Development and validation of analytical method for clopidogrel bisulphate and irbesartan by simultaneous equation spectroscopic method

Pankaj Savani*, Sudhanshu Chauhan, Vineet Jain, Hasumati Raj, Sagar Patel

Department of Quality Assurance, Shree Dhanvantary Pharmacy College, Kim, Dist: Surat, Gujarat – 394110, India

*For correspondence
Pankaj Savani,
Research Scholar
Department of Quality Assurance, Shree Dhanvantary Pharmacy College, Kim, Dist: Surat, Gujarat – 394110, India.
Email: pankajsavani75@gmail.com

ABSTRACT

Objective: The major approach take into consideration is to develop a simple, accurate, precise and reproducible method for development and validation of UV-visible spectrophotometric method for estimation of Clopidogrel Bisulphate and Irbesartan in synthetic mixture.

Methods: In linearity spectra of the Clopidogrel Bisulphate and Irbesartan to shows to possible a simultaneous equation method but the zero order linearity spectra was converted to first derivative and second derivative spectroscopic method to not shows any zero crossing point so that this method was not possible.

Results: In this spectroscopic method, for Clopidogrel Bisulphate 220 nm and 250 nm wavelengths were selected for measurement of absorptivity. Both the drugs show linearity in a concentration range of 10-50 μg/ml at their respective λmax with correlation coefficient (r²) of 0.9996 and 0.9998 for Clopidogrel Bisulphate and Irbesartan, respectively. Accuracy, precision and recovery studies were done by QC samples covering lower, medium and high concentrations of the linearity range. The relative standard deviation for accuracy, precision studies were found to be within the acceptance range (<2%). The limit of determination was 0.056 μg/ml and 0.075 μg/ml for Clopidogrel Bisulphate and Irbesartan, respectively. The limit of quantification was 0.172 μg/ml and 0.229 μg/ml for Clopidogrel Bisulphate and Irbesartan, respectively. Recovery of Clopidogrel Bisulphate and Irbesartan were found to be 99.58% and 99.66% respectively confirming the accuracy of the proposed method. % Assay was found to be 99.41% and 99.22% for Clopidogrel Bisulphate and Irbesartan, respectively.

Conclusions: It can be concluded from the study that assay results obtained by proposed method are in fair agreement and can be effectively applied for the estimation of these two drugs.

Keywords: Clopidogrel bisulphate, Irbesartan, Simultaneous estimation, Validation method
Introduction

The present study was aimed to develop simple, rapid, accurate and precise analytical method for simultaneous estimation of Clopidogrel Bisulphate (CLO) and Irbesartan (IRB). CLO and IRB are two widely used antithrombogenic agents and co-administration produces an enhanced therapeutic effect in many clinical conditions, particularly in high-risk patients with acute coronary syndromes and renal injury.\(^1\)

Antiplatelet drugs are used in platelet function of prophylaxis and thromboembolic disorders.\(^2\) They are therapeutic rather than prophylactic and work by activating the natural fibrinolytic system. CLO inhibits the ADP receptor blockers. As Fibrinogen Thienopyridine surface receptor on platelets is Inhibits selective irreversible P2Y\(_{12}\) Purinergic Receptor So Inhibits ADP, fibrinogen induced platelet Aggregation and Adenyl cyclase then result to reduce inflammation. IUPAN name of CLO Bisulphate Methyl 2- (2-Chlorophenyl) -2- (6,7-dihydro thieno [3,2-C] Pyridine- 5 (4H)-yl) Acetate sulphate.\(^3\)

\[\text{Figure 1: Structure of CLO.}\]

CLO is white crystalline powder. Solubility is given in practically insoluble in water, soluble in methanol, slightly soluble in 0.1 N HCl.\(^4\)

IRB is an Angiotensin II receptor Antagonist. It used mainly for the treatment of hypertension. It is an orally active nonpeptide tetrazole derivative and selectively inhibits Angiotensin II receptor type 2. Hypertension is the most common cardiovascular condition and its result to increased peripheral vascular smooth muscle tone, which leads to increased arteriolar resistance and reduced capacitance of the venous system. Angiotensin II receptor type1 antagonists have been widely used in treatment of diseases like hypertension, heart failure, cerebrovascular accidents, myocardial infarction and diabetic nephropathy. IUPAN name of IRB is 3-[(2'- (2H-tetrazol-5-yl) - (1,1'-biphenyl) -4-yl) methyl] -2- butyl-1,3-diazolspiro (4,4) non-1-en-4-one.\(^5\)

\[\text{Figure 2: Structure of IRB.}\]

IRB is white or almost white, crystalline powder. Solubility is given in practically insoluble in water, freely soluble in methanol, slightly soluble in 0.1 N NaOH.\(^6\)

Combination effect of Genetic Polymorphism on Clopidogrel Efficacy and Cardiovascular Events in the Clopidogrel in the Unstable Angina to Prevent Recurrent Events Trial and the Atrial Fibrillation Clopidogrel Trial with IRB for prevention of vascular events.\(^7\)

IRB in used in lower blood pressure in hypertension but its combination of antiplatelet drug such as a CLO to be used in anti-inflammatory renoprotective effect of chronic renal injury. Platelet activation and Angiotensin-II may each contribute to glomerular inflammation and fibrosis.\(^8\) The review of literature regarding quantitative analysis of CLO and IRB revealed that no attempt was made to develop analytical methods for CLO and IRB. Some spectrometric methods and chromatographic methods have been reported for the estimation of the individual drugs. The
focus of the present study was to develop and validate a rapid, stable, specific, and economic high performance liquid chromatographic method for the estimation of CLO and IRB in synthetic mixture.9

**Materials and Methods**

**Apparatus and instrument**

A double beam UV-Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2.0 nm, 1.0 cm quartz cells was used to measure absorbance of all the solutions.

Spectra were automatically obtained by UV-Probe system software.

An analytical balance (Sartorius CD 2250, Gottingen, Germany) was used for weighing purpose.

Sonicator (D120/2H, TRANS-O-SONIC) was used Sonication of solution.

All instruments and glass wares were calibrated.

**Reagents and material**

CLO raw material was received as gift sample from Cadila Healthcare LTD, Ankleshwar. IRB raw material was received as gift sample from CTX Life science, Surat. Methanol AR Grade (FINAR), Distilled water, HCl AR Grade, NaOH AR Grade (RANCHEM) was used for development purpose.

| Sr. No. | Drug            | Quantity (mg) | Quantity (mg) |
|---------|-----------------|---------------|---------------|
| 1       | Clopidogrel Bisulphate | 150           | 1500          |
| 2       | Irbesartan      | 150           | 1500          |
| 3       | Lactose         | 72.5          | 725           |
| 4       | Starch          | 48            | 480           |
| 5       | Anhydrous Silica| 0.5           | 5.0           |
| 6       | Magnesium Stearate| 4.0          | 40            |
| 7       | Talc            | q.s.          | q.s.          |

Table 1: Composition of formulation (synthetic mixture).

**Standard solution of CLO**

**Preparation of stock solution of CLO**

An accurately weighed quantity of CLO (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved, diluted up to mark with methanol to obtain standard solution having concentration of CLO (100 μg/ml).

**Standard solution of IRB**

**Preparation of standard stock solution of IRB**

An accurately weighed quantity of IRB (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved, diluted up to mark with methanol to obtain standard solution having concentration of IRB (100 μg/ml).

**Preparation of standard mixture solution (CLO + IRB)**

1.0 ml of standard stock solution of CLO (100 μg/ml) and 1.0 ml of standard stock solution of IRB (100 μg/ml) were pipetted out into 10 ml volumetric flasks and volume was adjusted to the mark with methanol to get 10 μg/ml of CLO and 10 μg/ml of IRB.

**Preparation of test solution**

The preparation of synthetic mixture was as per patent.10

All ingredients were shift and blend to make uniformity of mixing. Take synthetic powder equivalent to 10 mg of CLO in 100 ml volumetric flask. Dissolve in 25 ml of Methanol and Sonicated for 15 min. Dilute up to 100 ml with solvent shake vigorously. Filtered through Whatman filter paper No. 42 and further diluted. Finally the solution had concentration of 100 μg/ml for CLO and IRB, respectively. From that pipette out 1.0 ml in 10 ml volumetric flask and volume was made up to mark with Methanol to make final concentration of mixture 10 μg/ml for CLO and IRB, respectively.

**Calibration curves for CLO**

This series consisted of five concentrations of standard CLO solution ranging from 10 to 50

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µg/ml. The solutions were prepared by pipetting out Standard CLO stock solution (100 µg/ml). Then pipetting out (1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with methanol. A zero order spectrum of the resulting solution was recorded, measured the absorbance at 220.00 nm against a reagent blank solution (methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of CLO (Figure 3).

**Calibration curve for IRB**

This series consisted of five concentrations of standard IRB solution ranging from 10 to 50 µg/ml. The solutions were prepared by pipetting out Standard IRB stock solution (1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with methanol. A zero order spectrum of the resulting solution was recorded, measured the absorbance at 250.00 nm against a reagent blank solution (methanol). Calibration curve was prepared by plotting absorbance versus respective concentration of IRB (figure 3).

**Development and validation of spectroscopic simultaneous equation method**

**Selection of wavelength and method development for determination of CLO and IRB**

The standard solution of CLO and IRB were scanned separately between 200-400 nm, and CLO showed absorbance maxima at 220 nm and IRB at 250 nm (Figure 3).

**Validation parameters**

**Linearity and range**

The Zero order (figure 3) showed linear absorbance at 220 nm for CLO (10-50 µg/ml) and 250 nm for IRB (10-50 µg/ml) with correlation coefficient (r²) of 0.9996 and 0.9998 for CLO and IRB, respectively.

This method obeyed Beer’s law in the concentration range 10-50 µg/ml CLO and IRB, respectively (Table 2).

Correlation coefficient (r²) for calibration curve of CLO and IRB was found to be 0.9996 and 0.9998, respectively (Figure 3 and 7).

The regression line equation for CLO and IRB are as following,

\[
y = 0.028x + 0.048 \text{ for CLO} \quad (1)
\]

\[
y = 0.037x - 0.030 \text{ for IRB} \quad (2)
\]

**Precision**

**Intraday precision**

The precision of the developed method was assessed by analyzing combined standard solution containing three different concentrations 10, 30, 50 µg/ml for CLO and IRB, respectively. Three replicate (n=3) each on same day. Intraday precision data presented in Table 6.

These % RSD value was found to be less than 1.0 indicated that the method is precise.

**Interday precision**

The precision of the developed method was assessed by analyzing combined standard solution containing three different concentrations 10, 30, 50 µg/ml for CLO and IRB, respectively triplicate (n=3) per day for consecutive 3 days for inter-day precision. Interday precision data presented in Table 7.

These % RSD value was found to be less than 1.0 indicated that the method is precise.

**Accuracy**

Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, and 120%) of standard addition. The % recovery values are tabulated in Table 8 and 9.

Percentage recovery for CLO and IRB by this method was found in the range of 99.12 to 100.20% and 99.06 to 100.35%, respectively.

The value of % RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.
**Limit of detection and quantitation**

The LOD for CLO and IRB was conformed to be 0.056 μg/ml and 0.075 μg/ml, respectively.

The LOQ for CLO and IRB was conformed to be 0.172 μg/ml and 0.229 μg/ml, respectively.

The obtained LOD and LOQ results are presented in Table 10.

**Robustness and ruggedness**

The obtained Ruggedness and Robustness results are presented in Table 11.

The % RSD was found to be 0.243 - 0.979 % for CLO and 0.495 - 0.984% for IRB.

These % RSD value was found to be less than 1.0 indicated that the method is precise.

No significant changes in the spectrums were observed, proving that the developed method is rugged and robust.

**Application of the proposed method for analysis of CLO and IRB in synthetic mixture**

A zero order spectrum of the sample solution containing 20 μg/ml of CLO and 20 μg/ml of IRB was recorded and the absorbance at 220 nm and 250 nm were noted for estimation of CLO and IRB, respectively.

The concentration of CLO and IRB in mixture was determined using the corresponding calibration graph. The results from the analysis of synthetic mixture containing CLO (20 μg/ml) and IRB (20 μg/ml) in combination are presented in Table 12.

The percentage assay shows that there is no interference from excipients and the proposed method can successfully applied to analysis of commercial formulation containing CLO and IRB. The % assay values are tabulated in Table 12.

**Results and Discussion**

From the optical characteristics of the proposed method, it was found that the drug obeys linearity within concentration range of 10-50 μg/ml for both drugs CLO and IRB.

![Figure 3: overlain zero order spectra of CLO and IRB (Ratios: 1:1).](image)

![Figure 4: Calibration curve for CLO at 220 nm.](image)

![Figure 5: Calibration curve for CLO at 250 nm.](image)
Table 2: Calibration data for CLO at 220 nm and 250 nm *(n=6).

| Sr. No. | Concentration (μg/ml) | Abs. ± SD CLO (220 nm) | % RS D | Abs. ± SD CLO (250 nm) | % RS D |
|---------|-----------------------|------------------------|--------|------------------------|--------|
| 1       | 10                    | 0.3140 ± 0.0028        | 0.9    | 0.0918 ± 0.0007        | 0.8    |
| 2       | 20                    | 0.6221 ± 0.0038        | 0.6    | 0.1450 ± 0.0010        | 0.7    |
| 3       | 30                    | 0.8990 ± 0.0032        | 0.3    | 0.2008 ± 0.0019        | 0.9    |
| 4       | 40                    | 1.1681 ± 0.0023        | 0.1    | 0.2435 ± 0.0018        | 0.7    |
| 5       | 50                    | 1.4423 ± 0.0033        | 0.2    | 0.2836 ± 0.0028        | 0.9    |

Figure 6: Calibration curve for IRB at 220 nm.

Table 3: Calibration data for IRB at 220 nm and 250 nm *(n=6).

| Sr. No. | Concentration (μg/ml) | Abs. ± SD IRB (220 nm) | % RS D | Abs. ± SD IRB (250 nm) | % RS D |
|---------|-----------------------|------------------------|--------|------------------------|--------|
| 1       | 10                    | 0.5811 ± 0.0033        | 0.5    | 0.3542 ± 0.0023        | 0.6    |
| 2       | 20                    | 1.1970 ± 0.0021        | 0.1    | 0.7110 ± 0.0035        | 0.4    |
| 3       | 30                    | 1.8723 ± 0.0058        | 0.3    | 1.0991 ± 0.0041        | 0.3    |
| 4       | 40                    | 2.4338 ± 0.0024        | 0.1    | 1.4928 ± 0.0038        | 0.2    |
| 5       | 50                    | 2.8692 ± 0.0046        | 0.1    | 1.8508 ± 0.0033        | 0.1    |

Figure 7: Calibration curve for IRB at 250 nm.

Table 4: Average of absorptivity at 220 nm and 250 nm.

|                         | at 220 nm | at 250 nm |
|-------------------------|-----------|-----------|
| ax₁                     | 0.03010   | 0.00695   |
| ax₂                     | 0.0605    | 0.03638   |

From the result % assay was found 99.41% and 99.22% for CLO and IRB, respectively and % RSD is less than 2% which indicate that the method has good reproducibility. From the result shown in accuracy table it was found that the percentage recovery of pure drug from reanalysed solution of formulations were in between 98% - 102%, which indicate that the method is accurate and which reveals that commonly used excipient and additives present in the synthetic formulation did not interfere in proposed method.

Figure 8: Zero order spectra of CLO and IRB in combination (1:1 ratio).
Table 5: Mixture linearity for CLO and IRB (1:1 ratio).

| Sample No. | Concentration in mixture (μg/ml) | CLO Abs. + SD | IRB Abs. + SD | % RsD CLO (220 nm) | % RsD IRB (250 nm) |
|------------|----------------------------------|---------------|---------------|-------------------|-------------------|
| 1          | 10 10                            | 0.9925 ± 0.0093 | 0.4695 ± 0.0095 | 0.946 | 0.974 |
| 2          | 20 20                            | 1.6095 ± 0.0077 | 0.8791 ± 0.0085 | 0.946 | 0.974 |
| 3          | 30 30                            | 2.1716 ± 0.0087 | 1.3681 ± 0.0093 | 0.946 | 0.974 |
| 4          | 40 40                            | 2.6813 ± 0.0079 | 1.8293 ± 0.0080 | 0.946 | 0.974 |
| 5          | 50 50                            | 3.1891 ± 0.0091 | 2.2851 ± 0.0089 | 0.946 | 0.974 |

Table 6: Intraday precision data for estimation of CLO and IRB *(n=3).

| Conc. (μg/ml) | CLO Abs. * ± SD | % RsD | IRB Abs. * ± SD | % RsD |
|---------------|-----------------|-------|-----------------|-------|
| CLO 10        | 0.987 ± 0.0030  | 0.309 | 0.466 ± 0.0037  | 0.811 |
| CLO 30        | 2.169 ± 0.0060  | 0.277 | 1.367 ± 0.0045  | 0.335 |
| CLO 50        | 3.187 ± 0.0055  | 0.174 | 2.283 ± 0.0055  | 0.243 |
| IRB 10        | 20.07 ± 0.045   | 0.461 | 0.468 ± 0.0043  | 0.931 |
| IRB 30        | 2.171 ± 0.0060  | 0.276 | 1.368 ± 0.0055  | 0.402 |
| IRB 50        | 3.189 ± 0.0062  | 0.195 | 2.285 ± 0.0065  | 0.286 |

Table 7: Interday precision data for estimation of CLO and IRB *(n=3).

| Conc. (μg/ml) | CLO Abs. * ± SD | % RsD | IRB Abs. * ± SD | % RsD |
|---------------|-----------------|-------|-----------------|-------|
| CLO 10        | 0.994 ± 0.0045  | 0.461 | 0.468 ± 0.0043  | 0.931 |
| CLO 30        | 2.171 ± 0.0060  | 0.276 | 1.368 ± 0.0055  | 0.402 |
| CLO 50        | 3.189 ± 0.0062  | 0.195 | 2.285 ± 0.0065  | 0.286 |
| IRB 10        | 20.07 ± 0.030   | 0.461 | 0.468 ± 0.0043  | 0.931 |
| IRB 30        | 35.92 ± 0.045   | 0.276 | 1.368 ± 0.0055  | 0.402 |
| IRB 50        | 43.91 ± 0.026   | 0.195 | 2.285 ± 0.0065  | 0.286 |

The proposed method was simple, sensitive and reliable with good precision and accuracy. Hence this method can be used for the routine determination of CLO and IRB in synthetic mixture.

Figure 9: Calibration curve for CLO and IRB in combination.

Table 8: Recovery data of CLO *(n=3).

| Conc. (mg) | CLO from formulation (μg/ml) | Total amount of CLO found (μg/ml) | % Recovery (n=3) |
|-----------|-----------------------------|-----------------------------------|------------------|
| 20        | 20.04 ± 0.020               | 100.2                            | 0.9               |
| 20        | 35.90 ± 0.160               | 99.12                            | 0.9               |
| 20        | 39.91 ± 0.081               | 99.35                            | 0.9               |
| 20        | 43.96 ± 0.020               | 99.66                            | 0.9               |

Table 9: Recovery data of IRB *(n=3).

| Conc. (mg) | IRB from formulation (μg/ml) | Total amount of IRB found (μg/ml) | % Recovery (n=3) |
|-----------|-----------------------------|----------------------------------|------------------|
| 20        | 20.07 ± 0.030               | 100.3                            | 0.9               |
| 20        | 35.92 ± 0.045               | 99.06                            | 0.9               |
| 20        | 40.05 ± 0.065               | 99.90                            | 0.9               |
| 20        | 43.91 ± 0.026               | 99.33                            | 0.9               |
Conclusions

A new, simultaneous equation method has been developed for estimation of Clopidogrel Bisulphate and Irbesartan in synthetic mixture. The method was validated by employment of ICH guidelines. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. Ruggedness and robustness of method was confirmed as no significant were observed on analysis by subjecting the method to slight change in the method condition. Assay results obtained by proposed method are in fair agreement.

Acknowledgements

Authors are thankful Dr. Noolvi, Principal, Shree Dhanvantary Pharmacy College, Kim, Surat, for giving permission to carry out research work.

Funding: No funding sources
Conflict of interest: None declared

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Table 10: LOD and LOQ data of CLO and IRB *(n=10).

| Conc. (µg/ml) | Abs.* ± SD CLO | % RS D | Abs.* ± SD IRB | % RS D |
|--------------|----------------|--------|----------------|--------|
| CLO          | 0.9847 ± 0.0004 | 94     | 0.4625 ± 0.0008 | 3      |
| IRB          | 0.056           | 0.075  | 0.172           | 0.229  |

Table 11: Robustness and Ruggedness data of CLO and IRB *(n=3).

| No | Factor          | Level        | CLO Abs.* ± SD | % RSD | IRB Abs.* ± SD | % RSD |
|----|----------------|--------------|----------------|-------|----------------|-------|
| 1  | Change in      | UV-2450      | 0.992 ± 0.0025 | 0.253 | 0.474 ± 0.0045 | 0.966 |
|    | Instrument     | UV-1800      | 0.994 ± 0.0035 | 0.353 | 0.471 ± 0.0043 | 0.925 |
| 2  | Change in      | Analyst-1    | 0.991 ± 0.0040 | 0.407 | 0.467 ± 0.0030 | 0.653 |
|    | Analyst        | Analyst-2    | 0.994 ± 0.0030 | 0.301 | 0.470 ± 0.0041 | 0.885 |

Table 12: Analysis data of synthetic mixture *(n=3).

| Sr. No. | Drug | Formulation (synthetic mixture) (µg /ml) | % Assay* ± SD | USP limit (%) |
|---------|------|----------------------------------------|---------------|---------------|
| 1       | CLO  | 20                                     | 99.41 ± 0.401 | 97-101.5 %    |
| 2       | IRB  | 20                                     | 99.22 ± 0.152 | 98-102 %      |
Table 13: Summary of validation parameters.

| Sr. No. | Parameter                        | Clopidogrel Bisulphate | Irbesartan |
|---------|----------------------------------|------------------------|------------|
| 1       | Wavelength Max.                  | 220.00 nm              | 250.00 nm  |
| 2       | Linearity (µg/ml) (n=6)          | 10 to 50 µg/ml         | 10 to 50 µg/ml |
| 3       | Regression equation              | y = 0.028x + 0.048     | y = 0.037x - 0.030 |
| 4       | Correlation coefficient (r²)     | 0.9996                 | 0.9998     |
| 5       | Intraday Precision (% RSD) (n=3) | 0.174-0.309            | 0.243-0.811 |
| 6       | Interday Precision (% RSD) (n=3) | 0.195-0.461            | 0.286-0.931 |
| 7       | Accuracy (% Recovery) (n=3)      | 99.12-100.20           | 99.06-100.35 |
| 8       | LOD (µg/ml) (n=10)               | 0.056                  | 0.075      |
| 9       | LOQ (µg/ml) (n=10)               | 0.172                  | 0.229      |
| 10      | Robustness and Ruggedness (% RSD) (n=3) | 0.243-0.979 | 0.495-0.984 |
| 11      | Assay                            | 99.41                  | 99.22      |

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