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Research Article

RP-HPLC METHOD OF SIMULTANEOUS ESTIMATION OF ENALAPRIL MALEATE AND LERCANIDIPINE HCl IN SYNTHETIC MIXTURE

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ABSTRACT

A reversed-phase liquid chromatographic (RP-HPLC) method was developed for the simultaneous determination of Enalapril Maleate (EM) and Lercanidipine HCl (LH) in synthetic mixture. The analysis was carried out using kromasil C18, pre-packed column. Mobile phase, containing methanol:water:triethylamine (70:30:0.2) adjusted to pH 3.8 with orthophosphoric acid, was pumped at a flow rate of 1.0 mL/min with UV-detection at 209 nm. Retention time was 3.66 min and 7.42 min for Enalapril Maleate (EM) and Lercanidipine HCl (LH), respectively. The method was validated for linearity, accuracy, precision, and specificity. The method showed good linearity in the range of 20.0–45.0 µg/mL for both drugs. The detection limit of the proposed method was 0.46 and 0.19 µg/mL and the quantification limit was 1.4 and 0.58 µg/mL for Enalapril Maleate (EM) and Lercanidipine HCl (LH), respectively. The % recovery was within the range between 98.18% and 101.2% for Enalapril Maleate (EM) and % recovery was within the range between 99.8% and 100.94% for Lercanidipine HCl (LH). The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Enalapril Maleate (EM) and Lercanidipine HCl (LH) in synthetic mixture as well as in tablet dosage form.

Keywords: Enalapril Maleate, Lercanidipine HCl, RP-HPLC.

INTRODUCTION

Enalapril maleate (EM) is an ACE inhibitor used in the treatment of hypertension and heart failure. Enalapril shows its activity to enalaprilat to which it is converted after oral administration[1]. EM is the subject of a monograph in the Indian Pharmacopoeia(IP)[2], the United States Pharmacopoeia (USP)[3] and the British Pharmacopoeia (BP)[4]. The IP and USP recommends an HPLC method for the raw material and tablets with UV detection at 210nm while the BP recommends an aqueous titration for the raw material with sodium hydroxide and potentiometric end point detection and an HPLC method for the tablets. The therapeutic importance of EM initiated several reports for its determination, both in formulations and in biological fluids, viz: spectrophotometry[5–8], polarography[9], several HPLC methods[10], fluorimetry[11], flow injection chemiluminescence method[12], stripping voltammetry[13], and capillary electrophoresis.

Lercanidipine HCL (LH) is a dihydropyridine calcium-channel blocker used in the treatment of hypertension. LH is given by mouth as the hydrochloride in a usual initial dose of 10mg once daily before food intake, and increased if necessary[1]. There are several reports on the determination of LH, viz: spectrophotometry[14-16], voltammetry[17,18], HPLC methods[19–21] and capillary electrophoresis[22]. LH is a good option to

combine with angiotensin converting enzyme inhibitors to optimize control of blood pressure, even in patients with other cardiovascular risk factors. It has an agonistic effect, decreasing high blood pressure without increasing adverse events. LH and EM are established antihypertensive agents[23]. EM/LH 10mg/10mg or 20mg/10 mg, once daily, significantly reduced sitting diastolic blood pressure and sitting systolic blood pressure, relative to 10mg LH once daily. Fixed-dose LH/EM was generally well tolerated, with a tolerability profile similar to that of either of the individual drugs alone or placebo[24].

To the best of our knowledge, no HPLC method has been yet described for the simultaneous determination of the binary mixture of EM and LH. The aim of the present work is to develop a feasible, sensitive, and specific HPLC method for the analysis of the investigated drugs. Adaptation of the proposed procedures to the analysis of the synthetic mixtures is also an important task. Comparison of the suggested method is also investigated against reported methods.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of Analytical Reagent grade, and the solvents were of HPLC grade. Enalapril Maleate and Lercanidipine HCL standards were obtained from Torrent Pharma, Ahmedabad, India. HPLC grade water, methanol, orthophosphoric acid, and triethylamine (TEA) were purchased from S.D. Fine Chemicals, Mumbai, India.

Apparatus

Separation was performed with a Shimadzu LC 2010 CHT equipped with a Rheodyne injector valve with a 20.0 µL loop and a UV/VIS detector operated at 209 nm. LC solution software was applied for data collecting and processing. A Chemiline pH-meter was used for pH measurements.

Chromatographic Conditions

Kromasil C18 column (250mm x 4.6mm 5µ) was used in this study. The mobile phase was methanol:water:triethylamine (70:30:0.2) adjusted to pH 3.8 with orthophosphoric acid The Flow rate was 1.0 mL/min and UV detection was performed at 209 nm by UV detector and PDA detection at 209 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45-mm membrane filter (Millipore, Ireland).

Preparation of Solutions

Stock solutions containing 1.0 mg/mL of EM and LH were prepared in methanol and were used as working solutions (1000 µg/mL). Solutions were protected from light and were found to be stable for at least one week when kept in the refrigerator.

Study of Experimental Parameters

Different experimental parameters including, mobile phase composition, detection wavelength, and flow rate were intensively studied in order to specify the optimum conditions for the assay procedure. Variables were optimized by changing each, in turn, while keeping all others constant.

Construction of the Calibration Curve

Aliquots of the standard solutions covering the final working concentration range of 20.0–45.0 µg/mL for EM and LH were transferred into a series of 10mL volumetric flasks and diluted with the de-gassed mobile phase to the mark. 20 µl aliquots were injected (n=6) and eluted with the mobile phase under the reported chromatographic conditions. The calibration curves were constructed by plotting the peak area against the final concentration of the drug (µg/mL). Alternatively, the corresponding regression equations were derived.

Analysis of EM and LH Synthetic Mixtures

Synthetic Mixture containing EM 10 mg and LH 10 mg in combination were prepared in laboratory by using suitable excipients (prepared by mixing 10.0 mg EM, 10.0mg LH, 5.0 mg talc powder, 20.0 mg maize starch and lactose (excipient) to 200 mg tablet). The ratio of EM and LH in the samples was taken 1:1. For analysis, the drug mixture containing 10mg EM and 10mg LH shaken vigorously with de-gassed mobile phase for 15min.Then solution was filtered through a 0.45-mm membrane filter and then final volume of the solution was made upto 100ml with methanol to get the stock solution containing 100 µg/ml of EM and LH. Appropriate aliquots of EM and LH were taken within linearity range. The concentration of both drugs was determined using either the calibration curve or the corresponding regression equation.

VALIDATION

The method was validated for assay of EM and LH in accordance with ICH guidelines[25].

Linearity

In order to check the linearity for the developed method, solutions of six different concentrations ranging from 20.0-45.0 µg/ml were prepared for both drugs. The chromatograms were recorded and the peak areas were given in Table 1. A linear relationship between areas versus concentrations was observed in above linearity range. This range was selected as linear range for analytical method development for estimation of EM and LH.

Sensitivity

The sensitivity of measurement of EM and LH using the proposed method was estimated as the limit of quantification (LOQ) and the lowest concentration detected under these chromatographic conditions as the limit of detection (LOD). The LOD and LOQ were calculated by using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drug (n = 6), and B is the slops of the corresponding calibration plot. The limits of detection and quantification for EM were 0.46 µg/mL and 1.4 µg/mL respectively and those for LH were 0.19 µg/mL and 0.58 µg/mL respectively.

System suitability

Various system suitability parameters were also calculated. It was observed that all the values are within the limits which is shown in Table 2. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of LH and EM in tablet formulation. The results are furnished in Table 3.

Precision

Precision was measured by analysis of sample solutions six times at three different concentrations. Solutions containing 20, 30 and 40 µg/ml of EM and LH were subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table 4 and 5.

Accuracy

The accuracy of the method was determined by analysis of standard additions at three levels, i.e. multiple-level recovery studies. Reference standard at three different concentrations (80, 100, and 120%) was added to a fixed amount of pre-analyzed sample and the amounts of the drug were analyzed by the proposed method. Results from the recovery studies are given in Table 6.

Stability

The stability of LH and EM in standard and sample solutions containing determined by storing the solutions at ambient temperature (20±10^{0}C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48hrs, as during this time the results did not decrease below 98%. This denotes that LH and EM are stable in standard and sample solutions for at least 2days at ambient temperature.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

The RP-HPLC procedure was optimized with a view to develop accurate and stable assay method with the pure drugs EM and LH in a Synthetic mixture. Kromasil C18 (250mm x 4.6mm 5µ) column in isocratic mode, with mobile phase methanol:water:triethylamine (70:30:0.2) adjusted to pH 3.8 with orthophosphoric acid. The flow rate was 1mL/min and identical components were measured with PDA Detector at 209 nm. Linearity was assessed by plotting concentration vs. area which is shown in Fig 2 and Fig 3 respectively with is linear in the range of 20.0 – 45.0 µg/ml for EM and 20.0 – 45.0 µg/ml for LH with correlation coefficient 0.9992 and 0.9998 respectively with good linearity response greater than 0.998. The % recovery was found to be within limits of the acceptance criteria with recovery range 98.18% - 101.2% for EM and 99.8 - 100.94% for LH. The %RSD for intra-day and Inter-day precision is less than 2% for EM and LH. The detection

limit of the proposed method was 0.46 and 0.19 µg/mL, and the quantification limit was 1.4 and 0.58 µg/mL for Enalapril Maleate (EM) and Lercanidipine HCl (LH), respectively.Typical chromatogram of the sample is shown in Fig 5.The assay procedures were repeated for six times and the results were found to give 100.53% of EM and 99.46% of LH.

CONCLUSION

The Proposed study describes new and simple RP-HPLC method for the estimation of Enalapril Maleate and Lercanidipine HCL in synthetic mixture. The method was validated and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be used for quantification of Enalapril Maleate and Lercanidipine HCL in synthetic mixture as well as for routine analysis in quality control.

Table 1: Linearity data for EM and LH

S. No.	Concentration (µg/ml)	Mean * Peak Area of EM	Mean* Peak Area LH
1	20	1477956	2469907
2	25	1910340	3054029
3	30	2258412	3683817
4	35	2692906	4235779
5	40	3048749	4845028
6	45	3468662	5434644

*Average of six determinations

Table 2: System Suitability parameters for EM and LH

Parameter	EM	LH
λ_{max}	209 nm	209 nm
Linearity range(µg/mL)	20.0-45.0	20.0-45.0
Correlation coefficient (R^2)	0.9996	0.9991
Retention time (RT)	3.66 min	7.42 min
Theoritical plates	1975	2473
Capacity factor	0.59	2.17
Tailing factor	1.06	1.1
Resolution	3.31	6.15
Slope	78875	118557
Intercept	-87290	100900
LOD(µg/mL)	0.46	0.19
LOQ(µg/mL)	1.4	0.58

Table 3: Results of analysis of Synthetic mixture

Syntetic mixture	(Mean ±% R.S.D.)	
	EM	LH
%Conc. Estimated*	100.53±0.76	99.46±0.65

*Average of six determinations;
R.S.D.; Relative Standard Deviation.

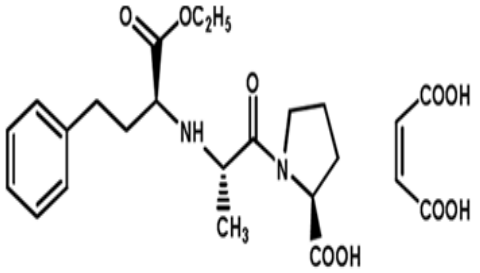

Fig. 1(A): Chemical structure of Enalapril Maleate

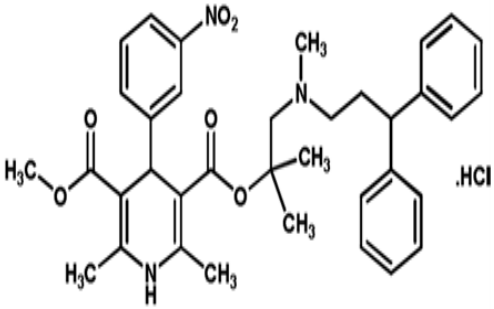

Fig. 1(B): Chemical structure of Lercanidipine Hydrochloride

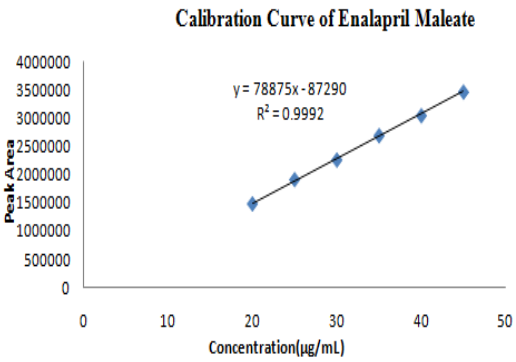

Fig. 2: Calibration Curve of Enalapril Maleate

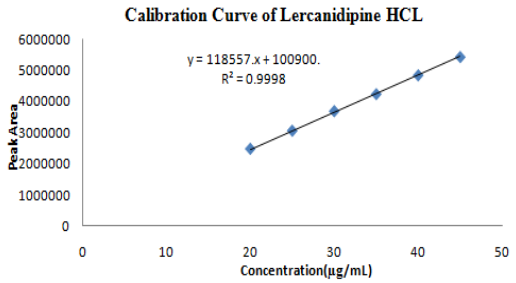

Fig. 3: Calibration Curve of Lercanidipine HCl

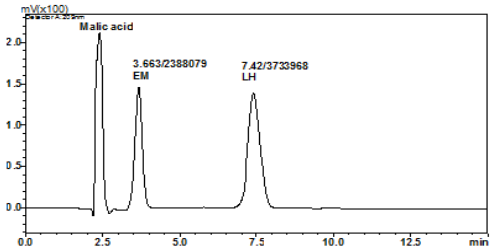

Fig. 4: Chromatogram obtained from Enalapril and Lercanidipine standard

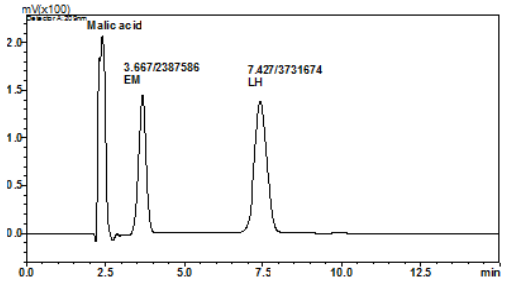

Fig. 5: Chromatogram obtained from Enalapril and Lercanidipine in synthetic mixture

Table 4: Intraday precision data for estimation of EM and LH

Conc. (µg/mL)	EM		LH		
	Peak Area Mean ± S.D. (n=6)	%RSD	Conc. (µg/mL)	Peak Area Mean ± S.D. (n=6)	%RSD
20	1470715±11087	0.7	20	2467239±6967	0.23
30	2270094±9729	0.42	30	3679636±19327	0.5
40	3005958±25269	0.81	40	4836252±22006	0.4

Table 5: Interday precision data for estimation of EM and LH

Conc. (µg/mL)	EM		LH		
	Peak Area Mean ± S.D. (n=6)	%RSD	Conc. (µg/mL)	Peak Area Mean ± S.D. (n=6)	%RSD
20	1474715±16593	1.12	20	2467733±24885	1.0
30	2279864±31426	1.37	30	3675568±47678	1.30
40	3011347±38858	1.29	40	4850523±53573	1.10

Table 6: Result from recovery studies

Spike level [%]	Mean recovery [%] (n=6)		RSD [%]	
	EM	LH	EM	LH
80	101.2	100.94	0.3	0.82
100	98.18	99.8	0.43	0.37
120	99.85	100.65	0.6	0.17

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