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Development and validation of a liquid chromatographic method for the determination of clopidogrel from pharmaceutical dosage form

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Abstract : A rapid, specific, precise and sensitive reversed-phase high-performance liquid chromatographic method was developed and validated for the determination of clopidogrel from pharmaceutical dosage form in the presence of flurbiprofen as internal standard. Clopidogrel and internal standard were analyzed on Purospher start C18, 5 μm column having 250 × 4.6 mm i.d., with mobile phase containing methanol : water (80 : 20; pH 3.4) was used. The flow rate was 1.0 mL min^{-1} and detector response was monitored at 235 nm. The retention time of clopidogrel and internal standard was 4.2 min and 6.4 min respectively. Calibration curve was linear in concentration range of 0.25-50.0 μg mL^{-1} with coefficient of determination (r^2) of 0.9994. Detection (LOD) and quantitation limits (LOQ) were 0.09 and 0.28 μg mL^{-1}, respectively. The intra-day accuracy ranged from 96.0-103.6% with a precision of 2.30-2.86%. Similarly, the inter-day accuracy was between 98.18 and 99.99% with a precision of 1.18-10.59%.

Keywords : Clopidogrel, liquid chromatography, HPLC-UV detection.

Introduction

Clopidogrel (Fig. 1) or (+)-(*S*)-methyl 2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3, 2-*c*]pyridin-5(4*H*)-yl) acetate sulphate, is a thienopyridine derivative. It is used for the reduction of atherosclerotic events including myocardial infarction, ischemic stroke and vascular diseases[1-6].

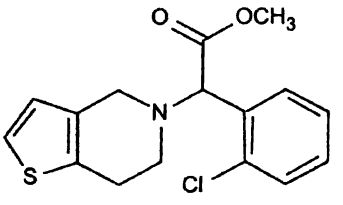

Fig. 1. Clopidogrel.

Literature survey revealed that few chromatographic methods for the determination of clopidogrel have been reported in pharmaceutical formulations[7,8] and plasma[9,10]. A nonenzymatic and enzymatic chiral inversion of clopidogrel has been investigated *in vitro* using ^{1}H NMR and a chiral HPLC spectrofluorimetric detection[11].

Previous reported methods[7,8] have more limitations or complexities due to use of complicated mobile phase (0.010 *M* sodium dihydrogen phosphate, pH 3.0) and acetonitrile (35 : 65, v/v), acetonitrile : phosphate buffer (50 : 50, v/v, pH 3.0), low or high flow rate (0.3 and 4.0 mL min^{-1}) and high LOD and LOQ values (0.97 μg mL^{-1} and 3.52 μg mL^{-1} and 0.12 and 0.39 μg mL^{-1}).

Therefore, the aim of this study was to develop a simple, rapid and reproducible method for the determination of clopidogrel from pharmaceutical dosage form. A comparison of published methods with ours is mentioned in Table 1.

Results and discussion

During the method development, some related parameters such as ratio of mobile phase, pH and wavelength were optimized to provide a sufficient separation. A pH value of 3.4 and detection wavelength of 235 nm was

Table 1. Comparison of published methods with developed method

Parameters	Aboul-Enein *et al.*[7]	Mitakos *et al.*[8]	Present method
Column	Monolithic silica column Chromolith performance 18e (100 × 4 6 mm i.d.)	BDS C8 (250 × 2 1 mm i.d., 5 µm)	Purospher start C18 (250 × 4 6 mm i.d., 5 µm)
Mobile phase	Acetonitrile · phosphate buffer (50 · 50, v/v, pH 3.0)	0 010 *M* Sodium dihydrogen phosphate acetonitrile (35 . 65, v/v, pH 3 0)	Methanol water (80 · 20, v/v, pH 3 40)
Flow rate (mL min^{-1})	4 0	0.30	1 0
Wavelength	235 (Photodiode detection)	235 (UV detection)	235 (UV detection)
Run time	–	3.08	4 2
LOD (µg mL^{-1})	0.97	0.12	0.09
LOQ (µg mL^{-1})	3.52	0 39	0 28
r^2	0.999	0.9991	0.9994

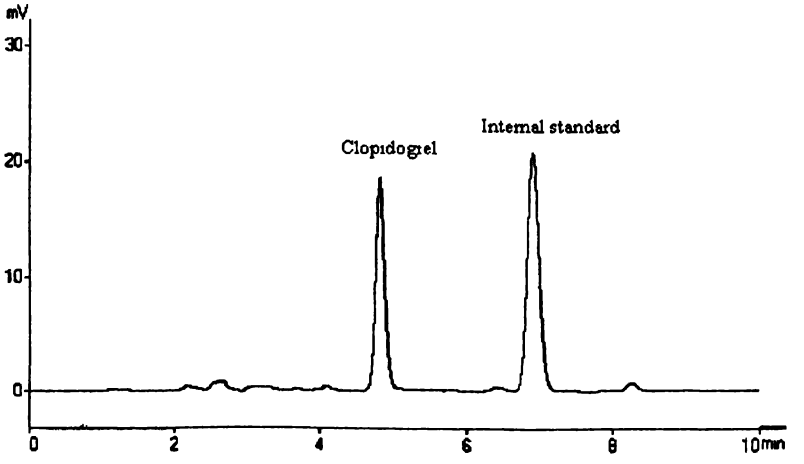

Fig. 2. Chromatogram of clopidogrel and internal standard (Flurbiprofen)

chosen for the optimum separation of the compounds, as this pH provided well resolved peaks. Chromatogram of clopidogrel with internal standard is shown in Fig. 2 where both the peaks are well resolved with retention time of 4.2 and 6.4 mL min^{-1} respectively.

Linearity of the analytical method was evaluated using pharmaceutical standards in the range of 0.25–50 µg mL^{-1} for three consecutive days at three determinations of each concentration. Calibration curves were constructed and linear relationship was obtained between the peak area ratio of clopidogrel to that of the internal standard and the corresponding concentration The regression and statistical parameters are shown in Table 2.

The reproducibilities of the proposed method were evaluated by recovery studies. In this study, the recovery assessment was made on the formulation samples instead

Table 2. Characteristic parameters of the calibration equations for the proposed method for determination of clopidogrel

Calibration range (μg mL^{-1})	0.25-50
Detection limit (μg mL^{-1})	0.09
Quantitation limit (μg mL^{-1})	0.28
Regression eq. $(Y)^a$:	
Slope (b)	3696.48
Standard deviation of the slope (Sb)	43.46
Relative standard deviation of the slope (%)	1.17
Confidence limit of the slope^b :	
Intercept (a)	3104.64
Standard deviation of the intercept (Sa)	1020.37
Confidence limit of the intercept :	
Coefficient of determination (r^2)	0.9994
Standard error of estimation	1865.51
Sum square residual	13920464
Degree of freedom (F)	7234.08

$^aY = a + bC$, where C is the concentration of compound in μg mL^{-1} and Y is the peak area.

^{b}95% confidence limit.

of preparing placebos. Thus, known amounts of clopidogrel formulation was spiked at different concentration levels at three replicates. Intra- and inter-day accuracy was calculated as the mean of the inter- and intra-day accuracy determinations. Intra- and inter-day accuracy ranged from 96.0–103.6% and 98.18–99.99% respectively (Table 3). The precision, expressed as a coefficient of variation (CV), was evaluated by calculating the intra- and inter-day coefficient of variation. The intra- and inter-day, percent coefficient of variation of the assay was found to be 2.30–2.86% and 1.18–10.59% respectively (Table 3).

Table 3. Intra- and inter-day precision and accuracy of the proposed method in pharmaceutical formulation

Concentration	Precision (CV, %)		Accuracy (%)	
(μg mL^{-1})	Intra-day	Inter-day	Intra-day	Inter-day
0.25	2.86	10.59	96.0	98.18
5.0	1.67	0.13	103.6	106.33
12.5	1.09	0.74	99.76	98.52
50	2.3	1.18	100.9	99.99

The limit of detection and quantification expressed as signal-to-noise ratio of 3 : 1 and 10 : 1 respectively were calculated as :

$$\text{LOD} = \frac{3.3\sigma}{S} \text{ and } \text{LOQ} = \frac{10\sigma}{S}$$

where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve.

The LOD and LOQ of clopidogrel by the proposed method were 0.09 μg mL^{-1} and 0.28 μg mL^{-1} respectively.

Specificity was evaluated by preparing drug and excipients solutions. The excipients include avicel ph, magnesium stearate, hydroxypropylmethyl cellulose, lactose and cornstarch. The developed method demonstrated good resolution and was found to be selective for clopidogrel in the presence of excipients.

The stability of clopidogrel in solution was determined for the samples stored at room temperature. The samples were checked after 3 successive days of storage and the data were compared with freshly prepared samples. In each case the coefficient of variation values of assay were found to be below 2.0%, indicating the stability of clopidogrel in the solution for 3 days.

To determine the robustness of the method, the experimental conditions like mobile phase ratio, pH of the mobile phase was altered slightly. Mobile phase of methanol and water with 80 : 20 combination and pH 3.4 was found suitable for the determination of clopidogrel and internal standard.

The system suitability parameters for the proposed method were calculated. These include number of theoretical plates, peak tailing and resolution. The number of theoretical plates were 6519 and the peak tailing found to be 1.20. The resolution of the method was found to be 3.57, indicating complete separation of the two components from each other with a well-defined baseline.

Experimental

Clopidogrel bisulphate reference standard was kindly donated by Getz Pharma (Pvt.) Ltd., Karachi, Pakistan. Clopidogrel tablets (labeled to contain 75 mg clopidogrel) were obtained from local market. Solvents used were of HPLC quality and chemicals were of analytical grade.

An HPLC system from Shimadzu (LC 10AT-VP) fitted with a Rheodyne injector (20-μL), UV detector (SPD 10A-VP) and Class-GC 10 software (Shimadzu, Kyoto,

Japan) for data collection and integration was used. The chromatographic separations of clopidogrel and internal standard were accomplished using a Purospher start, C18 (5 μm, 250 × 4.6 mm) column. The mobile phase was consisted of methanol and water, (pH was adjusted to 3.4 with orthophosphoric acid) in a combination of 80 : 20, v/v. Before use the mobile phase was degassed and filtered through a 0.45 μm filter. Mobile phase was pumped at a flow rate of 1 mL min^{-1} using isocratic pump system and eluents were monitored at 235 nm.

Stock solutions of clopidogrel and internal standard (100 μg mL^{-1}) were prepared by dissolving 10 mg in methanol. The stock solution of clopidogrel was subsequently used in the preparation of working standard (0.25–50 μg mL^{-1}) by further dilution with methanol and internal standard to 25 μg mL^{-1}. Stock solutions were kept in refrigerator at 4 °C.

The contents of twenty Norplat tablets (each containing 75 mg clopidogrel) were accurately weighed and finely grounded. A sufficient amount of tablet powder equivalent to 75 mg of clopidogrel was weighed, transferred to a 100 ml volumetric flask, magnetically stirred for 10 min and the final volume was made with methanol. This primary stock solution was filtered through Whatman filter paper number 42 and the filtrate was further diluted suitably to prepare a secondary stock solution. Aliquots of the secondary stock solution were diluted to a concentration of 0.25–50 μg mL^{-1} and the samples were analyzed using proposed method.

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