

Analytical Method Validation of Stability Indicating Hplc Method For Determination of Assay of Clopidogrel Bisulphate Tablets Usby HPCL Method



Chemistry

KEYWORDS : Clopidogrel bisulphate working standard, HPLC with UV detector.

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ABSTRACT

Clopidogrel is a thienopyridine class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease and cerebrovascular disease. The drug works by irreversibly inhibiting a receptor called P2Y12, an adenosine diphosphate (ADP) chemoreceptor on platelet cell membranes. Adverse effect includes hemorrhage, severe neutropenia, and thrombocytopenic purpura (TTP), which is important in activation of platelets and eventual cross-linking by the protein fibrin 1. The method is developed and validated by using ULTRON ES-OVM column (150 x 4.6 mm, 5 μm) containing Buffer: Acetonitrile mobile phase in (75 : 25). The flow rate is set at 1.0 ml/minute and the injection volume is 10 μl. The run time of clopidogrel was 15.0 min. The linearity of method is 0.50-150.60 μg/ml, the correlation coefficient is found to be 0.9999. Eluents were monitored by UV-detector at 220 nm. The method was validated as per ICH guideline for various parameters like precision, linearity, accuracy, ruggedness and robustness.

Introduction

Chemically it is (+)-(S)-methyl 2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridine-5(4H)-yl)acetate, used to inhibit blood clots in coronary artery disease, and peripheral vascular disease. Which is important in activation of platelets and eventual cross-linking by the protein fibrin¹. Clopidogrel bisulphate has the empirical formula C₁₆H₁₆ClNO₂S representing a molecular weight of 321.82 g/mol. It has been shown to prevent ischemic stroke, recent myocardial infarction or cardiovascular disease^{2,3,4}.

Experimental

Instruments and Apparatus

Waters alliance HPLC 2996 with a Waters photodiode array detector 2996 and column Ultron ES-OVM, 150 X 4.6 mm, 5 μm Mettler Toledo analytical balance and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

Reagents and materials

Working standard of clopidogrel bisulphate and USP clopidogrel related compound B used as standard of all experiment. Acetonitrile, HPLC grade Water, Methanol, and monobasic potassium phosphate for preparation of mobile phase and sample preparation.

Preparation of Standard Solution

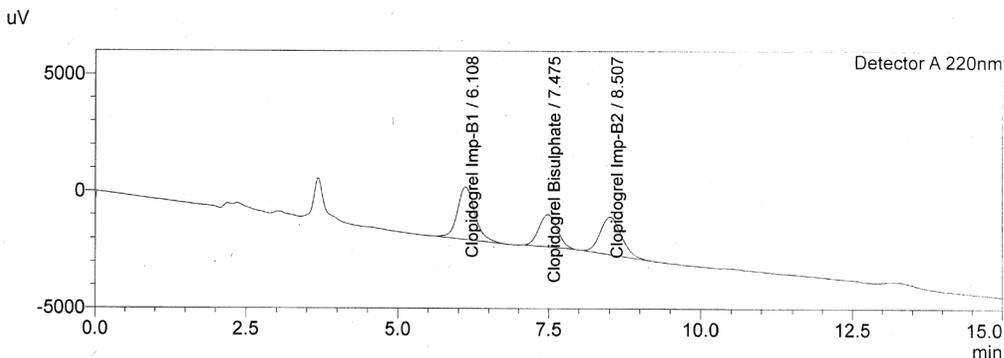
Transfer about 100 mg weight of Clopidogrel bisulphate WRS into 100 ml volumetric flask and dissolve in methanol and make up volume with methanol. Then 5 ml of above solution to a 50 ml volumetric flask and make up to mobile phase (100 ppm). This is standard solution.

System Suitability solution

Dissolve an accurately weighed quantity of USP Clopidogrel bisulphate related compound B RS in methanol, and mix with a Standard stock solution, to obtain concentration of about 1.0 and 0.5 mg per ml, respectively. Dilute this solution with mobile phase, and mix to obtain a solution having a final concentration of about 0.1 mg per ml of Clopidogrel related compound B (Fig.1).

Preparation of Sample Solution

Transfer the powder of Clopidogrel bisulphate equivalent to 100 mg to a 100 ml volumetric flask, add 50 ml of methanol. And sonicate for 10 minutes with intermittent shaking, and make up with methanol to mark and filter having a 0.45 μm filter paper and discarding the first 5 ml for rinsing then transfer 5 ml of above solution to a 50 ml volumetric flask and make up with mobile phase.



	Name	Retention Time	Area	% Area	USP Resolution
1	Clopidogrel Imp B1	6.108	45142	39.99	----
2	Clopidogrel Bisulphate	7.475	28964	25.66	2.52
2	Clopidogrel Imp B2	8.507	38773	34.35	1.71

Fig.1: System Suitability Chromatogram

Method Validation

Some analysis data are used in analytical validation.

Specificity

The Clopidogrel bisulphate Tablet was subjected to acid, base, oxidation, thermal degradation and photo degradation. For each degradation, a blank was prepared separately.

A blank, stressed sample for each degradation solutions were prepared and injected. The peak purity index for the main peak in all the degraded sample preparation was determined. (Table.1)

Table 1: Check for interference from forced degradation study

Condition and time	% Assay
Sample as is	100.05%
Acid degradation (1ml sample, 1N HCl, Heated in water bath at 80°C for 30 min)	100.42%
Alkali degradation (1ml sample, 1N NaOH, Heated at 80°C for 30 min)	101.09%
Peroxide degradation (1ml sample 30% H ₂ O ₂ , Heat in water bath at 80°C for 30 min)	99.67%
UV degradation (Sample, at 254nm for 24 hrs)	99.99%
Thermal degradation (Heat in water bath at 80°C for 15mins)	99.72%

Linearity and range

Linearity was determined at five levels over the range of 50% to 150% of test concentration. A standard linearity solution was prepared to attain concentration of 50%, 80%, 100%, 120% and 150% of the test concentration. Each linearity solution was injected in duplicate. The mean area at each level is calculated and a graph of mean area versus concentration is plotted. The correlation co-efficient (r^2), Y-intercept, slope of regression line, residual sum of squares are calculated. (Table.2)

Table 2: Linearity and Range

Sr.No.	Conc. (%)	Average area
1	50.18	875058.5
2	80.28	1422709
3	100.35	1737831
4	120.42	2057198.5
5	150.53	2619924.8
Correlation coefficient (r^2)		0.9995
Slope of regression line		17169.75
y-intercept		19559.79

Precision**Method Precision (Repeatability)**

Six replicate injection of the standard preparation were made into HPLC. And RSD of six replicate assays should be in limit 0.67 % found.

Intermediate precision (Ruggedness)

Intermediate precision was repeated on a different day; by different analyst, using a different HPLC system and different column using same lot of sample. calculated individual assay value, mean assay value % RSD, and overall % RSD record and Calculate the difference in the assay value of method precision and intermediate precision, calculate overall % RSD within 2%. (Table.3)

Table 3. Results of comparison Between Precision and Intermediate precision.

Sr. No.	Method Precision	Intermediate Precision
1	97.40	101.08
2	97.16	99.10
3	98.24	98.67
4	98.24	99.02
5	99.01	100.26
6	98.06	99.92
Mean	98.02	99.68
%SD	1.17	
%RSD	1.19	

Solution stability

This was evaluated by injecting initially a freshly prepared Clopidogrel Bisulphate standard solution and sample solution at different time intervals and results are % deviation from mean initial area count within limit. (Table.4)

Table 4: Solution stability for standard

Time (Hrs.)	Area of Clopidogrel bisulphate peak		% Deviation from mean Initial area count (Limit: $\pm 3.0\%$)	
	Standard	Sample	Standard	Sample
Initial	1750434.1	2363596.1	0.0	0.0
12	1756082.78	2369171.86	0.3	0.2
17	1754484.91	2357314.46	0.2	-0.3
22	1765918.97	2357750.03	0.9	-0.2
31	1766275.98	2358977.22	0.9	-0.2

Results and Discussion

The responses of sample solutions were measured at 220 nm for quantitation of Clopidogrel bisulphate by the proposed methods. The amount of Clopidogrel bisulphate present in the sample solutions was determined. The mobile phase consisting of Buffer: Acetonitrile (75:25), at a flow rate of 1.0 ml/min was found to be satisfactory to obtain good peak symmetry, better reproducibility and repeatability for Clopidogrel bisulphate. Quantification was achieved with UV and PDA detector at 220 nm based on peak area. The relative retention times were found to be 0.8 and 1.2 for two enantiomer of clopidogrel of related compound B, 1.0 for clopidogrel. Linear correlation was obtained between peak area and concentration for clopidogrel in the range of 50.18 - 150.53% con. (Table.2). The method was found to be specific as no significant change in the responses of clopidogrel bisulphate was observed after 31 hrs.

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