Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF CLOPIDOGREL IN TABLETS

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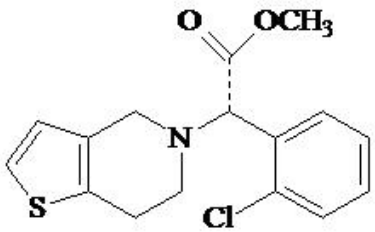
ABSTRACT

A Simple, sensitive and specific RP-HPLC method was developed and validated for the determination of clopidogrel in tablets. Isocratic chromatography was performed on a C18 column with acetonitrile-methanol-phosphate buffer 0.1M 80:10:10 (v/v/v) as mobile phase at a flow rate of 0.9 ml/min. UV detection was set at 240 nm. The method was validated with respect to accuracy, linearity, precision, selectivity, and robustness. All the parameters examined met the current recommendations of U.S.P (30) for analytical method validation. The method can be reliably used for routine quality control analysis and to determine the clopidogrel content of marketed tablets.

Keywords: Clopidogrel, RP-HPLC, Validation.

INTRODUCTION

Clopidogrel is an oral, thienopyridine class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease (figure 1). It is marketed by Bristol-Myers Squibb and Sanofi-Aventis with the trade name Plavix. It is an irreversible inhibitor of P2Y12, an adenosine diphosphate ADP chemoreceptor[1]. Blocking this receptor will inhibit platelet aggregation by blocking activation of the glycoprotein IIb/IIIa pathway.



(+)-(*S*)-methyl 2-(2-chlorophenyl)-2-(6,7-dihydrothieno [3,2-*c*]pyridin-5(4*H*)-yl)acetate

Figure 1: Structure of clopidogrel

Only few studies on determining clopidogrel content in pharmaceutical formulations have been published, involving Spectrophotometric and spectrodensitometric, voltametry as well as, HPTLC and HPLC methods[2-10]. Therefore, in the present investigation an attempt has been made to determine clopidogrel in dosages form using RP-HPLC without internal standard. Method validation procedure (linearity, precision, accuracy, selectivity and robustness) was based on the recommendations of U.S.P 30 for analytical method validation[11, 12].

MATERIALS AND METHODS

Clopidogrel working standard was obtained from the ministry of health- Syria and clopidogrel tablets were obtained from different local and regional manufactures.

Acetonitrile, methanol, and water of HPLC grade were purchased from SHAMLAB, sodium dihydrogen phosphate and phosphoric acid reagent grade from Riedel- de-hean.

A Jasco HPLC system equipped with a OPU-980 Intelligent HPLC gradient Pump, UV -970 Intelligent UV/VIS detector, manual injector and knauer C18 column (250 x 4.6mm, 5µm) was used. The mobile phase consisted of a mixture of acetonitrile-methanol-phosphate buffer (80:10:10, v/v/v). The flow rate was set to 0.9 ml/min. and the detection wavelength was set at 240 nm.

Preparation of standard and stock solutions

Stock solution of clopidogrel 1 mg/ml was prepared in methanol and diluted further with methanol to obtain standard solution of 100 µg/ml.

Preparation of Sample solutions

Twenty tablets (which were previously subjected to mass uniformity test) were weighed and finely powdered. A mass equivalent to 100 mg of clopidogrel was weighed and transferred in a 100 ml volumetric flask, mixed with methanol, and sonicated for 30 minutes. The solution was filtered through 0.45µ filter paper. The filtrate was transferred to a 100 ml volumetric flask and diluted to the mark with methanol. Aliquots of this solution (8, 10, 12 ml) were further diluted to 100 ml with methanol to obtain solutions containing 80,100,120 µg/ml respectively which were injected and chromatographed.

Accuracy

The accuracy of the method was assessed by determination of the recovery of the method at three

different concentrations (corresponding to 80,100, and 120% of the standard solution concentration) along with the excipients. Each concentration was injected in triplicate.

Linearity

Five concentrations were prepared containing 80, 90, 100, 110, and 120% of the standard solution concentration. Each solution was injected. Linearity was evaluated by linear-regression analysis.

Precision

Precision of the method was determined by performing repeatability and intermediate precision test. Repeatability of the method was checked by carrying out nine independent assays at three concentration levels. Intermediate precision was performed by analyzing the samples by two different analysts. Precision was determined as the relative standard deviations (RSD) of the drug recoveries at 80, 100 and 120 % concentration levels.

Selectivity

Selectivity of the method was assessed by preparing tablet powder without clopidogrel with the same excipients as those of in the commercial formulations. For RP-HPLC the solution was prepared using the same procedure as for the standard solution.

Robustness

Robustness of the method was performed by deliberately changing the flow rate of the mobile phase from 0.9 to 0.8 and 1.0 ml/min. The standard solution and different sample preparations were injected in each varied condition.

Detection limit and Quantification limit

The detection limit DL and Quantification limit QL were measured from the signal-to-noise ratio**.** The DL was defined as the concentration level corresponding to peak area of three times the baseline noise. The QL was defined as the lowest concentration level of a peak area with a signal-to-noise ratio higher than 10.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

In order to develop a suitable and robust LC method for the determination of clopidogrel, various chromatographic conditions were employed using different mobile phases. The system containing acetonitrile: methanol: 0.01*M* sodium dihydrogen phosphate buffer (pH 3.0 adjusted with orthophosphoric acid) (80:10;10, v/v/v) as a mobile phase at a flow rate of 0.9 ml/min was found to be satisfactory and gave well resolved peak for clopidogrel. A UV scan was performed and 240 nm was selected as a detection wavelength for estimation of clopidogrel using HPLC. Complete resolution of the peak with clear baseline separation was obtained. The retention time for clopidogrel was 5.21 min. (figure2).

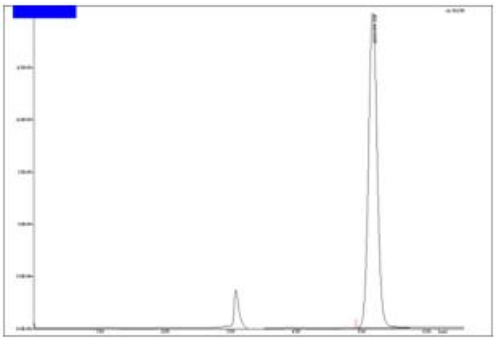


Figure 2: HPLC chromatogram of clopidogrel

System suitability

System suitability was performed before each validation run, five replicate injections of system suitability were performed. Retention time, area, asymmetry, theoretical plates, and capacity, for the five suitability injections were determined (table 1).

Table 1: System suitability for RP-HPLC method

Standard No	Retention time	Area	Asymmetry	plates	Capacity
1	5.185	2561182	1.075	8210	520
2	5.192	2653145	1.068	8139	520
3	5.205	2632279	1.066	8236	519
4	5.210	2674515	1.071	8184	518
5	5.212	2634005	1.085	8400	517
Average	**5.212**	**2629225**	**1.073**	**8233.8**	**518.8**
SD		**43358**			
RSD		**1.64**			

Table 2: Result of linearity of the proposed method

Standard No	Concentration		Area	
	µg /ml	%		
1	80	80	2238675	
2	90	90	2500922	
3	100	100	2669623	
4	110	110	2958938	R^2= 0.994
5	120	120	3225778	Y=24322X+28656

Table 3: Accuracy of the proposed method

Amount added µg/ml	Sets	Average amount recovered	Mean recovery %	% RSD
80	3	84.56	105.6	0.94
100	3	97.57	97.72	0.38
120	3	125.56	104.6	1.29

Table 4: Repeatability of the proposed method

Amount µg/ml	Sets	Average amount recovered	Mean % recovery	% RSD	% RSD
80	3	81.7	102.1	1.6	1.93
100	3	99.53	99.5	1.93	
120	3	120.46	100.9	1.9	

Table 5: Intermediate precision of the HPLC method

Amount µg/ml	*n*	Average amount recovered	Mean recovery %	% RSD	% RSD
80	3	83.43	104.2	0.2	2.0
100	3	100.83	100.8	1.7	
120	3	121.93	101.5	1.94	

Table 6: Selectivity of the proposed HPLC method

Amount µg/ml	Sets	Area	Average amount recovered	% recovery	Mean recovery %	% RSD
0	1	0	0	0	98.9	1.11
100	1	2488399	97.5	97.5		
100	2	2532070	99.2	99.2		
100	3	2552708	100.0	100.0		

Table 7: Robustness of the proposed HPLC method

Flow Rate	0.8 ml/min		1 ml/min	
Standard No.	Area	Asymmetry	Area	Asymmetry
1	2977393	1.000	2370334	1.009
2	2937596	0.994	2338355	1.012
3	2859140	0.994	2337780	1.008
4	2875139	0.987	2346308	1.013
5	2990602	0.986	2309289	1.008
Average	2927976		2340413	
SD	52887		19544	
RSD	1.8		0.8	
Sample	2921431	0.988	2379657	1.005
RT Std	5.76		4.58	
RT Sample	5.77		4.59	
Relative RT	1.002		1.001	

Table 8: Assay results for clopidogrel in marketed tablet dosage form by proposed HPLC method

Tablet	Amount claimed	Amount recovered	Mean % of label (claim)	% n=3 RSD
1	75	75	100	0.15
2	75	70.5	94	1.3
3	75	79.5	106	1.9
4	75	83.6	112.1	1.2
5	75	79.9	106.6	0.83
6	75	65.2	87	0.7
7	75	75.6	100.9	1.1

Validation of the developed methods

Calibration data for clopidogrel was shown in table 2. The linearity plot of clopidogrel was found to be linear. The linear equation and correlation coefficient were: Y=24322X+28656, 0.994 respectively (figure 3). This demonstrates the suitability of this method for the analysis of clopidogrel in tablets.

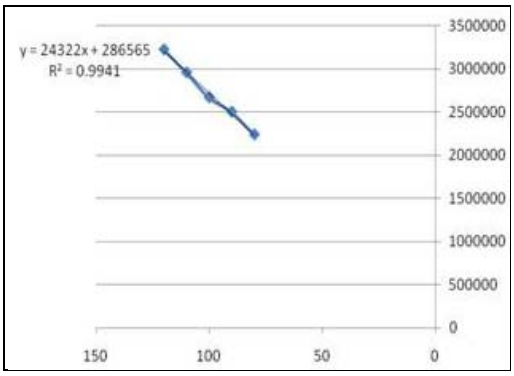


Figure 3: Relation between concentration and peak area of clopidogrel

The results of accuracy (table 3) showed that the method is accurate with percentage recovery of 97.7-105.6%, and acceptable RSD less than 2% at each level.

The percentage relative standard deviation of assay values for repeatability (n=9) was 1.93 (table 4) and for intermediate precision (n=9) was 2.0 (table 5).

The purity of analyte peak and the RSD value of < 2% (1.11 %) indicate that the method is selective for analysis of clopidogrel in its dosage form (table 6, figure 4).

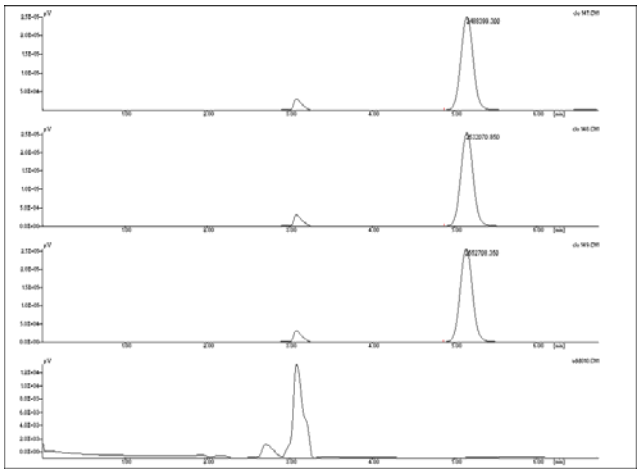


Figure 4: HPLC chromatogram of clopidogrel and excipients separately

The results of robustness test (table 7) indicated that the method is robust. The percentage relative standard deviation for the assay values (n=5) for clopidogrel peak were within the acceptance limit of 2%. The asymmetry peak was found to be <1.5, and the relative retention time of clopidogrel sample was 1.002, 1.001 (table 7).

The DL and QL were found to be 0.23µg/ml and 0.79µg/ml respectively.

Determination of clopidogrel in tablets

The developed method was applied for the determination of clopidogrel in tablets. The results of these assays ranged from 87% to 112% of the claim concentration (table 8). The results indicated that the method is selective for the assay of clopidogrel without the interference of the excipients used in the tablet.

CONCLUSION

An accurate, fast and precise isocratic reverse phase high performance liquid chromatographic method has been developed for the determination of clopidogrel in tablet dosage form. The developed method was found to be simple and have short run time which makes the method rapid. Several studies in the literature for the determination of the tested compound depends on UV, HPTLC or volumetric methods, few used HPLC upon then less used RP-HPLC. Nevertheless, the results of the study indicate that the developed HPLC method is simple, precise, accurate and less time consuming.

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