RESEARCH ARTICLE

Reversed phase liquid chromatographic conditions for simultaneous determination of antihypertensive formulations

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Rapid and simple reversed phase chromatographic conditions have been developed to separate pharmacologically active components of antihypertensive drug formulations (enalapril maleate + amlodipine besilate and ramipril + hydrochlorothiazide) on high-performance liquid chromatography (HPLC) and thin layer chromatography. The mobile phase consists of methanol–water (60:40 v/v) for reversed-phase thin layer chromatography (RPTLC). Detection was carried out by iodine vapors. The HPLC method was developed on a C-18 column with detection carried out by a UV detector at 215 and 220 nm for enalapril maleate + amlodipine besilate and ramipril + hydrochlorothiazide, respectively. The HPLC method was validated using data elements specificity, linearity and range, and accuracy and precision.

Key words: *Antihypertensive drug formulations, high-performance liquid chromatography and RPTLC, validation*

INTRODUCTION

Angiotensin-converting enzyme (ACE) inhibitor antihypertensive drugs are used to prevent and cure hypertension and congestive heart failure. In certain cases, single ACE inhibitor drugs do not respond sufficiently to reduce hypertension. Hence, these are used as combined dosage forms with other specific classes of drug compounds such as diuretics, calcium channel blocker antihypertensives, etc. ACE inhibitor enalapril maleate combined with amlodipine (calcium channel blocker antihypertensive) is used for the treatment of refractory hypertension coexisting with diabetes mellitus. Similarly, hydrochlorothiazide (diuretics) combined with ramipril increased the antihypertensive effect of the latter. Various analytical techniques have been applied frequently in the determination of the above drugs. Spectrophotometric methods have been reported for the assay of these drugs in commercial dosage forms.[1-5] Chromatographic methods such as gas chromatography (GC)[6] and high-performance liquid chromatography (HPLC)[7,8] have been developed for the analysis of ACE inhibitors in biological fluids as well as in pharmaceutical formulations. A liquid chromatographic method has been developed for the simultaneous determination of ramipril and hydrochlorothiazide in their dosage form using an LC-8 column.[9] Thin layer chromatographic (TLC) conditions for certain antihypertensive agents were developed on aminoplast layers with benzene–cyclohexane–methyl ethyl ketone (15:10:15 v/v) as the mobile phase.[10] The behavior of five ACE inhibitors and their active degradation products was studied in salting out chromatography on silica gel, cellulose, and polyacrylonitrile (PAN) with aqueous $(NH_4)_2SO_4$ solution in various concentrations as the mobile phase.[11] Liquid chromatographic separation using reversed-phase HPLC, column, and normal phase TLC of certain antihypertensive agents was carried out by Bhushan *et al*.[12] Silica gel and PAN sorbent layers have been reported for their TLC separation.[13] RP-18 silica gel plates have also been employed for the qualitative separation of these drugs.[14]

Literature suggests that although there are many methods of liquid chromatographic determination of single-dosage forms of antihypertensive drugs, very few analytical HPLC procedures for the simultaneous determination of combined dosage forms have been reported. Furthermore, there is no report on reversed-phase thin layer chromatography (RPTLC) of such formulations. Hence, in the present paper, RPTLC and HPLC conditions have been worked out for the analysis of two commercial formulations of antihypertensives viz. ramipril + hydrochlorothiazide and enalapril maleate + amlodipine besilate, which will be helpful in the determination of the above combinations in quality

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control laboratories. The HPLC method has been validated as discussed elsewhere.[15]

MATERIALS AND METHODS

Chemicals and reagents

All the solvents, e.g. methanol, liquid paraffin, chloroform, and acetone, used for RPTLC were of analytical grade from E Merck, Bombay, India. Dihydrogen potassium phosphate, dipotassium hydrogen phosphate, dihydrogen sodium phosphate, disodium hydrogen phosphate, and phosphoric acid used in buffer solution were also of analytical grade, from E Merck. TLC was performed on silica gel G (E Merck) containing calcium sulfate (13%), iron, and chloride (0.03% each). Drug formulations Amace (enalapril maleate 5 mg and amlodipine besilate 5 mg) and Cardace-H (ramipril 5 mg and hydrochlorothiazide 12.5 mg) were purchased from Systopic, J & K, India, and Aventis, Delhi, India, respectively. Acetonitrile used for HPLC was of HPLC grade. Reference standards of enalapril maleate, amlodipine besilate, hydrochlorothiazide, and ramipril for HPLC were kindly provided by Roorkee Research and Analytical Labs Pvt. Ltd., Roorkee, India.

RPTLC

Sample preparation

Crushed tablets of enalapril maleate, amlodipine besilate, and ramipril were extracted with methanol. Insoluble excipients were separated by filtration. The filtrate was evaporated to dryness and crystals were obtained. Purity checking of the above samples was carried out by melting point and spectra of the crystals. Standard solutions (10^{-2} M) of enalapril maleate, amlodipine besilate, and ramipril were prepared from the above-purified crystals. At the same time, a suitable number of crushed tablets of enalapril maleate (5 mg) + amlodipine besilate (5 mg) (sample A) and ramipril (5 mg) + hydrochlorothiazide (12.5 mg) (sample B) were extracted with a known volume of methanol and then filtered. The filtrate was used for spotting.

Chromatography

Plain thin silica gel layers were prepared by spreading the slurry of silica gel G (20 g) in distilled water (40 ml) with a stahl-type applicator and then drying them overnight at $60 \pm 2°$ in an oven. The reversed phase was obtained by impregnation of these silica gel layers in 5% paraffin liquid in chloroform for 18 h. Standard solutions of individual drugs were spotted on silica gel layers using capillaries along with the filtrate prepared from samples A and B. Chromatograms were developed up to 8-10 cm at room temperature in the mobile phase. Iodine vapors were used for detection.

HPLC

Apparatus

Quantitative HPLC was performed on gradient HPLC (Shimadzu Prominence, Japan) with pump LC20AT, UV detector SPD-20A, Luna C18 column, and LC solution software.

Chromatographic conditions

The mobile phases were acetonitrile–phosphate buffer (pH 6.5) (50:50 v/v) for sample A and acetonitrile–lans buffer (pH 6.8) (50:50 v/v) for sample B. They were filtered before use through a 0.22 μm membrane filter. The column flow rate was 1.0 and 0.8 ml/min for samples A and B, respectively, and the column temperature was 40°C. The volume of the injection loop was 20 μl.

Standard solutions

For HPLC analysis, the mixture of the standards was accurately weighed using an analytical balance (readability 0.001 mg). The weighed samples were dissolved in diluents. It was sonicated for 10 min and made up to 100 ml with the diluent to produce a stock solution containing 1.0 mg/ml (w/v) of enalapril maleate, 1.0 mg/ml (w/v) of amlodipine besilate, 0.5 mg/ml (w/v) of ramipril, and 1.5 mg/ml (w/v) of hydrochlorothiazide. Aliquots of the stock solutions were suitably diluted with the diluent to produce calibration solutions of suitable concentrations.

Sample preparation

Ten tablets of each formulation, viz. samples A and B, were weighed and finely powdered. The desired quantity was dissolved in 25 ml of the mobile phase, sonicated for 10 min, and filtered through a 0.22 μm membrane into a 25 ml volumetric flask. The residue in the filter was washed with the mobile phase and made up to the desired volume. The stock solution was suitably diluted to obtain the desired concentrations.

RESULTS AND DISCUSSION

Earlier work shows use of readymade silica gel plates for the quantitative determination of individual antihypertensive drugs. However, there is no report available for the separation of the chosen combinations on RPTLC. Therefore, efforts were made to separate the combinations of enalapril maleate + amlodipine besilate and ramipril + hydrochlorothiazide. Various proportions of the mobile phase methanol–water were employed. Finally, the mobile phase methanol–water (60:40, v/v) provided best separation results for sample A as well as for sample B. It was observed that as the amount of water increased, the retardation factor decreased. This behavior showed the lipophilic character of the drugs. Because both combinations have been separated in the mo0bile phase methanol–water (60:40, v/v), it can be recommended for the separation of all of the above drugs. The hR_F values have been given in Table 1.

Table 1: hR_F values of the selected antihypertensive drugs

Name of the drug	hR_F
Enalapril maleate	77
Amlodipine besilate	36
Ramipril	69
Hydrochlorothiazide	86

Note: $HR_F = R_F \times 100$

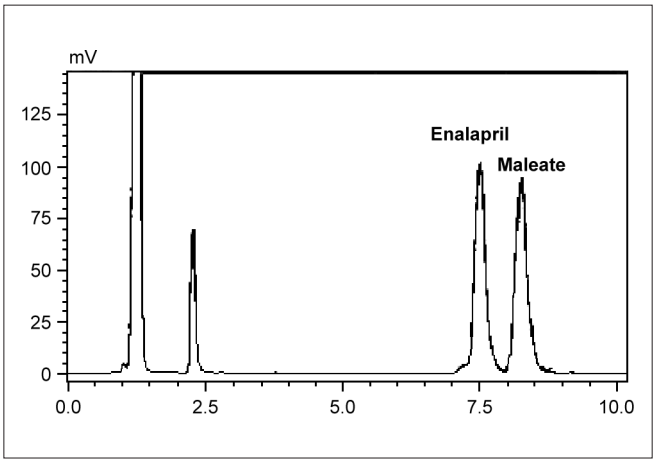

Figure 1: Chromatogram for separation of amlodipine besilate (Rt = 2.4) and enalapril maleate (Rt = 6 – 9) (sample A) (Rt = retention time)

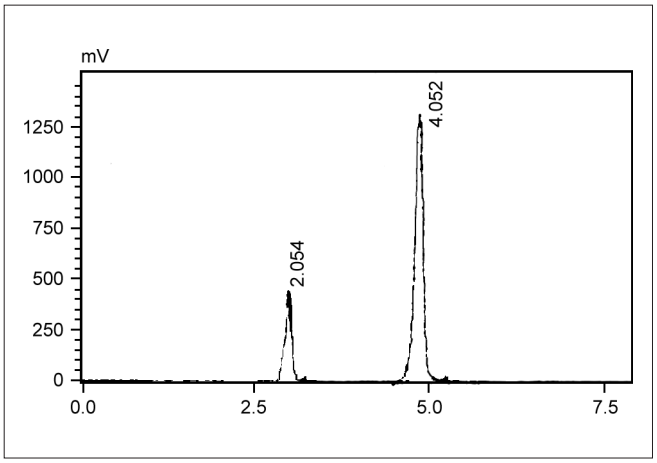

Figure 2: Chromatogram for separation of ramipril (Rt = 2.9) and hydrochlorothiazide (Rt = 4.8) (sample B)

HPLC conditions were also worked out for these drug formulations. Acetonitrile–phosphate buffer (pH 6.5) (50:50, v/v) was used as the mobile phase to separate sample A. The flow rate was adjusted to 1 ml/min and detection was carried out by a UV detector at a wavelength of 215 nm. For sample B, acetonitrile–lans buffer (pH 6.8) (50:50, v/v) was used as the mobile phase (lans buffer is prepared by dissolving 1.3 g of KH_2PO_4 and 0.5 g of K_2HPO_4 in 1000 ml of distilled water). Detection was carried out at a wavelength of 220 nm with a flow rate of 0.8 ml/min. Retention time for individual drug components can be determined from the HPLC chromatogram. The chromatogram has been shown in Figures 1 and 2.

Specificity, linearity and range, and precision and accuracy are the data elements that were studied for validation.

Specificity

All samples were analyzed in the presence of various excipients commonly used in dosage forms and compared with data of the reference solution. The above excipients did not interfere with the assay procedure as no interfering peaks were found in the chromatogram. Hence, the method is specific in the presence of excipients in commercial tablets.

Linearity and range

The calibration curve for these drug combinations was constructed by plotting the response versus concentration graph. Linearity was evaluated by regression analysis Amlodipine besilate and enalapril maleate showed the linearity in the range of 100-510 μg/ml. Ramipril and hydrochlorothiazide showed the linearity in the range of 50-250 and 90-450 μg/ml, respectively. The correlation coefficient for these compounds ($R^2 > 0.998$) suggests that the method provides excellent correlation between peak area and response.

Precision

The method was investigated for interday precision in similar conditions at different concentrations for each drug. Method precision was performed by seven different solutions of the same concentration. Each solution was injected in triplicate under same conditions in interdays and the mean value of the peak area response for each solution was taken. The relative standard deviation based on the response of seven triplicate injections was found to be 0.51, 0.519, 0.518, and 0.53% for enalapril maleate, amlodipine besilate, ramipril, and hydrochlorothiazide, respectively, which is less than 2%. Therefore, the above data indicate that the developed HPLC method is precise.

Accuracy

Accuracy of the method was determined by recovery experiments. The recovery studies for both the combinations carried out at the three levels, each in triplicate, and the percentage recovery were 99.65, 99.59, 100.35, and 100.93% for enalapril maleate, amlodipine besilate, ramipril, and hydrochlorothiazide, respectively. Recovery was within the range of 100 ± 2%, which indicates accuracy of the method.

CONCLUSION

The developed RPTLC method on homemade silica gel layers for the separation of the two combinations is rapid and cost effective. As expected, the mobile phase developed for RPTLC was more polar than that for RPHPLC. Application of the HPLC method has permitted the determination of each drug component with high recovery. The designed HPLC procedure fulfills the validation requirement and could be applied for the analysis of other similar formulations of the above drugs.

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