SIMULTANEOUS ESTIMATION OF AMLODIPINE AND CLOPIDOGREL IN BULK AND MARKETED FORMULATION BY Q-ABSORBANCE RATIO METHOD


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<th>ARTICLE INFO</th>
<th>ABSTRACT</th>
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<td><strong>Article history</strong></td>
<td>A simple UV spectrophotometric method has been developed for the simultaneous analysis of Amlodipine and Clopidogrel in tablet dosage form is based on the absorbance ratio method. The acidic methanol was used as solvent. The two wavelengths were selected 239.4 nm as isosbestic point and 361.8 nm as absorption maxima for Amlodipine for the analysis. Amlodipine and Clopidogrel obeyed Beer-Lambert’s law in the concentration range of 1-3 μg/ml and 30-90 μg/ml with coefficient of correlation 0.9970 and 0.9960 at 239.4 nm respectively. Amlodipine was obeyed Beer-Lambert’s law in the concentration range 1-3 μg/ml with coefficient of correlation 0.999 at 361.8 nm. Recovery of the developed method was found to be in the range of 100.60% for CL and 100.7% for AM. This proposed method was statistically validated in accordance with ICH guidelines. This method was found to be accurate, precise and rugged as indicated by low values (&lt;2%) of % RSD. This method was also found to be rapid and economical can successfully be applied for the routine analysis of bulk and combined tablet dosage form.</td>
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**Keywords:**
- Amlodipine besylate (AM)
- Clopidogrel bisulphate (CL)
- Q- Absorbance ratio method
- Marketed formulation

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Please cite this article in press as Salunke et al. Simultaneous Estimation of Amlodipine and Clopidogrel in Bulk and Marketed Formulation by Q-Absorbance Ratio Method. Indo American Journal of Pharm Research.2013:3(4).
INTRODUCTION

Amlodipine besylate (AM) [Figure 1] chemically is 3-Ethyl- 5-Methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-2 chlorophenyl)-6- methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzenesulphonate is a long-acting calcium channel blocker used for hypertension and angina pectoris.\textsuperscript{[1,2]}

![Figure 1: Structure of Amlodipine besylate](image)

Clopidogrel bisulphate (CL) [Figure 2] is a prodrug and it is congener of ticlopidine\textsuperscript{[3]}. It is chemically methyl (+)-(S)-(o-chlorophenyl)-6, 7-dihydrothieno-[3, 2-c] pyridine 5(4H)-acetate.\textsuperscript{[4]} It is anti-platelet agent and alters surface receptors on platelets and inhibits adenosine diphosphate (ADP) as well as fibrinogen induced platelet aggregation. It is used in myocardial infarction, unstable angina, in coronary angioplasty, stents and in bypass implants.\textsuperscript{[5]}

![Figure 2: Structure of Clopidogrel bisulphate](image)

Amlodipine besylate and Clopidogrel bisulphate was estimated by various methods individually or in combination with other drugs. Official method for estimation of Amlodipine is high performance liquid chromatography and UV spectrophotometry.\textsuperscript{[6]}

Literature survey revealed various methods as Reverse phase chromatography, UV spectrophotometric methods for determination of Amlodipine in combination with other drugs\textsuperscript{[7-11]} and spectrophotometric method\textsuperscript{[12, 13]}, HPTLC method\textsuperscript{[14]}, fast liquid chromatography\textsuperscript{[15-19]} Ultra-Performance Liquid Chromatography\textsuperscript{[20]} for estimation of Clopidogrel alone or in combination with other drugs. For estimation of Amlodipine besylate and Clopidogrel bisulphate together RP-HPLC method have been developed.\textsuperscript{[21, 22]} The reported methods are applicable for the estimation of either for Amlodipine besylate or Clopidogrel bisulphate individually or in combination with other drugs from pharmaceutical dosage forms. The present works describes the developed and validate spectroscopic method, which can quantify these components simultaneously from a combined dosage form.

MATERIALS AND METHODS

Materials:

All the chemicals and solvents used were of AR grade and the pure drugs Amlodipine besylate and Clopidogrel bisulphate were gift samples obtained from Koparan Laboratories Ltd., Pune and Emcure

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Pharmaceuticals, Pune respectively and were used as reference standards. The tablet formulation was purchased from local market.

**Instrumentation**

UV experimentation was performed on Shimadzu1800 UV-visible spectrophotometer equipped with Photo Diode Array (PDA) detector, with 1 cm quartz cell.

**Selection of Common Solvent**

Acidic methanol was selected as common solvent for developing spectral characteristics of drug. The choice of the solvent was made after evaluating the solubility in different solvents and as per literature survey.

**Preparation of Standard Stock Solutions**

**Clopidogrel Standard Stock Solution:**

An accurately weighed Clopidogrel (100 mg) was dissolved in acidic methanol (50 mL) in 100 mL volumetric flask and volume was made up to the mark using acidic methanol to get final concentration (1000 µg/ml).

**Amlodipine Standard Stock Solution:**

An accurately weighed quantity of Amlodipine (100 mg) was dissolved in acidic methanol (50 mL) in 100 mL volumetric flask and volume was made up to the mark using acidic methanol to get final concentration (1000 µg/ml).

**Study of Spectra and Selection of Wavelength:**

The aliquot portions of standard stock solutions of CL and AM were diluted appropriately with acidic methanol to obtain concentration 10 µg/mL of both drugs. The solutions of both drugs were scanned separately in the range of 400 – 200 nm. The overlain UV absorbance spectrum of CL and AM is shown in Fig. 3. From the overlain spectrum the wavelengths selected for estimation of drugs were 239.4 nm as isobestic point and 361.8 nm as λ max of Amlodipine.

![Fig. 3 Overlain Spectra of CL and AM](image-url)
Study of Linearity Curves

The aliquot portions of standard stock solutions of CL and AM were diluted appropriately with acidic methanol to get a series of concentration from 30–90 μg/mL and 1–3 μg/mL respectively. The absorbance of these drugs was measured at 239.4 and 361.8 nm respectively and calibration curves were plotted as concentrations versus absorbances.

Q-Absorbance method uses the ratio of absorbance at two selected wavelengths, one at isoabsorptive point and other being the λ max of one of the two drugs. CL and AM have λ max at 241 nm and 361.8 nm respectively and isoabsorptive point 239.4 nm. The wavelengths selected for analysis were 239.4 and 361.8 nm, respectively. E (1%, 1cm) values of CL and AM were determined at 239.4 and 361.8 nm. The concentration of two drugs in mixture was calculated by using following equations:

\[
\frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A}{ax1} = C_{CL} \quad (1)
\]

\[
\frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A}{ay1} = C_{AM} \quad (2)
\]

Where,

\[
Q_m = \frac{\text{Absorbance of sample at 361.8 nm}}{\text{Absorbance of sample at 239.4 nm}}
\]

\[
Q_x = \frac{E (1\% \text{ } 1\text{cm}) \text{ of CL at 361.8 nm}}{E (1\% \text{ } 1\text{cm}) \text{ of CL at 239.4 nm}}
\]

\[
Q_y = \frac{E (1\% \text{ } 1\text{cm}) \text{ of AM at 361.8 nm}}{E (1\% \text{ } 1\text{cm}) \text{ of AM at 239.4 nm}}
\]

‘A’ is the absorbance of mixture at 239.4 nm and ax1 (39000), ax2 (13500) and ay1 (29370), ay2 (0) are absorptivities E (1%, 1 cm) of CL and AM at 239.4 nm and 361.8 nm and Qm= A2/A1, Qy = ay2/ay1 and Qx = ax2/ax1.

Analysis of Marketed Formulation by Proposed Method:

Twenty tablets were accurately weighed and were reduced to fine powder and mixed thoroughly. A quantity of tablet powder equivalent to CL (10 mg) was transferred to 10ml volumetric flask and dissolved in acidic methanol with vigorous shaking and sonicate it for 30mins. The volume was made up to the mark using acidic methanol. The solution was filtered through Whatman filter paper no. 41. The aliquot portion of filtrate was further diluted to get CL (60μg/ mL) and AM (2μg/ mL) respectively.
The absorbance of sample solution was measured at 239.4 nm and 361.8 nm and the results are shown in Table No. 1.

Table No. 1: Results of Application of Proposed Method for Analysis of Marketed Formulation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Label Claimed</th>
<th>% Label Claim ± SD</th>
<th>%RSD</th>
</tr>
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<tbody>
<tr>
<td>Numlopar</td>
<td>Clopidogrel 75mg</td>
<td>100.60 ±0.3</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Amlodipine 2.5mg</td>
<td>100.70 ±1.7</td>
<td>1.67</td>
</tr>
</tbody>
</table>

Validation of Method:
Accuracy:
Accuracy of each of the proposed method was ascertained on the basis of recovery studies performed by standard addition method as shown in the table no.2

Precision:
Precision of the analytical method is expressed as the series of the measurement. It was ascertained by replicate estimation of the drug by the proposed method as shown in table no.2.

Linearity and Range:
The suitable aliquots were taken to obtain 1, 1.5, 2, 2.5, 3μg/ml from Amlodipine besylate stock solution. The suitable aliquots were taken to obtain 30, 45, 60, 75, 90 μg/ml from Clopidogrel bisulphate stock solution. The results are shown in table no 04 and 05 and fig. no. 4 and 5 respectively.

Repeatability:
Repeatability was ascertained by getting the sample analyzed by different analyst and carrying out analysis for no. of times. The results are shown in table no 02.

RESULTS AND DISCUSSION
In acidic methanol, AM and CL obeyed linearity in the concentration range of 01-03 μg/ml and 30-90 μg/ml respectively at λ max with correlation coefficient (r² > 0.9998 and 0.9996) in both the case. Marketed brand of tablet were analyzed. The amounts of AM and CL determined by absorption ratio Method was found to be 100.70 and 100.60, respectively. In this method precision was studied as repeatability (% RSD < 2) and inter and intra-day variations (%RSD < 2) for both drugs. The accuracy of method was determined by calculating mean percentage recovery. It was determined at 80,100 and 120 % level. The ruggedness of the methods was studied by two different analysts using the same operational and environmental conditions. The % recovery, repeatability data, ruggedness data were presented in Table-2.
Table 2: Validation Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AM</th>
<th>CL</th>
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<tbody>
<tr>
<td>Working wavelengths</td>
<td>239.4</td>
<td>361.8</td>
</tr>
<tr>
<td>Linearity Range(μg/mL)</td>
<td>1-3</td>
<td>1-3</td>
</tr>
<tr>
<td>Precision [%RSD]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday [n=3]</td>
<td>0.001-0.002</td>
<td>0.002-0.004</td>
</tr>
<tr>
<td>Intraday [n=3]</td>
<td>0.002-0.003</td>
<td>0.001-0.004</td>
</tr>
<tr>
<td>Repeatability [n=6]</td>
<td>1.67</td>
<td>0.72</td>
</tr>
<tr>
<td>Ruggedness [n=6]</td>
<td></td>
<td></td>
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<tr>
<td>Analyst I [n=6]</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Analyst II [n=6]</td>
<td>1.35</td>
<td>0.59</td>
</tr>
<tr>
<td>% Recovery [n=3]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD</td>
<td>0.66-1.00</td>
<td>0.59-0.99</td>
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CONCLUSION

The developed spectrophotometric method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in two-component tablet dosage form of Amlodipine besylate and Clopidogrel bisulphate. The developed method was validated according to ICH guidelines for linear relation including coefficient of correlation, repeatability, accuracy and precision. The RSD for all parameters was found to be less than 2, which indicates the validity of method. The developed method can be used for routine quantitative simultaneous estimation of Amlodipine besylate and Clopidogrel bisulphate in pharmaceutical preparation.

ACKNOWLEDGEMENT

The authors are thankful to Koparan Laboratories Ltd. Pune and Emcure Pharmaceuticals, Pune for providing gift samples of Amlodipine besylate and Clopidogrel bisulphate respectively.
REFERENCES


