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DEVELOPMENT AND VALIDATION OF A UV-VISIBLE SPECTROSCOPIC METHOD FOR CLOPIDOGREL BISULFATE: A COMPARATIVE STUDY OF SINGLE POINT STANDARDIZATION, STANDARD ABSORPTIVITY, AND CALIBRATION CURVE APPROACHES

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ABSTRACT
UV spectrophotometric techniques play a crucial role in pharmaceutical analysis for the estimation of active compounds. This study presents the development and validation of a UV-Visible Spectroscopy method for Clopidogrel Bisulphate using methanol as a solvent. The method adheres to Beer-Lambert's law and ICH guidelines, exhibiting a maximum absorbance at 227 nm. Excellent linearity (R^2 = 0.997) was observed over the tested concentration range, with precise LOD and LOQ values confirming the method's sensitivity. Additionally, the method demonstrated high precision, accuracy, and robustness, making it a reliable choice for routine pharmaceutical quality control applications.

KEYWORDS: Clopidogrel Bisulphate, methanol, ICH guidelines, Linear.

1.0 INTRODUCTION[1,2,3,4,5]

An irreversible inhibitor of the P2Y12 adenosine diphosphate receptor on platelet cell membranes, Clopidogrel Bisulphate (CDB) is an antiplatelet agent used to prevent blood clots in a number of conditions, including peripheral vascular disease, coronary artery disease, and cerebrovascular disease. The usage of clopidogrel is linked to a number of severe adverse medication events. cardiovascular edema, different types of hemorrhage, and neutropenia. According to a review of the literature, liquid chromatography methods have been documented for the measurement of clopidogrel bisulphate in biological samples, pharmaceutical dosage forms, and pure drugs. Therefore, the authors have made an effort to create a straightforward, quick, accurate, and precise approach for estimating these medications in tablet dosage forms. Verification of the usefulness of the created.

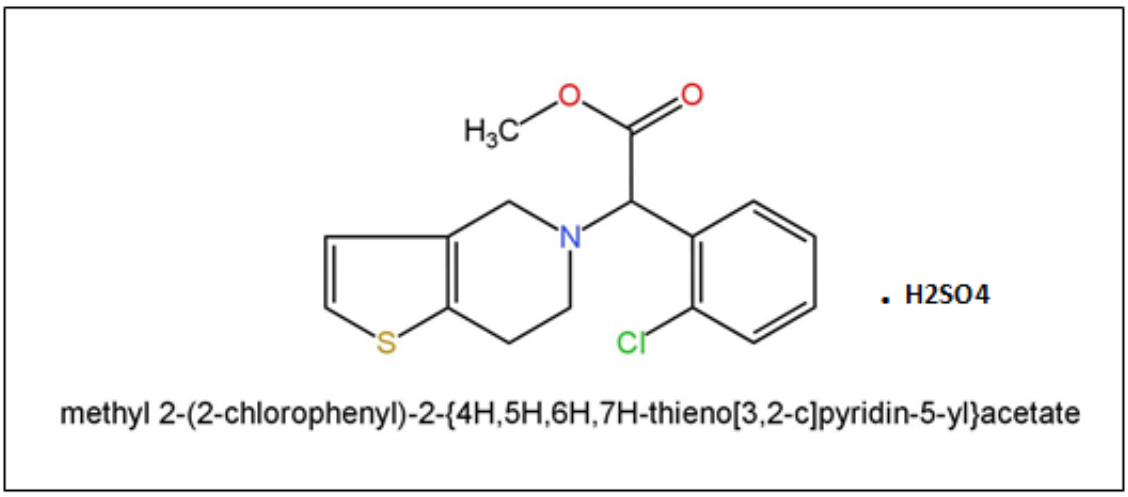

Fig. No. 01: Structure of CDB.

2.0 MATERIAL AND METHOD

2.1 Instrumentation

A Systronics (India) Limited UV-visible spectrophotometer, Model AU-2707, with a 1 cm matched cell and a double beam and double detector configuration, was used for the study. DS/BCOP/MACH/2022/01 is our internal instrument ID number. UV Professional double beam is the program that is utilised. An ultrasonicator cleaner was used to degas the mobile phase. For the weighing, an electronic balance (Egale scale) was implemented.

2.2 Material or Chemicals[5,6]

An analytically pure sample of CDB was procured as a gift sample from Ajanta Pharmaceutical grade PPIC department Mumbai were purchased. Tablet formulation (Clopitab), manufactured by Lupin Ltd. India was procured from a local pharmacy with labeled amount75mg/ tablet.

3.0 METHODOLOGY[7]

3.1 Selection of suitable solvent

The drug solubility, stability, and absorbance maxima of the chemical in the specific solvent were taken into consideration when choosing the solvent. The solubility of 10 mg of CDB was assessed using distilled water, 0.1N hydrochloric acid, 0.01N sodium hydroxide, methanol, ethanol, and phosphate buffer pH 6.8. Methanol is the more effective solvent from the list above. Methanol is therefore utilised as a solvent.

3.2 Preparation of standard stock solution

50 mg of pure CDB was weighed, put in a 50 ml volumetric flask, and dissolved in methanol. To get a final concentration of 1000 ppm, it was thoroughly dissolved and diluted with diluent. A 10-ppm solution was made from the stock solution using methanol, which served as the working standard.

3.3 Determination of λmax

The wavelength of the UV spectrum was selected for the investigation of CDB. The UV spectra of clopidogrel bilsulphate, which was synthesized at a concentration of 10 ppm, were scanned between 200 and 400 nm in order to determine the wavelength maximum. It was found that the absorbance maximum against methanol was **227** nm.

3.4 Estimation of CDB[8,9]

Table No. 1: Absorbance.

Sr. No.	Concentration	Absorbance
1	10	0.189
2	20	0.391
3	30	0.582
4	40	0.768
5	50	0.956
6	Tablet 10 ppm	0.184

3.4.1 Standard Absorptivity Method

Prepare standard solutions of CDB in methanol at concentrations ranging from 10 ppm to 50 ppm. Measure the absorbance of each solution at 227 nm using a UV-visible spectrophotometer. Calculate the absorptivity (a) using Beer-Lambert's law:

A = abc

Where:
A = Absorbance, **a** = Molar Absorptivity, **b** = Path length (1 cm), **c** = Concentration in ppm

The standard absorptivity method is based on the principle of Beer-Lambert's law, which states that absorbance is directly proportional to concentration by determining the absorptivity coefficient (a), unknown sample concentrations can be estimated accurately. This method is widely used due to its simplicity and ability to provide reliable quantitative results. By using formula it was noted that the percentage purity of drug by using this method was found 97.3 %. (By using Table No. 1)

3.4.2 Single Point Standardization Method

Prepare a standard solution of CDB in methanol at a known concentration (e.g., 10 ppm). Prepare the test sample solution in methanol. Measure the absorbance of both the standard and test sample at 227 nm using a UV-visible spectrophotometer. Calculate the concentration of the test sample using the equation:

Ctest = (Atest X Cstd) / Astd

Where, Ctest = concentrations of sample
Cstd = concentrations of standard
Atest = absorbance of the sample
Astd = absorbance of the standard

The Single Point Standardization Method is a direct and simple approach for estimating the concentration of an unknown sample by comparing its absorbance with that of a standard solution of known concentration. It is particularly useful for quick and routine analysis where high precision is not required. By using formula it was noted that the percentage purity of drug by using this method was found 97.3 %. (By using Table No. 1)

3.4.3 Calibration Curve Method

Prepare standard solutions of in methanol at concentrations ranging from 10 ppm to 50 ppm.

Measure the absorbance of each solution at 227 nm using a UV-visible spectrophotometer. Plot a calibration curve by graphing absorbance against concentration. Determine the equation of the calibration curve using linear regression analysis. Use the calibration curve equation to estimate the concentration of unknown samples by interpolating their absorbance values.

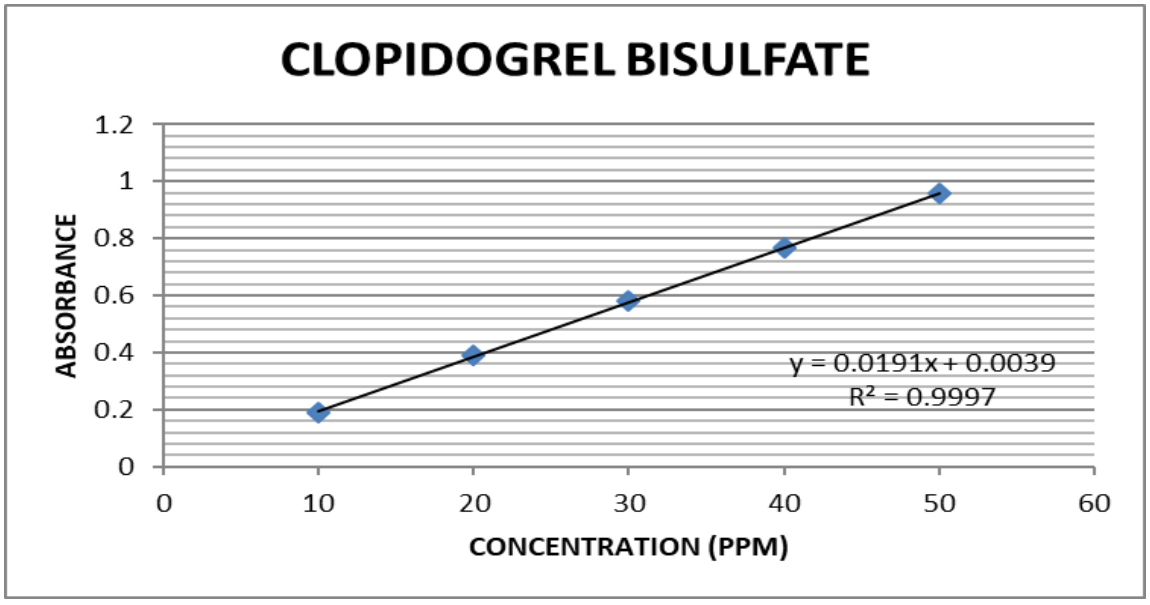

Fig. No. 02: Calibration curve for CDB.

The Calibration Curve Method is based on Beer-Lambert's law, where absorbance is directly proportional to concentration. By constructing a standard calibration curve, the concentration of unknown samples can be accurately determined. This method ensures high precision and reliability in quantitative analysis. By using equation of line after calculating by using formula it was noted that the percentage purity of drug by using this method was found 97.3%.

4.0 Method of Validation[10]

4.1 Linearity[5-8]

Using methanol as a blank, the absorbance of each concentration was measured at 227 nm using this method. New aliquots were made from standard stock-2 solution ranging from 10-50 ppm. The regression coefficient (R2) from the linearity curve was found to be 0.9997, indicating excellent linearity.

Table No. 02: Linearity results.

Sr. No.	Concentration	Absorbance	Calculation	Values
1	10	0.189	**Mean**	0.5772
2	20	0.391	**SD**	0.3021
3	30	0.582	**RSD**	0.5233
4	40	0.768	**%RSD**	52.33%
5	50	0.956	**Slope**	0.0191
6	Tablet(10ppm)	0.184	**Intercept**	0.0039

4.2 Precision[11-13]

The drug's duplicate concentration was computed for the intraday research twice in the same day. The drug's concentration was measured over two days in a row in an inter-day study, indicating the laboratory's fluctuation over a number of days. The results are shown below. The intra-day precision is shown in Table 2, and the inter-day precision is shown in Table 3.

Table No. 03: Observation table for Intra Day Precision.

Sr. No.	Time	Concentration	Absorbance
1	0 Hrs	20 PPM	0.391
2	2 Hrs	20 PPM	0.395
3	4 Hrs	20 PPM	0.387
4	8 Hrs	20 PPM	0.394
5	12 Hrs	20 PPM	0.391
6	24 Hrs	20 PPM	0.398
Mean			**0.392**
SD			**0.0038**
RSD			**0.0096**
%RSD			**0.96**

Table No. 04: Observation table for Inter Day Precision.

Sr. No.	Time in hrs.	Conc.in ppm	Day 1	Day2	Day 3
1	0 Hrs	20 PPM	0.391	0.387	0.398
2	2 Hrs	20 PPM	0.395	0.381	0.389
3	4 Hrs	20 PPM	0.387	0.388	0.387
4	8 Hrs	20 PPM	0.394	0.389	0.395
5	12 Hrs	20 PPM	0.391	0.391	0.393
6	24 Hrs	20 PPM	0.398	0.393	0.387
Mean			**0.392**	**0.388**	**0.3915**
SD			**20.00**	**0.0041**	**0.0045**
RSD			**51.02**	**0.010**	**0.011**
%RSD			**0.51**	**0.0001**	**0.0011**

4.3 Accuracy[13-15]

Using recovery trials at several replication levels for 80%, 100%, and 120%, the suggested method's accuracy was evaluated. The pre-analyzed formulation was mixed with a known quantity of pure medication to create the sample solutions. In the clopidogrel, the mean percentage recovery was computed and reported.

Table No. 05: Accuracy.

Spiked Level (%)	Added Concentration	Measured Concentration	% Recovery
80	8 ppm	7.9	98.8
100	10 ppm	10.1	101
120	12 ppm	12.2	101.7

***Result shows the mean of 3 readings**

4.4 Ruggedness[16-20]

To get the % RSD, the absorbance of each of the two different analysts who conducted the analysis was noted. Table No.06 provides the results.

Table no. 06: Ruggedness.

Sr. No.	Analyst - 1		Analyst - 2	
	Concentration	Absorbance	Concentration	Absorbance
1	20 PPM	0.391	20 PPM	0.387
2	20 PPM	0.395	20 PPM	0.394
3	20 PPM	0.387	20 PPM	0.389
4	20 PPM	0.394	20 PPM	0.391
5	20 PPM	0.391	20 PPM	0.393
6	20 PPM	0.398	20 PPM	0.395
1	**Average**	**0.3926**	**Average**	**0.3915**
2	**SD**	**0.00382**	**SD**	**0.00281**
3	**RSD**	**0.00973**	**RSD**	**0.00717**
4	**% RSD**	**0.973**	**% RSD**	**0.717**

4.5 Robustness[21-23]

The robustness of the approach was assessed using three different wavelengths of analysis. The results were displayed in Table No.07 after the relative absorbance was recorded.

Table No. 07: Robustness.

Sr. No.	Concentration	Wavelength		
		226 nm	227 nm	229 nm
1	20 PPM	0.393	0.391	0.388
2	20 PPM	0.395	0.395	0.386
3	20 PPM	0.389	0.387	0.392
4	20 PPM	0.392	0.394	0.394
5	20 PPM	0.387	0.391	0.391
6	20 PPM	0.388	0.398	0.391
Average		**0.39**	**0.392**	**0.39**
SD		**0.0031**	**0.0038**	**0.0028**

RSD	**0.0079**	**0.0096**	**0.0071**
% RSD	**0.7948**	**0.9693**	**0.7179**

4.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)[24-28]

LOD is the lowest amount of analyte that can be detected but not necessarily quantified under stated conditions and LOQ is the lowest amount of analyte that can be quantitatively determined with acceptable precision and accuracy.

Table No. 08: LOD and LOQ.

Concentration	Absorbance at 227 nm
10 ppm	0.189
10 ppm	0.187
10 ppm	0.191
MEAN	0.189
S.D.	0.002
LOD	0.3455
LOQ	1.0471

$$LOD = \frac{3.3\ X\ \sigma}{S} \qquad LOQ = \frac{10\ X\ \sigma}{S}$$

Where, σ = Standard deviation of the response

S = Slope of the calibration curve

LOD and LOQ were calculated as be 0.3455µg/ml and 1.0471µg/ml, respectively.

5.0 Estimation of CDB from Tablet Dosage Form

Weigh and finely grind 20 tablets. Weigh a portion equivalent to 100 mg of CDB. Dissolve in 100 mL methanol (Sonicate if needed). Filter the solution to remove excipients. Take 1 mL of the filtrate and dilute to 10 mL. Again, from this take 1Ml and dilute to 10 mL (final concentration ~10 ppm). Check the absorbance of this resulting solution at 227 nm. By using formula, it was noted that the percentage purity of drug was found 97.3 %.

Table No. 9: Assay of Marketed Formulation.

Concentration	Absorbance at 227 nm	Specifications
10 ppm	0.189	**Brand Name:** Clopitab
10 ppm	0.187	**Label Claim:** 75 mg
10 ppm	0.191	**Manufacturer:** Lupin Pvt. Ltd. India
Mean	0.189	**Manufacturing date:** Feb 2024
SD	0.002	**Expiry Date:** Jan 2026
RSD	0.01058	**Batch No:** J800399
%RSD	1.058	**% Purity of drug:** 97.3%

6.0 DISCUSSION

CDB was scanned in the UV area between 200 and 400 nm with methanol as a solvent in order to develop the UV-spectrophotometric method. The wavelength maximum was recorded at 227 nm. This approach has been validated in accordance with ICH criteria. CDB is analysed and determined using a variety of characteristics, including linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ. 50 mg of medication was carefully weighed and transferred in 50 ml of volumetric flask which was filled up with the help of methanol as a solvent to get 1000 ppm concentration (stock-1). One ml of the stock-1 solution mentioned above was taken and put into a ten milliliter volumetric flask, which was then diluted with methanol to obtain 100 parts per million. For linearity investigations, several concentrations between 10 and 50 ppm were generated from the above mentioned solution, and the linearity curve's R2 value was determined to be 0.997. It was found that the mean percentage recovery came within the range suggesting that the method had been established accurately when the accuracy criterion was evaluated at 80%, 100%, and 120%. Since the percentage RSD for the parameter's intra-day and inter-day precision levels was less than 2, the devised approach was considered precise. The linearity curve was used to calculate the parameter LOD and LOQ, which came out to be 0.3455 µg/ml and 1.0471 µg/ml.

7.0 CONCLUSION

For CDB, a straightforward UV-spectrophotometric technique has been created and verified in accordance with ICH guidelines. According to our investigation's results, the suggested UV-spectrophotometric approach was incredibly sensitive, accurate, and reasonably priced when compared to the previously published techniques. It was determined that the suggested UV-spectrophotometric method could be used to determine the amount of CDB in bulk.

8.0 CONFLICT OF INTEREST

The authors declare no conflicts of interest relevant to this article.

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