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

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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 isoabsorptive 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, isoabsorptive, 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 1ȹ-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-dicarboxylate benzene sulphonate. The British Pharmacopoeia examines amlodipine besylate by liquid chromatography. Reversed phase HPLC (Zarghi *et al*., 2005; Naidu *et al*., 2005; Bahrami and Mirzaeei, 2004; Tatar and Atmaca, 2001; Shang, 1996), HPTLC (Meyyanathan and Suresh, 2005) and UV-spectrophotometric method (Rahman and Nasrul 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 nm quartz cells was used. The chemicals used were of analytical grade. The commercially available tablets of

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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 I: (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.

$$A_1 = 568.80\ C_{ena} + 422.91\ C_{amlo} \text{ ---(at } \lambda_{209}) \quad (1)$$

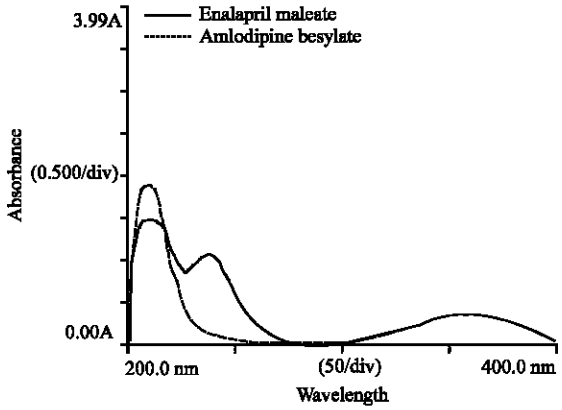

Fig. 1: Overlain spectra of enalapril maleate and amlodipine besylate

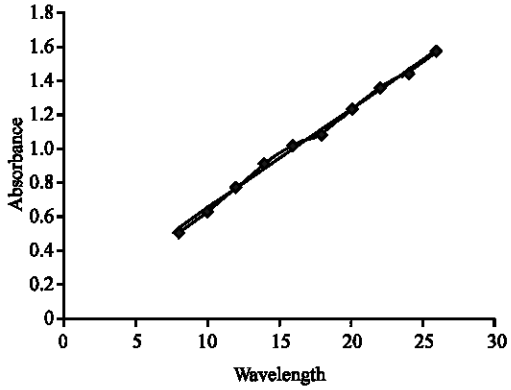

Fig. 2: Calibration curve of enalapril at 209 nm

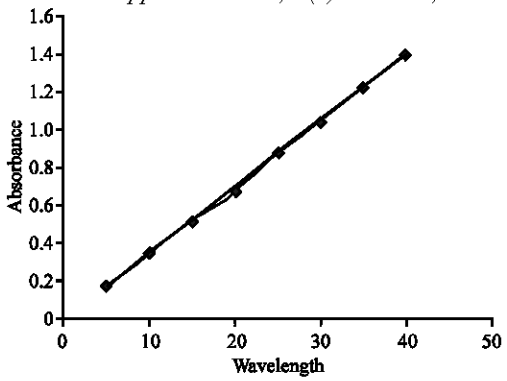

Fig. 3: Calibration curve of amlodipine at 238 nm

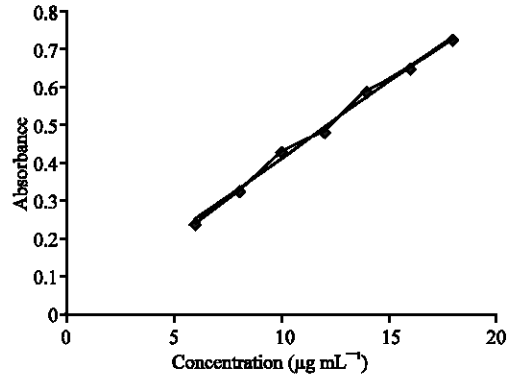

Fig. 4: Calibration curve of amlodipine and enalapril at 219 nm

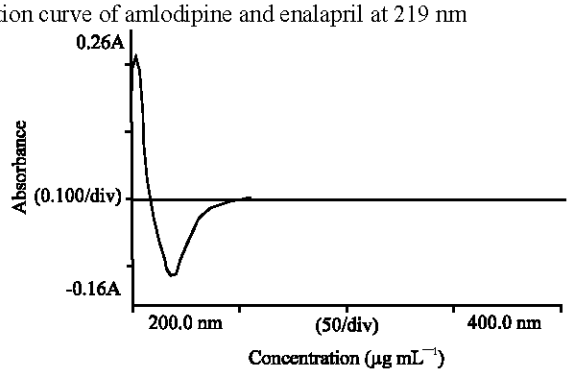

Fig. 5: First order derivative spectra of enalapril maleate

$$A_2 = 19.61C_{ena} + 310.12C_{amlo} \text{ ---(at } \lambda_{238}) \quad (2)$$

A_1 and A_2 are absorbances at 209 nm and 238 nm, respectively and C_{ena} and C_{amlo} are concentrations of enalapril maleate and amlodipine 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 colleques 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.

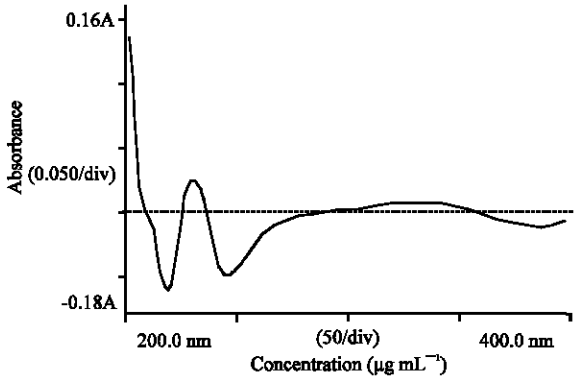

Fig. 6: First order derivative spectra of amlodipine besylate

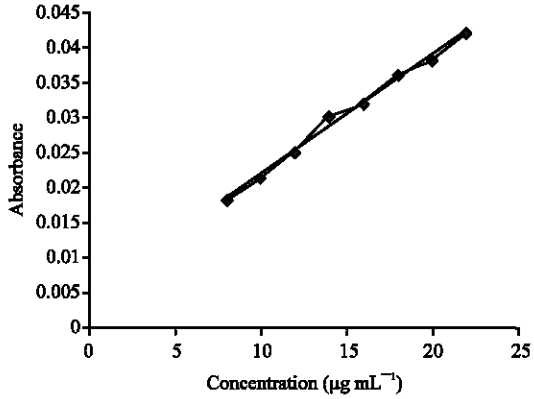

Fig. 7: Calibration curve of enalapril at 227 nm

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:

$$A_1 = 411.25\ (Cx + Cy) = 411.25\ Cx\ (0.7.64/Qm - 0.7540) \quad (3)$$

Where, Qm is A_2/A_1 and A_1, A2 are the absorbances at 219 and 238 nm, respectively. Cx and Cy are concentrations of enalapril and amlodipine resprectively.

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.

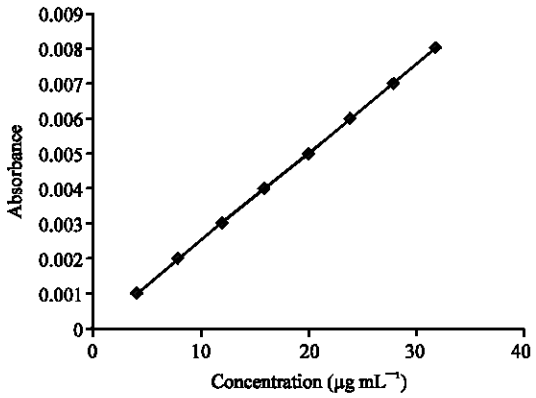

Fig. 8: Calibration curve of amlodipine at 327.5 nm

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

Methods	Tablet brand	Tablet composition	Label claim* mg/tab	Recovery mg/tab±SD* (%)	SE*	RSD* (%)	Percentage range of error with in 95% confidence limit*
I	A	Enalapril	5	99.06±0.204	0.0021	0.022	±0.0147
		Amlodipine	5	99.15±0.069	0.0049	0.068	±0.0249
	B	Enalapril	5	98.94±0.204	0.0037	0.169	±0.0368
		Amlodipine	5	98.97±0.139	0.0058	0.107	±0.0247
II	A	Enalapril	5	99.68±0.025	0.0061	0.101	±0.0879
		Amlodipine	5	99.22±0.098	0.0089	0.087	±0.1354
	B	Enalapril	5	99.16±0.164	0.0341	0.046	±0.0312
		Amlodipine	5	99.32±0.179	0.0267	0.125	±0.3809
III	A	Enalapril	5	99.78±0.011	0.0129	0.139	±0.1204
		Amlodipine	5	99.34±0.057	0.0127	0.019	±0.3697
	B	Enalapril	5	99.88±0.031	0.0381	0.016	±0.0197
		Amlodipine	5	99.87±0.042	0.0324	0.036	±0.2570

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

Table 2: Compilation of results of drug recovery study

		Enalapril maleate		Amlodipine besylate	
Methods	Tablet brand	Recovery (%)*	Standard deviation*	Recovery* (%)*	SD
I	A	99.04	0.0169	99.36	0.0099
	B	98.99	0.0205	99.03	0.0108
II	A	98.74	0.0378	98.79	0.0357
	B	98.89	0.0245	99.11	0.0174
III	A	99.91	0.0109	99.29	0.0009
	B	99.87	0.0062	99.92	0.0014

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

$$y = 0.0017x + 0.0054 \text{ --- (At } \lambda_{227}) \quad (4)$$

$$y = 0.0003x + 0.0004 \text{ --- (At } \lambda_{327.5}) \quad (5)$$

Where, x is the concentration in μg mL^{-1}, y is the peak amplitude of the first-derivative curves at 227 and 327.5 nm for enalapril and amlodipine, respectively.

Estimation from Tablets

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

excipients were separated by filtration. The supernatant liquid was transferred to 100 mL volumetric flask and the volume made up with methanol. The solution so obtained was suitably diluted with methanol so that the concentration (10 μg mL^{-1}) can be read directly from the calibration curve and the absorbances were taken at different wavelengths as stated above. Using the Eq. (1-5) the concentrations were determined (Table 1).

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 (isoabsorptive 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.

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