Development and Validation of Simultaneous Estimation of Enalapril Maleate and Amlodipine Besylate in Combined Dosage Forms

Gopal Garg, Shailendra Saraf and Swarnlata Saraf
Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.) 492 010, India

Abstract: Simple, sensitive and specific spectrophotometric methods were developed and validated for quantization of enalapril and amlodipine in tablet dosage form. Three new analytical methods were developed based on the simultaneous estimation of drugs in a binary mixture without prior separation. In simultaneous equation method, the drugs were determined by using the absorptivity values of enalapril and amlodipine at selected wavelengths, viz., 209 and 238 nm, respectively. Second method is based on the determination of graphical absorbance ratio at two selected wavelengths, one being the isosorptive point for the drugs (219 nm) and the other being the absorption maximum of amlodipine (238 nm); in this method both the drugs obeyed the Beer-Lambert’s law in the concentration range of 6-18 µg mL⁻¹. The third method is based on the derivative spectrophotometric method at zero crossing wavelengths. These methods are simple, accurate and rapid and they require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

Key words: Enalapril, amlodipine, derivative, isosorptive, simultaneous equation

INTRODUCTION

Enalapril maleate is used as an anti hypertensive drug, chemically it is 1-[N-[(S)-1-carboxy-3-phenylpropyl]-L-alanyl]-L-proline 1α-ethyl ester, maleate. Official methods for the quantitative estimation of enalapril maleate is, UV-spectrophotometric (Prasad et al., 1999; Walily et al., 1995; Carlucci et al., 1993) and capillary electrophoresis (Zhi et al., 1992) has been reported. Amlodipine besylate is calcium antagonist and chemically, it is 3-ethyl-5-methyl-(4 RS)-2-[(2-amino ethoxy) methyl]-4(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylic benzene sulphonate. The British Pharmacopoeia examines amlodipine besylate by liquid chromatography. Reversed phase HPLC (Zarghi et al., 2005; Naidu et al., 2005; Bahrani and Mirzaee, 2004; Tatar and Atracca, 2001; Shang, 1996), HPTLC (Meyyanathan and Suresh, 2005) and UV-spectrophotometric method (Rahman and Nasrud Hoda, 2003; Basavaiah et al., 2003; Sridhar et al., 1997) are few of the methods reported in literature for the simultaneous analysis of amlodipine besylate with various drugs from their respective formulations. Although enalapril and amlodipine are commonly used in dual drug therapy as a potent anti hypertensive drug, yet no method is so far reported for their simultaneous estimation. A successful attempt has been made to estimate these two drugs simultaneously by spectrophotometric analysis.

MATERIALS AND METHODS

Shimadzu 1700 Pharmaspec UV-visible spectrophotometer with a matched pair of 10 mm quartz cells was used. The chemicals used were of analytical grade. The commercially available tablets of

Corresponding Author: Dr. Swarnlata Saraf, Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.) 492 010, India Fax: +91-7712262832

278
enalapril and amlodipine were procured from local market. Enalapril maleate and amlodipine besylate received as gift sample from Ranbaxy Labs. Dewas, were used as such without further purification.

**Preparation of Standard Solutions**

Solutions of enalapril and amlodipine were prepared by dissolving accurately weighed 100 mg each of standard enalapril and standard amlodipine in 100 mL methanol separately. Working standard solutions (A) and (B) were further prepared by taking 1 mL of stock solution of enalapril and amlodipine in 10 mL volumetric flasks and made up the volume with methanol.

**Methods of Analysis**

**Method 1: (Based on Simultaneous Equation Method)**

In Fig. 1 Enalapril shows absorption maxima at 209 nm and amlodipine shows at 238 nm. The calibration curves for enalapril and amlodipine were prepared in the concentration range of 8-26 μg mL⁻¹ (Fig. 2) and 5-40 μg mL⁻¹ (Fig. 3), respectively at both the wavelengths i.e., 209 and 238 nm. The absorptivity coefficients were determined for both the drugs at both the wavelengths and following equations were made.

\[
A = 568.80 C_{ma} + 422.91 C_{mb} \quad \text{---(at } \lambda_{209})
\]

![Graph showing absorbance vs wavelength for enalapril maleate and amlodipine besylate](image1)

**Fig. 1: Overlaid spectra of enalapril maleate and amlodipine besylate**

![Graph showing calibration curve of enalapril at 209 nm](image2)

**Fig. 2: Calibration curve of enalapril at 209 nm**
Fig. 3: Calibration curve of amlopidine at 238 nm

Fig. 4: Calibration curve of amloidipine and enalapril at 219 nm

Fig. 5: First order derivative spectra of enalapril maleate

$$A_s = 19.61C_{m} + 310.12C_{mbs} \quad \text{at } \lambda_{238}$$  \tag{2}

$A_s$ and $A_c$ are absorbances at 209 nm and 238 nm, respectively and $C_m$ and $C_{mbs}$ are concentrations of enalapril maleate and amloidipine besylate, respectively. The concentrations of both the drugs in the mixture were determined by Eq. (1 and 2).

**Method II: Graphical Absorbance Ratio Method**

This method is based on the method used by Ghanem and his colleagues which makes use of the iso-absorptive point of the two drugs i.e. the wavelength of equal absorptivity of the two components of the mixture.
Fig. 6: First order derivative spectra of amlodipine besylate

Fig. 7: Calibration curve of enalapril at 227 nm

The iso-absorptive point was 219 nm in this case. The other wavelength selected is the absorption maximum of one of the components. In this case it was 238 nm, the absorption maximum of amlodipine. The concentrations of the two components are related to the ratio of the absorbance at these two wavelengths. The absorbance of the mixture was noted at 219 and 238 nm. Calibration curves of enalapril and amlodipine were plotted in the concentration range 6-18 µg mL\(^{-1}\) (Fig. 4) (range for which Beer-Lambert's law followed). The absorptivity coefficients were determined for both the drugs and the average value was taken. These values and the absorbance ratio were used to develop equations as given:

\[
A_t = 411.25 \times (C_x + C_y) = 411.25 \times C_x \left(0.764/Q_{m} - 0.7540\right) \tag{3}
\]

Where, \(Q_m\) is \(A_2/A_1\) and \(A_1, A_2\) are the absorbances at 219 and 238 nm, respectively. \(C_x\) and \(C_y\) are concentrations of enalapril and amlodipine respectively.

Method III: Derivative Spectrophotometric Method

Upon examining the first-derivative spectra of the two drugs, it can be noticed that enalapril maleate can be determined at 227 nm (Fig. 5) where amlodipine has no contribution and amlodipine can be determined at 327.5 nm (Fig. 6) where enalapril shows a zero crossing. Calibration curves for enalapril and amlodipine were prepared in the concentration range of 8-22 µg mL\(^{-1}\) (Fig. 7) and 4-32 µg mL\(^{-1}\) (Fig. 8), respectively at wavelengths i.e., 227 and 327.5 nm.
Fig. 8. Calibration curve of amiodipine at 327.5 nm

Table 1: Compilation of results of statistical analysis of commercial formulations

<table>
<thead>
<tr>
<th>Methods</th>
<th>Tablet brand</th>
<th>Tablet composition</th>
<th>Label claim (%)</th>
<th>Recovery mg/tab (SD) (%)</th>
<th>SE (SD) (%)</th>
<th>RSD (SD) (%)</th>
<th>Percentage range of error with 95% confidence limit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A</td>
<td>Enalapril</td>
<td>5</td>
<td>99.06±0.204</td>
<td>0.0021</td>
<td>0.022</td>
<td>±0.0147</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Anlodipine</td>
<td>5</td>
<td>99.15±0.069</td>
<td>0.0049</td>
<td>0.068</td>
<td>±0.0249</td>
</tr>
<tr>
<td>II</td>
<td>A</td>
<td>Enalapril</td>
<td>5</td>
<td>98.94±0.204</td>
<td>0.0037</td>
<td>0.169</td>
<td>±0.0368</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Anlodipine</td>
<td>5</td>
<td>98.97±0.159</td>
<td>0.0058</td>
<td>0.107</td>
<td>±0.0247</td>
</tr>
<tr>
<td>III</td>
<td>A</td>
<td>Enalapril</td>
<td>5</td>
<td>99.68±0.025</td>
<td>0.0061</td>
<td>0.101</td>
<td>±0.0879</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Anlodipine</td>
<td>5</td>
<td>99.22±0.098</td>
<td>0.0089</td>
<td>0.087</td>
<td>±0.1354</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>Enalapril</td>
<td>5</td>
<td>99.16±0.164</td>
<td>0.0341</td>
<td>0.046</td>
<td>±0.0312</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Anlodipine</td>
<td>5</td>
<td>99.32±0.179</td>
<td>0.0267</td>
<td>0.125</td>
<td>±0.3809</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>Enalapril</td>
<td>5</td>
<td>99.78±0.011</td>
<td>0.0129</td>
<td>0.139</td>
<td>±0.1204</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Anlodipine</td>
<td>5</td>
<td>99.54±0.057</td>
<td>0.0127</td>
<td>0.019</td>
<td>±0.3697</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>Enalapril</td>
<td>5</td>
<td>99.88±0.031</td>
<td>0.0381</td>
<td>0.016</td>
<td>±0.0197</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Anlodipine</td>
<td>5</td>
<td>99.87±0.042</td>
<td>0.0324</td>
<td>0.036</td>
<td>±0.2570</td>
</tr>
</tbody>
</table>

*: Average of nine determinations; SD = Standard Deviation, %RSD = Relative Standard Deviation and SE = Standard Error. Statistical calculations were carried out by SPSS (official software).

Table 2: Compilation of results of drug recovery study

<table>
<thead>
<tr>
<th>Methods</th>
<th>Tablet brand</th>
<th>Enalapril</th>
<th>Recovery (%)</th>
<th>Standard deviation (%)</th>
<th>Amlodipine</th>
<th>Recovery (%)</th>
<th>Standard deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A</td>
<td>99.04</td>
<td>0.0169</td>
<td>99.36</td>
<td>0.0099</td>
<td>0.0108</td>
<td>0.0174</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>98.99</td>
<td>0.0209</td>
<td>99.03</td>
<td>0.0108</td>
<td>0.0108</td>
<td>0.0174</td>
</tr>
<tr>
<td>II</td>
<td>A</td>
<td>98.74</td>
<td>0.0378</td>
<td>98.79</td>
<td>0.0357</td>
<td>0.0378</td>
<td>0.0357</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>98.89</td>
<td>0.0245</td>
<td>99.11</td>
<td>0.0174</td>
<td>0.0245</td>
<td>0.0174</td>
</tr>
<tr>
<td>III</td>
<td>A</td>
<td>99.91</td>
<td>0.0109</td>
<td>99.29</td>
<td>0.0009</td>
<td>0.0109</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>99.87</td>
<td>0.0062</td>
<td>99.92</td>
<td>0.0014</td>
<td>0.0062</td>
<td>0.0014</td>
</tr>
</tbody>
</table>

*: Readings are average of nine determinations, statistical calculations were carried out by SPSS (official software).

\[
y = 0.0017x + 0.0054 \quad (At \lambda_{227})
\]

\[
y = 0.0003x + 0.0004 \quad (At \lambda_{327.5})
\]

Where, \(x\) is the concentration in \(\mu g\ mL^{-1}\), \(y\) is the peak amplitude of the first-derivative curves at 227 and 327.5 nm for enalapril and amiodipine, respectively.

**Estimation from Tablets**

Twenty tablets were weighed and average weight determined. Powder equivalent to 5 mg of enalapril and 5 mg amiodipine was extracted quantitatively with small amount of methanol. Insoluble
RESULTS AND DISCUSSION

In the first method, the content of enalapril and amlodipine was directly found from the Eq. 1 and 2. Two wavelengths of respective absorbance maxima i.e., 209 nm for enalapril and 238 nm for amlodipine were used for the analysis of the drugs. In the second method, the absorbance ratio and the absorptivity coefficients were determined and the values were substituted in the Eq. 3 to give the results. In this method the primary requirement for developing a method for analysis is that the entire spectra should follow the beer’s law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelength used for analysis of both the drugs were 219 nm (isosbestic point) and 238 nm (wavelength maxima of amlodipine). In the third method the absorbance of the one drug was taken at the zero crossing point of the other drug and the values were substituted in the Eq. 4 and 5. The validation parameters were studied at all the wavelengths for all the methods. Accuracy was determined by calculating the recovery and the mean was determined (Table 2). The value of recovery is very close to 100% show the accuracy of the methods. Precision was calculated as repeatability (standard deviation and relative standard deviation) for both the drugs. The percent recovery obtained indicates non-interference from the excipients like starch, magnesium stearate etc. if used in the formulations. The value of confidence level was under the standard value show the significance of data. By observing the validation parameters, all the methods were found to be specific, accurate and precise. Hence these methods can be employed for routine analysis of these two drugs in combinations.

CONCLUSION

The main advantage of the proposed methods is its suitability for routine determination of amlodipine and enalapril from their marketed formulations. The proposed methods are economic, simple, sensitive, precise and reproducible and do not require any expensive or sophisticated apparatus, in contrast with the reported chromatographic methods.

ACKNOWLEDGMENTS

Thanks are extended to The Director, Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur (C.G.) for providing necessary facilities for research work and AICTE New Delhi for financial assistance under the scheme RPS and MODROB. We are also grateful to Ranbaxy Labs. Dewas for providing gift samples of enalapril and amlodipine.

REFERENCES


