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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CLOPIDOGREL IN TABLET DOSAGEFORM BY UV SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple UV-Spectroscopic method was developed and validated for analysis of Clopidogrel formulation. Clopidogrel, an antiplatelet agent structurally and pharmacologically similar to ticlopidine, is used to inhibit blood clots in a variety of conditions such as peripheral vascular disease, coronary artery disease, and cerebrovascular disease. The drug samples were analyzed by UV spectroscopy using 0.1 n HCL as solvent. The average content of drug present in the formulation was found to be 74.71 mg (99.62%). The percentage recovery of clopidogrel sample was found within the limit 96.48%- 105.76%.

Key Words: Clopidogrel, UV-Spectroscopic method, Validation, ICH guidelines

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INTRODUCTION

The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological ones. Of them, physical and physicochemical methods are used mostly. Physical methods of analysis involve the studying of the physical properties of a substance. They include determination of the solubility, transparency or degree of turbidity, color density or specific gravity (for liquids), moisture content, melting, freezing and boiling points. [3]Physicochemical methods are used to study the physical phenomenons that occur as a result of chemical reactions. Among the

physicochemical methods are optical refractometry, polarimetry, emission and fluorescent methods of analysis, photometry including photo colorimetry, spectrophotometry, nephelometry and turbidimetry; electrochemical (potentiometry, amperometry, coulometry, voltametry, polarography) and chromatography (column, paper, thin layer, gas-liquid, high performance liquid chromatography) methods are generally preferable. Methods involving nuclear reactions such as nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are becoming more and more popular. The chemical methods include the gravimetric and volumetric procedures, which are based on complex formation, acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have been widely used in pharmaceutical analysis whenever the existing amounts are in milligram level and the interferences are negligible. The methods (U.V, HPLC, GLC, NMR and Mass Spectroscopy) of choice for assay involve sophisticated equipment, which are used to analysis bulk drugs and pharmaceutical formulations (1).

The extensive literature survey (2-8) carried out and revealed that several methods have been reported for the simultaneous estimation of clopidogrel and combination with other drugs. However there is no method reported for the individual analysis of the clopidogril by UV-VISIBLE SPECTROSCOPY method in pharmaceutical formulations. Hence, an attempt was made to develop a simple, rapid, accurate, precise and validated method for the estimation of individual dosage forms.

MATERIALS AND METHODS

MATERIALS

Drug Sample

Clopidogrel was obtained as a gift sample from Dr.REDDY'S Pharmaceuticals Pvt.Ltd., Hyderabad.

Formulation Used

″CLOPILET″ tablets containing 75 mg of Clopidogrel was purchased from local Pharmacy.

Chemicals and Solvents

Distilled water, Hydrochloric acid (0.1N), Ethanol, Methanol, Dihydrogen potassium phthalate, Dihydrogen sodium phosphate, Phosphate buffer 6.8 were purchased from Merck Specialties Ltd, Mumbai.

METHODS

UV Spectrophotometric Method

Selection of Solvent

The solubility of was determined in a variety of solvents as per Indian Pharmacopoeia standards. Solubility test for Clopidogrel was carried out in different polar and non-polar solvents. From the solubility studies, 0.1 N HCL was selected as suitable solvent for proposed method.

Selection of λ_{max}

The standard stock solution was further diluted with distilled water to get 10 µg/ml concentration. The solution was scanned between 200 and 400 nm range using distilled water as blank. From the UV Spectra 219 nm was selected as λ_{max} for analysis of Clopidogrel Stability of the Clopidogrel in water was studied by measuring the same solution at this λ_{max} in different time intervals. It was observed that Clopidogrel in water was stable for more than 2 hours.

Calibration Graph

In this aliquots of stock solution of Clopidogrel (0.4-1.0ml of 1000 µg /ml) were transferred in to 10 ml volumetric flask and made up to the mark with distilled water. The absorbance of different concentration solutions were measured at 219 nm against blank. The samples were found to be linear from 40-100 µg /ml The calibration curve was plotted using concentration Vs absorbance. The curve obtained was linear in the concentration range of 40-100 µg /ml.

Recovery Studies

To the pre-analyzed formulation, a known quantity of standard solution (2,4 and 6 µg/ml solution) was added and the contents were mixed well, finally made up to the volume with distilled water. Absorbance was measured at 219 nm. Amount present was calculated from slope and intercept.

Validation

The validation of the assay procedure was carried out using the following parameters such as Specificity, Accuracy, Precision, Linearity, Limit of Detection (LOD), Limit of Quantitation (LOQ), Robustness, Ruggedness and Sample Solution Stability were carried out according to ICH guidelines (9, 10).

RESULTS AND DISCUSSION

UV-Spectroscopic Studies

The solubility of clopidogrel was determined in a variety of solvent ranging from non polar to polar using essentially a method of Schefter and Higuchi. The drug was found to be freely soluble in distilled water, and very soluble in methanol and acetonitrile.

10 mg of clopidogrel raw material was accurately weighed and transferred into the 100 ml volumetric flask and dissolved in minimum quantity of 0.1 N HCL and made up to 100 ml with distilled water, resulting in 100 mcg/ml of drug concentration. It was scanned in the range of 200-400 nm and it shows constant λ_{max} at 219 nm this is shown in Fig-1. The linearity of the drug clopidogrel was found, its calibration curve was constructed and is shown in Fig-2, the optical characteristics such as Beer's law limit (40-100µg/ml), sandell's sensivity (0.021855), correlation coefficient (0.9996), slope(0.002619) and intercept(0) , molar absortivity ($1.2810x10^2$), were calculated and shown in Table-1.

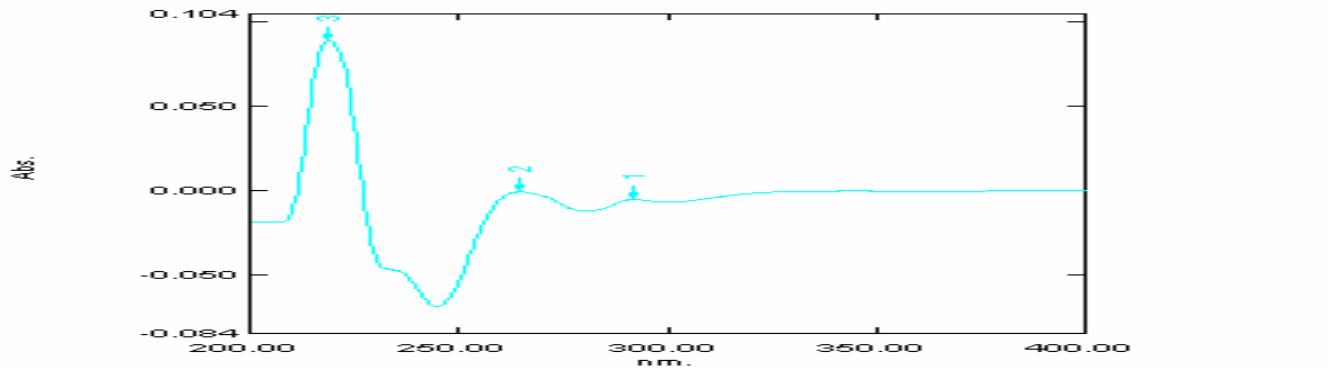

Fig-1 Ultra violet absorption spectrum of clopidogrel showing absorbance at 219 nm.

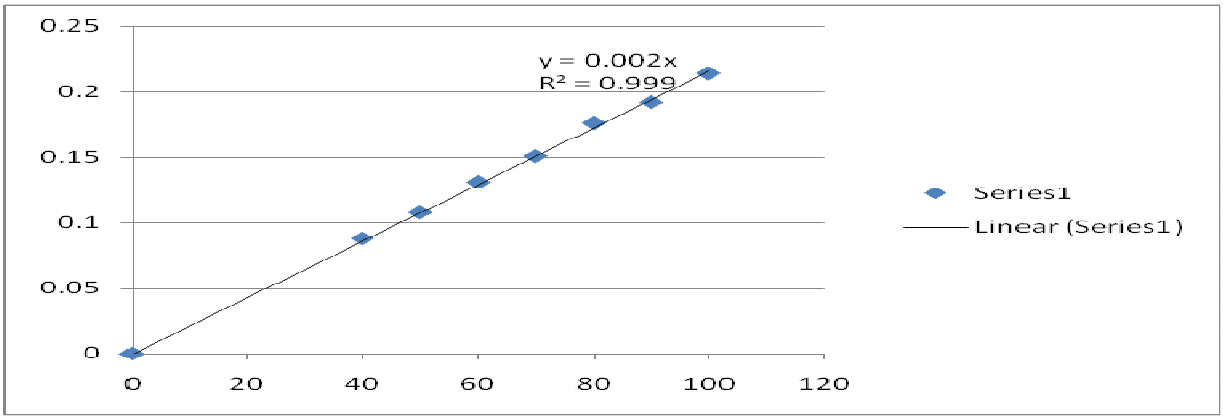

Fig-2 Calibration curve of Clopidogrel by UV method

Table-1 Optical Characteristics of Clopidogrel in UV Method

PARAMETERS	METHOD VALUES
λ_{max}(nm)	219
Beer's law limit(µg/ml)	40-100
Sandell's sensitivity (µg/cm^2/0.001 AU)	0.021855
Molar absorbtivity(L mol^{-1} cm^{-1})	1.2810x10^2
Correlation Co-efficient (r)	0.9996
Regression equation (Y=mx+c)	Y=0.0021x+0
Slope(m)	0.002
Intercept(c)	0
LOD(µg/ml)	0.0064
LOQ(µg/ml)	0,0211
Standard error of mean of regression line	0.001484

The limit of detection and limit of quantification were determined from the linearity studies. The limit of detection was found to be 0.0064µg/ml and the limit of quantification was found to be 0.0211µg/ml. Table-2 shows the result of formulation quantification on repeatability also found to be within the limits 98-100.09% (99.62±0.224)%

Table-2 Assay of Clopilet tablets

TABLET SAMPLE	LABEL CLAIM, MG/TABLET	ACTUAL CONTENT FOUND, MG±S.D	PERCENT ACTUAL CONTENT FOUND, ±S.D	% RECOVERY ± SD
Clopilet-75mg	75mg	74.71±0.167	99.62±0.224	99.64 ±0.236

To evaluate the accuracy of the method, known amount of pure drug (10, 20 and 30 µg/ml solution) was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. The percentage recovery of clopidogrel sample was found with in the limit 96.48%- 105.76% Mean of SD 99.78 (%RSD 0.43,), Mean of SD 99.41 RSD(%0.26,) Mean of SD 99.94 (%RSD 0.57). These values were given in Table-3.

Table-3 Recovery studies of Clopidogrel

RECOVERY	TARGETIN µG/ML	SPIKED IN µG/ML	TOTAL IN µG/ML	AMOUNT FOUND IN µG/ML	%RECOVERY	Mean	%RSD
50%	20	10	30	29.818	99.39	99.78	0.43
	20	10	30	30.07	100.24		
	20	10	30	29.92	99.73		
100%	20	20	40	39.707	99.27	99.41	0.26
	20	20	40	39.707	99.267		
	20	20	40	39.890	99.72		
150%	20	30	50	49.66	99.32	99.94	0.57
	20	30	50	50.03	100.07		
	20	30	50	50.22	100.44		

Precision of the method was studied by making repeated analysis of the sample and it was carried out three times in a day and repeated for 3 days. The percentage standard deviation for intra-day and inter-day analysis was found for recovery and **%** RSD 0.11 and **%** RSD 0.17

CONCLUSION

The proposed analytical methods are simple, reliable, rapid, sensitive, reproducible and accurate for the estimation of Clopidogrel. The method adopted for our studies is Simple UV-Spectroscopic method. The drug samples were analyzed by UV spectroscopy using 0.1 n HCL as solvent and the average content of drug present in the formulation was found to be 74.71 mg (99.62%). The above methods do not suffer from any interference due to common excipients. Therefore it was shown that the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing Clopidogrel. Thus the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry.

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