. It has been reported that this drug would reduce rates of major cardiovascular adverse events[1]. It is approved for the reduction of atherosclerotic events in patients with stroke, myocardial infection, cardiovascular disease and acute coronary syndrome. Its action may be related to an adenosine diphosphate (ADP) receptor on platelet cell membranes. It specifically and irreversibly inhibits the platelet P2Y12 subtype of the ADP receptor, which is important in the aggregation of platelets and cross-linking by the protein fibrin[2]. As a result, activation of the glycoprotein IIb/IIIa complex, which is involved in platelet activation and stabilization of the platelet aggregate, is also inhibited[3]. Clopidogrel base has not been used as a commercial drug, since it is an oil phase which is difficult to purify and handle. Thus, Clopidogrel bisulphate, a salt form, has been mainly used in commercial products (Plavix; Sanofi-Aventis Korean Co.) because of its crystalline form and improved solubility of about 90mg/mL [4, 5]. However, this commercial drug has been reported to be unstable under accelerated moisture and heat conditions[6], resulting in the production of significant amounts of degradants [7]. At accelerated storage conditions of 40ºC/ 75%RH, this compound gets hydrolyzed to form a degradant, which has no biological activity. Moreover, it can be transformed into an isomer, racemized degradant, with much less pharmacological activity[8]. During the development of drug products, predictability is a desirable attribute for assuring quality of the drug product[9]. In the development of pharmaceutical products, many different types of formulations or dosage forms are examined prior to settling on the final market image of the product. As seen in figure 1, the molecule is a thienopyridine derivative containing an assymetric carbon leading to the existence of two enantiomers (R and S). Studies from Sanofi–Synthelabo showed that the active compound clopidogrel is the S-enantiomer[10]. This implies that the content of the R-enantiomer must be carefully controlled in Clopidogrel bulk substance and drug products, as a regulatory requirement[6].

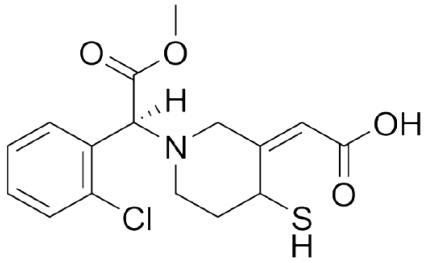

Figure 1
Structure of Clopidogrel

According to the International Conference on Harmonization (ICH) guideline (ICH Q3B) entitled “Impurities in New Drug Products”, the level of impurities contained in the drug product should also be carefully controlled and the upper level of impurities should be determined and supported by adequate data in the dossier filed for registration of a new drug [12]. Clopidogrel bisulphate is marketed in the name of Plavix and contains hydrogenated castor oil, low substituted hydroxypropylcellulose, mannitol, microcrystalline cellulose and polyethylene glycol 6000 as inactive ingredients. The objective of present study is to

develop a stable Clopidogrel tablet formulation using Polyplasdone XL (PVPP XL) and evaluate the tablet physico-chemical properties[14] against innovator tablet containing LHPC.

MATERIALS AND METHODS

(i) Formulations

Two different formulations were developed and both are containing Clopidogrel Bisulphate with label claim of 75 mg of Clopidogrel free salt. Details of the formulations are given in Table 1 .

Table 1
Details of Clopidogrel Tablet formulation

Details of Ingredients	Weight of tablet in mg	Percentage
Active	98	31.6
MCC	50	16.1
Mannitol	102	32.9
Superdisintegrant*	10	3.2
PEG 6000	30	9.7
Aerosil	5	1.6
Talc	5	2.6
Adventia Prime Pink	10	3.2
Total weight	310	

Superdisintegrant: Polyplasdone XL (PVPP XL) and Low substituted hydroxylpropyl-cellulose (LHPC).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

In order to achieve separation among two isomers of Clopidogrel, different HPLC parameters like type, capacity, pH of buffers, ratio of buffers, organic modifier and flow rate were optimized[15]. Complete analysis was performed on a chiral column, ULTRON ES-OVM, 4.6 mm × 150 mm, 5µM. The effect of composition and pH of the mobile phase on the retention time of Clopidogrel was studied. An increase in the percentage of acetonitrile decreases the retention of Clopidogrel. On increasing acetonitrile concentration to more than 75%, Clopidogrel is eluted with the solvent front. While acetonitrile concentration of lower than 15% was used, the elution of Clopidogrel peak was observed to be seriously delayed. In addition, the effect of pH in the chromatographic elution of the drug was investigated by changing pH values of aqueous component of the mobile phase from 6.0 to 2.5 using potassium phosphate buffer. Clopidogrel is a weak base (pKa 4.5), thereby at pH below 4.5 its retention decreases due to protonization. 10mM phosphate buffer mixed with methanol in ratio of 75:25 was chosen as mobile phase for optimum separation of R and S-isomers, since the analyte peaks were well defined and resolved[5, 6]. The optimum wavelength for detection was at 220nm, at which the best detector response was obtained for all the substances. The HPLC system consisted of Waters Alliance pump (Waters Corporation, MA, USA) equipped with a Waters PDA 2996.

(i) Instrument parameters

Mobile phase	: Degassed Buffer and Methanol mixture in volumes of 75:25mL
Buffer	: 10 mM Potassium dihydrogen phosphate buffer prepared by dissolving1.36 g KH_2PO_4 in 1000 mL of water and degassed through 0.45µM filter.
Diluent	: Mobile phase
Flow rate	: 1 mL min^{-1}
Column	: ULTRON ES-OVM, 4.6 mm × 150 mm, 5µM (Agilent)
Column temperature	: 25°C
Injection volume	: 10 µL
Detector wavelength	: 220 nm
Run time	: 20 minutes
Retention Time	: About 4.5 ± 0.5 minutes

(ii) Preparation of Standard Stock Solution

Standard of Clopidogrel bisulphate solution was prepared by dissolving 100 mg of drug in 100 mL volumetric flask with methanol. 5 mL of this standard stock solution was transferred into 50 mL volumetric flask and made upto the mark with mobile phase. The prepared solution was mixed well for 15 minutes using cyclomixer. This yielded standard solution with nominal concentration of 75ppm of Clopidogrel free base (without bisulphate).

(iii) Preparation of Sample Solution

The samples were prepared by finely powdering 20 tablets from each lot using mortar and pestle. Powder equivalent to one tablet weight contain 98.0 mg of Clopidogrel bisulfate which is the molar equivalent of 75 mg of Clopidogrel base was dissolved in 100 mL of methanol and mixed well. This sample solution was centrifuged at 2000 rpm for 10 minutes and filtered. From this 5 mL was pipetted out and the volume was made to 50 mL with diluent. This solution is 75 ppm of Clopidogrel free base. The standard, sample and placebo solutions were introduced as triplicate injections. The assay of the samples was determined by comparing the area counts of the sample with that of the reference standard.

(iv) Dissolution test

The dissolution test was performed using USP type II (Paddle apparatus) with 1000 mL HCl buffer solution of pH 2.0 as dissolution media at 37±0.5ºC. The speed of the paddle was adjusted to 50 rpm. Clopidogrel tablets at an equivalent dose of 75 mg Clopidogrel free base were placed into the dissolution tester. At pre-determined time intervals of 0, 10, 20, 30, 45 and 60 minutes, 10 mL of the medium was sampled and filtered through a membrane filter (0.45 µm). The concentration of Clopidogrel in the filtrate was analyzed using Waters HPLC.

RESULTS AND DISCUSSION

(i) Tablet Characteristics

The prepared matrix tablets were characterized immediately for hardness, friability, disintegration and drug content. The hardness of both LHPC and PVPP XL containing tablets showed 55 Newtons with variation was less than ±2 Newtons from tablet to tablet. It means that the applied compression force is good enough to give the desired hardness and without chipping and capping. Another test of friability of tablets showed less than 1% w/w variation. It indicates that the tablets were compressed
properly and ability of the compressed tablet to avoid fracture and breaking apart during

transportThe dissolution rate of a drug from a tablet results, a combination of the physico-chemical properties of the drug itself and the disintegration of the tablet. It is therefore of value to study the disintegration step in isolation, in order to examine the influence of physiologically relevant media on the preliminary step in the overall dissolution process. Additianally, disintegration is frequently used as an initial screen for formulations in development. Clopidogrel tablets formulated using PVPP XL and LHPC were checked for disintegration time and a marked difference was observed between the tablets containing of PVPP XL and LHPC.

(ii) In-Vitro Drug Release Profile

According to the percentage drug release results presented in Table 2, it can be clearly seen that 80% release of drug was observed in PVPP XL containing tablets within 15 minutes whereas drug release was slow in tablets formulated with LHPC at accelerated condition of 40ºC/75%RH after six months time.

Table 2
Dissolution data of Clopidogrel tablets

Period months	Storage condition details	Superdisintegrant containing			
		T 50 DATA in minutes		T 80 DATA in minutes	
		LHPC Tablet	PVPP XL Tablet	LHPC Tablet	PVPP XL Tablet
Zero		10:62	3:19	16:88	6:96
One	40ºC/75%RH	12:71	2:15	24:91	7:00
	30ºC/65%RH	11:52	2:78	18:65	7:88
	25ºC/60%RH	11:41	3:35	18:74	7:26
Two	40ºC/75%RH	16:03	2:78	28:71	9:02
	30ºC/65%RH	13:71	4:54	24:89	11:32
	25ºC/60%RH	14:73	3:69	28:06	8:99
Three	40ºC/75%RH	14:65	2:48	27:04	7:03
	30ºC/65%RH	11:77	3:80	18:99	7:48
	25ºC/60%RH	10:90	3:36	17:35	8:40
Six	40ºC/75%RH	13:92	3:15	26:50	7:81
	30ºC/65%RH	12:15	2:17	22:17	6:55
	25ºC/60%RH	13:22	3:60	25:24	10:43

However, both the tablets (with either PVPP XL or LHPC) showed drug release at 30 minutes in line with US Pharmacopeia limits [Not less than 80% (Q) of the labeled amount of Clopidogrel should be dissolved in 30 minutes]. It means that both tablets are stable for three years at room temperature. The obtained data is presented in Table 2.

3.3 Assay and Percentage Purity

Assay of the tablets was carried out using validated HPLC method. The Assay of PVPP XL and LHPC containing tablet showed greater than 99.0% at accelerated condition (40°C/75% RS-isomer) in both formulations because it is important to monitor R-isomer of clopidogrel in tablets.

Table 3
Data on assay of Clopidogrel tablets by HPLC

Storage Period months	Storage conditions	Superdisintegrant containing	
		PVPP XL Tablet	LHPC Tablet
Zero		101.2	100.9
One	40ºC/75%RH	99.3	100.8
	30ºC/65%RH	99.8	100.8
	25ºC/60%RH	100.2	100.5
Two	40ºC/75%RH	99.9	100.4
	30ºC/65%RH	99.5	100.2
	25ºC/60%RH	100.0	100.0
Three	40ºC/75%RH	99.8	100.2
	30ºC/65%RH	99.3	100.1
	25ºC/60%RH	99.9	100.6
Six	40ºC/75%RH	99.1	99.2
	30ºC/65%RH	99.0	99.0
	25ºC/60%RH	99.7	99.4

The R-isomer in both tablet formulation was found to be less than 2.5% at three different ICH condition after six months storage. Detailed results of assay and purity are summarized in Tables 3 and 4. The purity of the tablets was consistent from day zero to six months time point at all three ICH conditions. Similarly, R-Isomer also consistent in tablets containing of PVPP XL as compare to LHPC tablets (equivalent innovator formula) from day zero to six months.

Table 4
Percentage Purity of Clopidogrel tablets

Storage Period months	Storage conditions	Superdisintegrant containing			
		PVPP XL		LHPC	
		Clopidogrel S-Isomer S-Isomer	Clopidogrel R-Isomer R-Isomer	Clopidogrel S-Isomer S-Isomer	Clopidogrel R-Isomer R-Isomer
Zero		97.5	2.4	97.0	2.3
One	40ºC/75%RH	95.2	2.2	96.4	2.2
	30ºC/65%RH	93.9	2.1	96.6	2.2
	25ºC/60%RH	95.5	2.2	95.9	2.2
Two	40ºC/75%RH	98.2	1.7	98.2	1.8
	30ºC/65%RH	98.2	1.8	98.3	1.7
	25ºC/60%RH	98.2	1.8	98.3	1.7
Three	40ºC/75%RH	96.4	2.5	96.8	2.2
	30ºC/65%RH	96.1	2.5	96.7	2.4
	25ºC/60%RH	94.9	2.1	96.7	2.0
Six	40ºC/75%RH	95.0	2.5	95.8	2.4
	30ºC/65%RH	96.3	2.5	96.5	2.5
	25ºC/60%RH	96.3	2.4	96.4	2.3

CONCLUSION

Tablets of Clopidogrel were formulated using disintegrants of low substituted hydroxypropylcellulose (to match as closely as possible to Plavix) and Polyplasdone XL using wet granulation process. The tablets formulated with two different disintegrants showed almost similar percentage of drug release and assay on stability test. Clopidogrel R-isomer was detected in both LHPC (innovator equivalent formulation) and PVPP XL containing tablet formulations and it was found to be less than 2.5% at six months stability test.

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