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A NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CLOPIDOGREL IN TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, specific, accurate and precise Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method was developed for the estimation of Clopidogrel in tablet dosage form. Inertsil C18 ODS(150mm ×4.6mm) at ambient temperature (100×2.1mm ID) 5µm was the column used. Methanol: Water in the ratio of 60:40(v/v) was the optimized mobile phase.The flow rate was 1ml/min with the end effluents were monitored at 225nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity. The retention time of Clopidogrelwas found to be 2.929minutes with injection volume of 20µL respectively. Limit of Detection (LOD)and Limit of Quantification(LOQ) of Clopidogrel were found to be 0.00471 and 0.01429 respectively.

INTRODUCTION

Clopidogrel is anAromaticheteropolycyclic compound as well as inhibitor of adenosine diphosphate (ADP) induced platelet aggregation.It is also known as methyl (2S)-2-(2-chlorophenyl)-2-{4H,5H,6H,7H-thieno[3,2-c]pyridin-5-yl}acetate or an anti-platelet drug**.** Clopidogrel prevents binding of Adenosine Diphosphate (ADP) to its platelet receptor, impairing the ADP-mediated activation of the glycoprotein GPIIb/IIIa complex. It is proposed that the inhibition involves a defect in the mobilization from the storage sites of the platelet granules to the outer membrane. The drug specifically and irreversibly inhibits the P2Y12 subtype of ADP receptor, which is important in aggregation of platelets and cross-linking by the protein fibrin. No direct interference occurs with the GPIIb/IIIa receptor. As the glycoprotein GPIIb/IIIa complex is the major receptor for fibrinogen, its impaired activation prevents fibrinogen binding to platelets and inhibits platelet aggregation. It also acts as anti-depressant drug[2-4]. Clopidogrel is marketed under the brand name Clopilet and also used in the management of depression. Literature review revealed that analysis of Clopidogrelhas beencarried out bysome UV- spectrophotometric methods [5,6] and one HPLC method [7] for the estimation of Clopidogrel in tablet dosage form.The present study was mainly aimed to develop stable, simple,specific,rapid, accurate,precise chromatographicmethodwith high sensitivity and to acquire better resolution, speed for the estimation of Clopidogrel in tablet dosage form

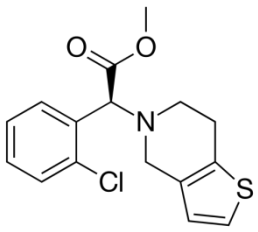

Fig.1 chemical structure of Clopidogrelbisulphate[1]

MATERIALS AND METHODS

Pharmaceutically pure sample of Clopidogrelbisulphate was obtained as gift sample from Chandra laboratories pvt ltd, Prashanth nagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point, ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification.HPLC-grade water, methanol was obtained from standard reagents pvtltd.A tablet formulation of Clopidogrel bisulphate (75 mg label claim) was procured from local market (Clopilet, Sun Pharma, India)

INSTRUMENTATION

The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatograph with SPD-20A prominence UV-visible detector and Spinchrom software , reverse phase C18 column (Inertsil ODS 3V C18, 5µ, 150 mm × 4.6 mm) as stationary phase.The output signal was monitored and processed using software. The pH of the solutions was measured by a pH meter (Thermo scientific). Thermo Electron Corporation double beam UV-visible spectrophotometer (vision pro-software) was used. In the sample preparation, an ultrasonic instrument (Digital Ultrasonic cleaner) was used for sonification. Shimadzu analytical balance AY-220, Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Chromatographic Conditions

The method was developed using Reverse phase C18 column (Inertsil ODS 3V C18, 5µ, 150 mm × 4.6 mm) as stationary phase (column) with isocratic elution mode. The mobile phase consists of methanol, water in a ratio of 60:40% (v/v). Degassing of the mobile phase was done by ultra-sonication for 10 min followed by filtration through the membrane filter. The flow rate was 1ml/min and the effluents were monitored at 225nm.The total run time of method was set at 6 min.

Determination of Working Standard Wavelength (λ_{max})

10 mg of the Clopidogrelbisulphate standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with methanol to give a concentration of 10 µg/ml.The above prepared solution is scanned in UV between 200-400 nm using methanol as blank. The λmax was found to be 220nm (Fig.2).

CONSTRUCTION OF STANDARD GRAPH

Preparation of mobile phase: 60 volumes of methanol and 40 volumes of water were mixed and sonicated for 15 min and filtered through a 0.45 µ membrane filter.

Preparation of standard solution: About 10mg of standard Clopidogrelbisulphate was weighed and transferred into 10ml volumetric flask,to this 10ml of Mobile phase was added.The flask was shaken and volume was made up to the mark with mobile phase to give a primary stock solution containing 1000µg/ml of Clopidogrelbisulphate.From the above solution 2ml of solution was pipetted out into a 10 ml volumetric flask and volume was made up to mark with mobile phase .

Preparation of working standard solution: The stock solution equivalent to 20ppm and 70ppm were prepared,sonicated and filtered through 0.45µ membrane.

METHOD DEVELOPMENT

The main objective of this chromatographic method is to develop a new method with minimum run time and less solvent consumption for the estimation of Clopidogrel in Tablet Dosage Form

Trial 1: First trail was carried out using reverse phase Phenomenex C18(100*2.2mm I.D.), 1.8microns column with a mobile phase of

100% pure degaussed methanolin isocratic mode by maintaining the flow rate of 0.8ml/min. Peak tailing was seen and peak shapes were not found to be good.

Trial 2: Second trial was carried out using C18 column (Inertsil ODS 3V C18, 5µ, 150 mm × 4.6 mm) column with a mobile phase composition of methanol and acetonitrile in the ratio of 90:10(%v/v) in isocratic mode by maintaining the flow rate of 1.5ml /min. In this trial peak shape was found to be bad.

Trial 3: Third trial was carried out usingC18 column (Inertsil ODS 3V C18, 5µ, 150 mm × 4.6 mm)columnwith a mobile phase composition of methanol, water, acetonitrile mixed in the ratio of60:20:20(%v/v/v) in isocratic mode by maintaining the flow rate of 2ml/min. In this trial, peak splitting was observed.

Trial 4: Fourth trial was carried out using C18 column (Inertsil ODS 3V C18 5µ, 150 mm × 4.6 mm) with a mobile phase composition of methanol and water in a ratio of 60:40(%v/v) in isocratic mode by maintaining the flow rate of 1ml/min. Clopidogrel peak was observed at Rt of 2.922 min with good efficiency, peak shape and better resolution. Different trials were carried out in different ratios using different mobile phases, from whichfirst threetrials were found to be futile. Fourth trial has given significant results pertaining to efficiency, peak shape and resolution.So, this trial was considered and validated according to ICH guidelines. The chromatogram of Clopidogrel is shown in Fig3.

Assay: Preparation of sample solution

For the estimation of the drug in tablet formulation, twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 10 mg of Clopidogrelbisulphate was accurately weighed and the powder was transferred to 10 ml volumetric flask and shaken vigorously with mobile phase and sonicated for 15 min and volume made up to the mark with mobile phase. The solution was shaken vigorously and filtered using whatmann filter no.41. From the above filtered clear solution 1ml of sample pipetted out into a 10 ml volumetric flask and volume made up to the mark with mobile phase to give a solution containing 100µg/ml of Clopidogrelbisulphate. Five replicates of each of sample and standard solutions were injected and their average peak areas were taken.

Method validation: Method validation was performed according to ICH guidelines with respect to system suitability, precision, linearity, specificity, accuracy, and limit of detection, limit of quantification, robustness and ruggedness

System Suitability: To verify that the analytical system is working properly,accuracy and precision results were evaluated bytaking 100µg/ml of Clopidogrelwhich was injected six times and chromatograms were recorded for the same.

Precision: Method precision was determined by injectingsample solutions of clopidogrel(1000µg/ml)for six times and the %RSD of drugs was calculated.

Linearity and Range: Linearity solutions were prepared from stock solution at 5 concentration level from 50 to 150µg/ml for Clopidogrel.Theslope, y-intercept and correlation coefficient were calculated.

Specificity: The effect of excipients and other additives usually present in the Clopidogreltablet dosage form in the determination under optimum conditions was investigated. The specificity of RP-HPLC method was established by injecting the blank and placebo solution in to the RP-HPLC system.

Accuracy: Accuracy of the method was determined by recovery studies. To the formulation (pre- analyzed sample), the reference standards of the drugs were added at the level of 50, 100, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug.

Limit of Detection (LOD) and Limit of Quantification (LOQ) : The limit of detection was defined as the lowest concentration of Clopidogrelresulting in a peak height greater or equal to three times from background noise.

Table 1**:** Optimised Chromatographic Conditions.

Mobile phase	Methanol: water (60:40)% v/v
Column	Inertsil ODS 3V C18, 5µ, 150 mm × 4.6 mm
Flow rate	1 ml/min
Column Temperature	30ºC
Sample Temperature	15ºC
Wave length	225 nm
Injection volume	20 µl
Run time	6 min
Retention time of clopidogrel	2.922 min

Table 2: Results for Assay of Clopidogrel

Clopidogrel		
	Standard Area	Sample Area
Injection-1	3438.240	3440.119
Injection-2	3444.826	3425.326
Injection-3	3430.172	3436.149
Injection-4	3429.948	3445.016
Injection-5	3390.253	3440.107
Average Area	3426.6878	3430.143
Assay (% purity)	99.99	

Table 3: Results for System Suitability of Clopidogrel

Ss	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.921	2102936.24	11003	1.126
2	2.920	2102846.85	11040	1.126
3	2.922	2103011.54	11020	1.130
4	2.923	2102942.85	11038	1.127
5	2.921	2103038.45	11060	1.128
Mean	2.9214	2102955.18	11032	1.128
SD	0.00114	74.7604	-------	-------
% RSD	0.03928	0.00355	-------	-------

Table 4: Data of Repeatability (System precision)

	Injection	Peak Areas of Clopidogrel	%Assay
Concentration 40ppm	1	2102965.54	100.22
	2	2102912.84	100.22
	3	2102886.52	100.22
	4	2103045.69	100.23
	5	2102946.46	100.22
Statistical Analysis	Mean	2102951.41	100.22
	SD	60.8501	0.00290
	% RSD	0.00289	0.00290

Table 5: Data of Repeatability (Method precision)

	Injection	Peak Areas of Clopidogrel	%Assay
Concentration 40ppm	1	2102877.32	100.22
	2	2102956.23	100.22
	3	2102997.12	100.23
	4	2103022.22	100.23
	5	2103075.84	100.23
	6	2103124.45	100.23
Statistical Analysis	Mean	2103008.86	100.23
	SD	87.4487	0.00418
	% RSD	0.00415	0.00417

Table 6: Linearity data of Clopidogrel

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	52296
20	1051491.45	y-Intercept	6338
30	1577236.65	Correlation Coefficient	0.999
40	2102982.87	-	-
50	2628727.42	-	-
60	3154473.86		
70	3649285.27	--	

Table 7**:** Recovery Results for Clopidogrel

%Recovery	**Amount present (ppm)**	**Amount found (ppm) ***	**Percent Recovery ***	**% Mean Recovery**
50	20	19.98	99.92	100.2266
100	40	40.09	100.23	
150	60	60.31	100.53	

Table 8:Limit of Detection and Limit of Quantification of RP-HPLC Method for Clopidogrel

S.N.	**Parameters**	**Formula**	**Value(ppm/ml)**
1	LOD	3.3×(SD/slope)	0.00471
2	LOQ	10×(SD/slope)	0.01429

Table 9: Results for Robustness of Clopidogrel

Chromatographic changes		Retention time (min)	Tailing factor	% Assay
Flow rate(mL/min)	0.8	2.50	1.114	99.50
	1.0	2.79	1.113	99.91
	1.2	3.50	1.123	100.5
Wavelength(nm)	218	2.91	1.70	100.1
	220	2.95	1.75	98.7
	222	2.90	1.65	99.3

Table 10: Results for Ruggedness of Clopidogrel

Clopidogrel	%Assay
Analyst 01	100.23
Analyst 02	100.22
% RSD	0.00451

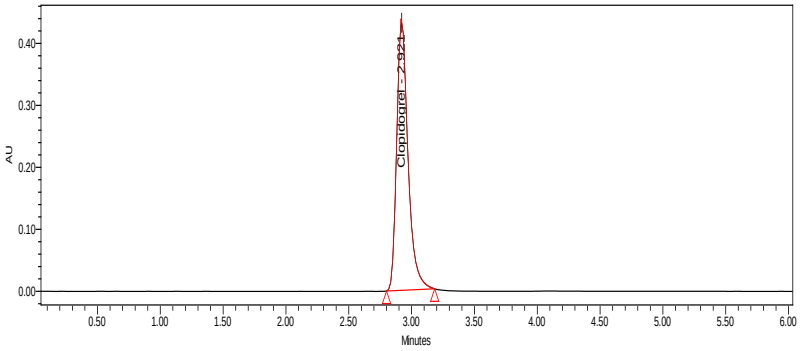

Fig.3: Optimised Chromatogram of Clopidogrel by using Mobile Phase

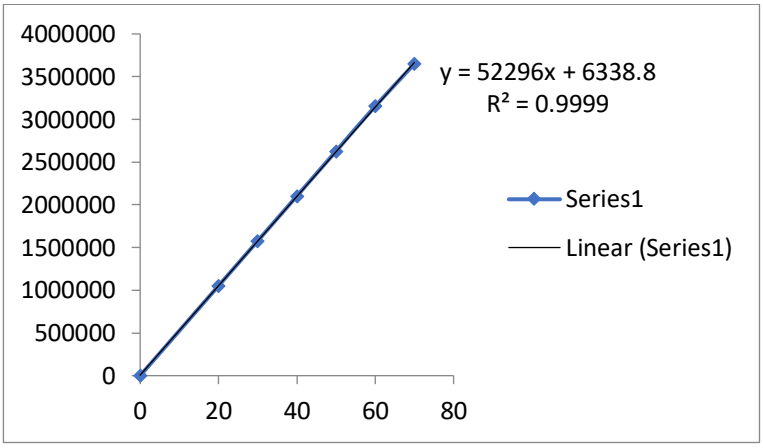

Fig.4: Linearity graph of clopidogrel

The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio) for the determination of LOQ, the percentage deviation and %RSD should be less than 20%.

Robustness: To demonstrate the robustness of the method, solution was prepared as per test method and injected at different variable conditions like flow rate and temperature.

Ruggedness: The intermediate precision is also known as Ruggedness which is determined by calculating analyst to analyst variation i.e., assay is performed by two analystthe sample concentrations of 1000µg/ml of ofClopidogrelwere injected two times into the RP-HPLC system and the %RSD for the assay of two replicate injections was calculated.

RESULTS

Method Development:

Optimised Chromatographic Conditions: The Clopidogrel peak was observed at 2.929 min with good efficiency, peak shape and good resolution. So, this trial was considered and validated according to ICH guideline

Optimisation: After carrying out initial trials, the stationary phase, mobile phase, flow rate, column temperature and wavelength of detection were optimised and optimised method conditions are given in Table 1.

Assay: The percentage assay for the drug was found to be within the limits. The percentage purity of Clopidogrel was found to be within the limits*i.e*99.30% ± 0.84and the results are presented in Table 2

Method Validation

System Suitability: The plate count and tailing factor results were found to be satisfactory and are found to be within the limits .The results are presented in Table 3.

Precision: The method precision was evaluated with six sample replicate injections and the %RSD of six determinations of Clopidogrelfor method precision was found to be within the acceptance criteria of less than 2.0% and it is presented in Tables 4 and 5.

Linearity: Linear detector response for the peak areas of clopidogrelwere observed in concentration range between 20 and 70ppm. Calibration curves were constructed by plotting the peak area versus concentration and the regression equations were calculated. The results obtained are listed in Table 6, and these results indicate that the current method is linear for clopidogrelin the specified range above with a correlation coefficient better than 0.999.The results of linearity are presented in table 6

Specificity: After injecting sample and placebo solution ofClopidogrel, it was observed that diluents or placebo peaks were not interfering with the Clopidogrel

Accuracy: The percentage recovery of Clopidogrelwere found between 98% and 102%. The results of the method validation study for accuracy are presented in Table 7.

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were experimentally verifiedby injection of Clopidogrelat appropriate concentrations. The LOD and LOQ of Clopidogrel were found to be 0.00471 and 0.01429respectively.The results are presented in Table 8.

Robustness: From the observation the tailing factor was found to be within the limits on small variation of flow rate and temperature and these results are presented in a Table 9.

Ruggedness: From the observation, between two analysts assay values are not greater than 2.0%, hence the method was rugged and these are given in Table 10.

DISCUSSION

This study presents the development and validation of RP-HPLC method for determination of Clopidogrel in tablet dosage form using the methanol and water as mobile phase in different ratios as mobile phase. As the concentration of methanol was increased, the retention time of Clopidogrel was reduced and peak shape has improved. Hence, the mobile phase is optimized to methanol: water in a ratio of 60:40% (v/v) after using combinations of different solvents in different ratios .The run time of this method with other drug combinations was short i.e., 6 min when compared to the reported methods were the run times were about 7-9 min and optimized optimised chromatographic conditions (Table 1). The reduced retention times have two advantages firstly, the solvent consumption is decreased and secondly multiple determinations can be carried out in a short time. The percentage assay of drug is found to be within the limits(Table 2). System suitability parameters were carried out to prove that the overall system performed well, it was calculated by different parameters like resolution, retention, tailing factor and theoretical plates. The plate count and tailing factor results is found to be satisfactory (Table 3). Precision of the method is performed for the drug and the optimised results (Tables4 and 5). In linearity the parameters like slope, intercept and correlation coefficient (0.999) were found to be within the limits (Table 6).Accuracy of the method was determinedby recovery studies at 50, 100 and 150% and the mean of concentrations were within the acceptance limits and the results of accuracy studies(Table 7). The least concentration of the sample was prepared with respect to the base line noise and signal to the noise ratio was measured (Table 8). Robustness of the method is performed by deliberately changing the experimental conditions like flow rate and wavelength. From the above observation the tailing factor was found to be within the limits on small variations of flow rate and wavelength (Table 9). Ruggedness is performed by two **analysts and it is observed that, %RSD of theassay values is not greater than 2.0%, hence the method** was rugged (Table 10.). Moreover, the method showed good accuracy, linearity, precision.

CONCLUSION

A RP-HPLC method was developed and validated for the estimation of Clopidogrel in tablet dosage form. The method was found to be precise, accurate and sensitive allowing

multiple determinations in a very short time. The method was found to be suitable for routine analysis in quality control and R&D.

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REFERENCES

1. Clopidogrel bisulphate: Indian pharmacopeia, Government of India, Ministry of Health and Family Welfare, Indian pharmacopeia commission, Ghaziabad.
2. ICH, Q2A validation of analytical procedure:Methodology International Conference on Harmonization, Geneva, October, 7(3), 1994, 1-5.
3. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
4. https://en.wikipedia.org/wiki/Antiplatelet_drug.
5. Pravin B. Cholke, Raihan Ahmed S Z Chemate and Jadhav K R et al. Development and Validation of Spectrophotometric Method for Clopidogrelbisulfate in pure and in film coated tablet dosage form, Archives of Applied Science Research, 4(1), 2012, 59-64.
6. Dwarakanadha Reddy P et al, Design, development and *In vitro* characterization of Clopidogrel bisulfate floating drug delivery system, J Compr Phar 2015;2(2):48-56
7. SuhasGurav, Venkatamahesh R et al.Development and Validation of Derivative UV- Spectropotometric Methods for Quantitative Estimation of Clopidogrel in Bulk and Pharmaceutical Dosage Form, International Journal of ChemTech Research, 4(2), 2012, 501
8. Jose Kurien and P Jayasehar. Stability indicating HPLC determination of Clopidogrelbisulfate in pharmaceutical dosage forms, PharmacieGlobale (IJCP), 4(1), 2013, 1-5.