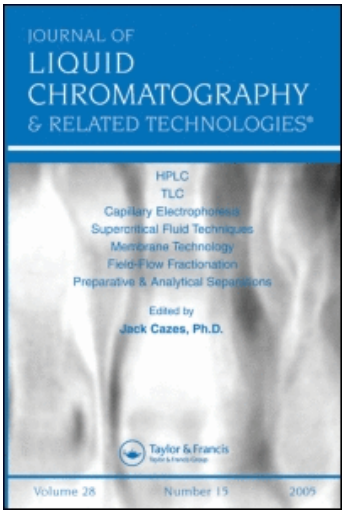

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SIMULTANEOUS *IN VITRO* HPLC DETERMINATION OF ENALAPRIL MALEATE AND LERCANIDIPINE HCL

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SIMULTANEOUS *IN VITRO* HPLC DETERMINATION OF ENALAPRIL MALEATE AND LERCANIDIPINE HCL

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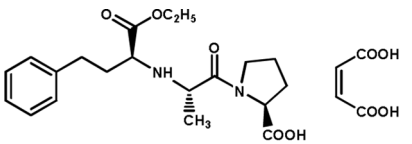
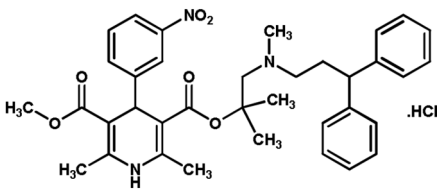
□ *A reversed-phase liquid chromatographic (RP-HPLC) method was developed for the simultaneous determination of a binary mixture of enalapril maleate and Lercanidipine HCl in pure form, synthetic mixture, and synthetic tablets. The analysis was carried out using Hibar® C18, pre-packed column. Mobile phase, containing acetonitrile:methanol:water:orthophosphoric acid (35:35:30:0.2) adjusted to pH 3.0 with triethylamine, was pumped at a flow rate of 1.0 mL min^{-1} with UV-detection at 210 nm. The method showed good linearity in the range of 5.0–100.0 μg mL^{-1} for both drugs. The detection limit of the proposed method was 0.80 and 0.93 μg mL^{-1}, and the quantitation limit was 2.42 and 2.80 μg mL^{-1} for ENM and LER, respectively.*

Keywords enalapril maleate, HPLC, lercanidipine HCl

INTRODUCTION



Enalapril maleate, $C_{20}H_{28}N_2O_5,C_4H_4O_4$



Lercanidipine Hydrochloride; $C_{36}H_{41}N_3O_6$,HCl

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Enalapril maleate (ENM) is an ACE inhibitor used in the treatment of hypertension and heart failure. Enalapril owes its activity to enalaprilat to which it is converted after oral administration.[1] ENM is the subject of a monograph in both the United States Pharmacopoeia (USP)[2] and the British Pharmacopoeia (BP).[3] The USP recommends an HPLC method for the raw material and tablets with UV detection at 210 nm 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 ENM initiated several reports for its determination, both in formulations and in biological fluids, viz: spectrophotometry,[4–9] fluorimetry,[10] flow injection chemiluminescence method,[11] polarography,[8] stripping voltammetry,[12] several HPLC methods,[9,13–19] and capillary electrophoresis.[20–22]

Lercanidipine HCl (LER) is a dihydropyridine calcium-channel blocker used in the treatment of hypertension. LER is given by mouth as the hydrochloride in a usual initial dose of 10 mg once daily before food intake, and increased if necessary.[1] There are several reports on the determination of LER, viz: spectrophotometry,[23–28] voltammetry,[29,30] HPLC methods,[31–43] and capillary electrophoresis.[44]

LER 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. LER and ENM are established antihypertensive agents.[45] ENM/LER 10 mg/10 mg or 20 mg/10 mg, once daily, significantly reduced sitting diastolic blood pressure and sitting systolic blood pressure, relative to 10 mg LER once daily. Fixed-dose LER/ENM was generally well tolerated, with a tolerability profile similar to that of either of the individual drugs alone or placebo.[46]

To the best of our knowledge, no HPLC method has been yet described for the simultaneous determination of the binary mixture of ENM and LER.

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 and synthetic co-formulated tablets is also an important task. Comparison of the suggested method is also investigated against reported methods.

EXPERIMENTAL

Chemicals

All the chemicals used were of Analytical Reagent grade, and the solvents were of HPLC grade. LER was kindly provided by Recordati Industria Chimica e Farmaceutica S.p.A. via Mediana Cistema, Milan, Italy

(Lot No. 03000630) and was used as received. ENM was purchased from Sigma-Aldrich Chemie (GmbH, Steinheim, Germany, Lot No. 38H0500, EC No. 278-375-7) and was used as received. Acetonitrile, methanol, orthophosphoric acid, and triethylamine (TEA) were purchased from Sigma-Aldrich Chemie (GmbH, Steinheim, Germany).

Apparatus

Separation was performed with a Perkin Elmer™ Series 200 Chromatograph equipped with a Rheodyne injector valve with a 20.0 µL loop and a UV/VIS detector operated at 210 nm. Total Chrom Workstation (Massachusetts, USA) was applied for data collecting and processing. Mobile phase was degassed using Merck solvent L-7612 degasser. A Consort P-901 pH-meter was used for pH measurements.

Chromatographic Conditions

Hibar® C18, pre-packed column RT (150 mm × 4.6 mm ID, Lichrosorb® RP-18 5 µm particle size) was used in this study. The mobile phase was acetonitrile:methanol:water:orthophosphoric acid [35:35:30:0.2] adjusted to pH 3.0 with TEA. The Flow rate was 1.0 mL min^{-1} and UV detection was performed at 210 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45-µm membrane filter (Millipore, Ireland).

Preparation of Solutions

Stock solutions containing 1.0 mg mL^{-1} of ENM and LER were prepared in methanol and were used as working solutions (1000 µg mL^{-1}). Solutions were protected from light and were found to be stable for at least one week when kept in the refrigerator.

METHOD

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 Graphs

Aliquots of the standard solutions covering the final working concentration range of 5.0–100.0 $\mu g\,mL^{-1}$ for ENM and LER were transferred into a series of 10 mL volumetric flasks and diluted with the de-gassed mobile phase to the mark. Twenty microliter aliquots were injected (in triplicate) 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 ($\mu g\,mL^{-1}$). Alternatively, the corresponding regression equations were derived.

Analysis of ENM and LER Synthetic Mixtures

Aliquots of ENM with LER standard solutions in a 1:1 and 2:1 ratios of ENM:LER were transferred into a series of 10 mL volumetric flasks and diluted with the de-gassed mobile phase to the volume and analyzed as described under construction of the calibration graph. The concentration of both drugs was determined using either the calibration curve or the corresponding regression equation.

Analysis of ENM and LER Synthetic Tablets

Ten synthetic tablets (prepared by mixing 10.0 mg ENM, 10.0 mg LER, 5.0 mg talc powder, 20.0 mg maize starch and lactose (excipient) to 200 mg/tablet) were weighed and powdered. An accurately weighed amount of the powder equivalent to 25.0 mg of ENM and LER was transferred into a conical flask, 15 mL of methanol were added and the solution was sonicated for 10 min then filtered into 25 mL volumetric flask. The conical flask was washed with a few milliliters of methanol; the washing was passed into the same volumetric flask which was then completed to the volume with methanol to give a working solution of 1.0 $mg\,mL^{-1}$. Aliquots covering the final concentration range (5.0–100.0 $\mu g\,mL^{-1}$) were transferred into 10.0 mL volumetric flasks. The general procedure was then applied as under construction of calibration graph, and the nominal content of tablets was determined either from a previously plotted calibration graph or using the corresponding regression equation.

RESULTS AND DISCUSSION

Development and Optimization of Method

The chromatographic experimental variables affecting the peak shape and retention of both drugs ENM and LER were carefully studied in order

to obtain the most suitable chromatographic conditions that provide the least peak broadening and symmetry as well as satisfactory resolution of both drugs. Optimization of separation was approved by measuring the highest number of theoretical plates and the best resolution factor.

Optimization of the Chromatographic Performance and System Suitability

Two symmetrical peaks (Figure 1) for both drugs were obtained after optimization of the following variables:

UV-Detection

The UV absorption spectrum of ENM in methanol showed one maxima at 210 nm, while that of LER in methanol showed four maxima at 355, 237, 219, and 206 nm (Figure 2). 210 nm was chosen for the detection to assure the highest sensitivity with a reasonable response for both ENM and LER.

Mobile Phase

Several modifications in the mobile phase composition were performed in order to study the possibilities of improving the efficacy of the chromatographic system and resolution of the eluted peaks. These modifications

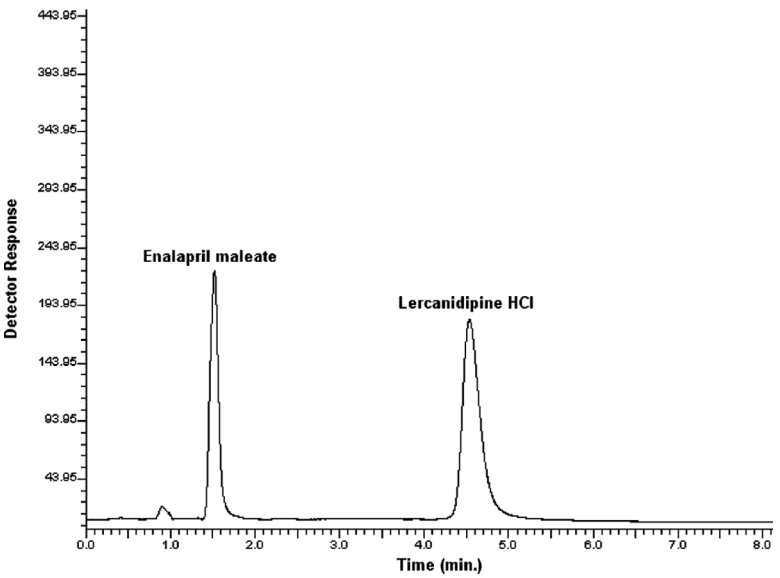

FIGURE 1 Typical chromatogram of ENM (50.0 μg mL^{-1}) and LER (50.0 μg mL^{-1}) under the optimum chromatographic conditions.

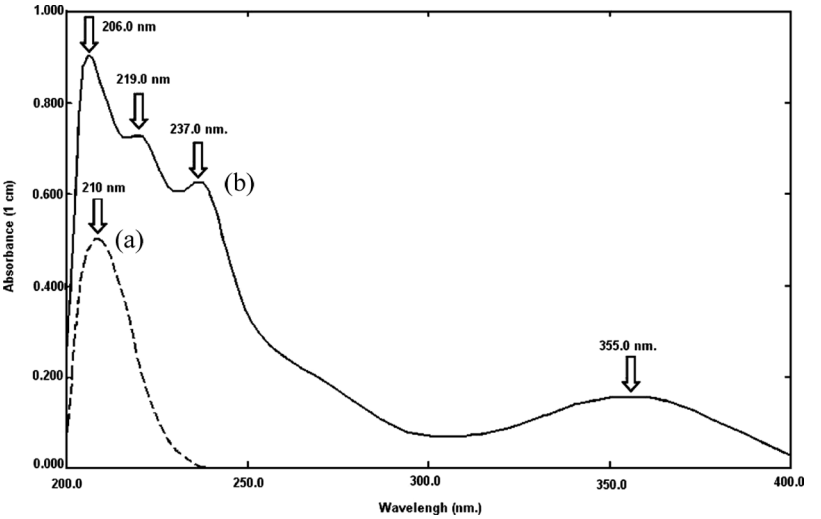

FIGURE 2 Absorption spectra of ENM ($10.00\,\mu g\,mL^{-1}$) (a) and LER ($16.00\,\mu g\,mL^{-1}$) (b) in methanol.

included changing the proportions of the mobile phase components, and the pH and the flow rate. D_m, R_r, N, and R were measured versus each variable. The optimum chromatographic performance was achieved when using a mobile phase composed of acetonitrile:methanol:water:orthophosphoric acid [35:35:30:0.2], adjusted to pH 3.0 using TEA. A summary of the study could be presented as follows:

Ratio of Mobilephase Components

As shown in Table 1, different ratios were tried and the optimum ratio was found to be 35:35:30:0.2 acetonitrile:methanol:water:orthophosphoric acid, respectively.

However, ENM and LER have octanol:water partition coefficient (*Log P*) values of 2.45 and 6.42,[38,47] respectively. This difference in lipophilicity was expressed as difference in their elution order.

pH of the Mobile Phase

The pH of mobile phase was changed over the range of 2.6–3.4 using TEA. Generally, the retention of both drugs was increased upon increasing the pH. The study revealed that the optimum pH was found to be 3.0 as it provides the best peak shape for both drugs with reasonable resolution. The results were presented in Table 1.

The pK_a values of ENM are 2.97 and 5.35 and of LER is 9.0,[39,48] so they are still in the unionized form up to pH 3.0; thus, the differences in their elution is expressed only as a difference in their lipophilicity.

TABLE 1 Effect of the Chromatographic Conditions on the Chromatographic Performance of the Simultaneous HPLC Determination of ENM and LER

Parameter		Number of Theoretical Plates (N)	
		ENM	LER
Ratio of mobile phase (Acetonitrile:methanol: water:orthophosphoric acid)	**40:35:25:0.2**	1080	1331
	30:35:35:0.2	735	1692
	35:25:40:0.2	1296	1176
	35:35:30:0.2	**1415**	**1947**
	35:30:35:0.2	1299	1626
	35:40:25:0.2	500	1170
pH of the mobile phase	**2.6**	1347	1400
	2.8	1348	1820
	3.0	***1415***	***1947***
	3.2	546	1703
	3.4	463	1245
	2.6	1347	1400
Flow rate of the mobile phase	**0.6**	1081	1625
	0.8	1316	1873
	1.0	***1415***	***1947***
	1.2	1064	1407
	1.4	830	1387
	1.5	756	1315

Flow Rate

The flow rate was changed over the range of 0.6–1.5 mL min^{-1} and a flow rate of 1.0 mL min^{-1} was the optimum for good separation in a reasonable time. The results obtained were shown in Table 1.

Analytical Performance

The peak area-concentration plots for ENM and LER were linear over the concentration range cited in Table 2. Linear regression analysis of the data gave the following equations:

$$A = 38.84 + 28.84\ C \quad (r = 0.9999) \qquad [\text{For ENM}]$$

$$A = 114.01 + 42.49\ C \quad (r = 0.9999) \qquad [\text{For LER}]$$

Where A is Peak area, C is the concentration of the drug (μg mL^{-1}) and r is correlation coefficient.

Statistical analysis[48] of the data illustrated in Table 2, shows acceptable value of the correlation coefficient ($r > 0.999$) of the regression equation, small values of the standard deviation of residuals ($S_{y/x}$), intercept (S_a), and slope (S_b), and small value of the percentage relative standard

TABLE 2 Analytical Data for the Simultaneous HPLC Determination of ENM and LER

Parameters	ENM	LER
Concentration range ($\mu g\,mL^{-1}$)	5.0–100.0	
Correlation coefficient	0.9999	0.9999
Slope	28.8400	42.4900
Intercept	38.8400	114.0100
LOD ($\mu g\,mL^{-1}$)	0.8000	0.9300
LOQ ($\mu g\,mL^{-1}$)	2.4200	2.8000
$S_{y/x}$	11.8500	20.2700
S_a	6.9600	11.9200
S_b	0.1300	0.2200
%RSD	1.0200	0.9900
%Er	0.3200	0.3100

deviation (RSD%) and the percentage relative error (%Er). All these data proved the linearity of the calibration graph.

Validation of the Method

The proposed method was tested for specificity, robustness, linearity, precision, accuracy, limit of quantification (LOQ), and limit of detection (LOD).

Specificity

The specificity of the method was investigated by observing any interference encountered from common tablet excipients. It was shown that these compounds did not interfere with the results of the proposed method.

Robustness

Maintaining the resolution of the two main drug peaks from each using different mobile phase composition and different pH values (Table 1) proves the robustness of the proposed method.

Linearity

Linear regression equations were obtained. The regression plots showed a linear dependence of peak area on drug concentration over the range cited in Table 2. The small values of the RSD% and Er% point out to the low scattering of the points around the calibration curve and high precision of the proposed method.

Accuracy and Precision

To prove the accuracy of the proposed method, the results of the assay of ENM and LER in pure form and synthetic co-formulated tablets were compared with those obtained from the reported comparison

methods.[9,27] Moreover, several synthetic mixtures of ENM and LER in 1:1 and 2:1 ratios were also analyzed. Statistical analysis of the results obtained by the proposed and comparison methods using Student's *t*-test and variance ratio *F*-test showed no significant differences between them regarding accuracy and precision, respectively (Table 3).

The results of the inter-day and intra-day accuracy and precision of the method were summarized in Table 4. The inter-day and intra-day precisions were examined by analysis of ENM and LER in concentrations 20.0, 40.0, and 70.0 $\mu g\,mL^{-1}$ each three times a day for three consecutive days. The precision of the proposed method was fairly high, as indicated by the low values of SD and RSD (%). Also, the inter-day and intra-day accuracy was proved by the low values of Er (%).

The Limit of Quantification (LOQ) and Limit of Detection (LOD)

The limit of quantification (LOQ) and the limit of detection (LOD) were determined according to ICH Q2 (R1) recommendations[49] and the results were abridged in Table 2.

TABLE 3 Application of Proposed HPLC Method for the Simultaneous Determination of ENM and LER in Synthetic Mixtures

	Proposed Method			
	Amount Taken ($\mu g\,mL^{-1}$)			
Drug Determined	ENM	LER	% Found	Reference Method[9]
ENM	**10**	10	100.25	
	30	30	99.34	
	60	60	99.16	
	50	25	100.42	
	40	20	100.92	
	80	40	99.50	
$\overline{X} \pm SD$		99.93 ± 0.70		100.09 ± 0.83
t			0.50 (2.26)	
F			1.41 (5.19)	
	Proposed Method			Reference Method[27]
LER	10	**10**	99.58	
	30	**30**	99.97	
	60	**60**	100.45	
	50	**25**	101.10	
	40	**20**	100.66	
	80	**40**	99.75	
$\overline{X} \pm SD$		100.25 ± 0.58		100.00 ± 0.97
t-value			0.63 (2.26)	
F-value			2.80 (5.19)	

Each result is the average of three separate determinations.
Figures between brackets are the tabulated *t* and *F*-values at ($P = 0.05$).

TABLE 4 Evaluation of the Accuracy and Precision Data of the Proposed HPLC Method for the Simultaneous Determination of ENM and LER

Drug	Amount Added ($\mu g\,mL^{-1}$)	Found ($\mu g\,mL^{-1}$)*	$\overline{X} \pm SD$	RSD (%)	Er (%)
ENM	Intra-day				
	20	19.88	99.69 ± 0.35	0.38	0.20
	40	19.92	99.83 ± 0.91	0.91	0.53
	70	20.01	99.72 ± 0.77	0.77	0.54
	Inter-day				
	20	20.16	99.90 ± 0.80	0.80	0.44
	40	19.86	100.36 ± 0.72	0.72	0.42
	70	19.92	100.21 ± 0.55	0.55	0.32
LER	Intra-day				
	20	19.94	100.43 ± 0.67	0.67	0.39
	40	20.11	99.79 ± 0.89	0.89	0.51
	70	20.21	99.76 ± 0.74	0.74	0.43
	Inter-day				
	20	20.07	100.13 ± 0.55	0.55	0.32
	40	19.90	100.45 ± 0.57	0.57	0.33
	70	20.12	100.33 ± 0.73	0.73	0.42

*Each result is the average of three separate experiments.

TABLE 5 Application of Proposed HPLC Method for the Simultaneous Determination of ENM and LER in Synthetic Coformulated Tablet

	Proposed Method			
	Amount Taken ($\mu g\,mL^{-1}$)	Amount Found ($\mu g\,mL^{-1}$)	Found (%)	Reference Method[9]
Preparation	ENM			
Synthetic tablet (10 mg ENM + 10.0 mg LER/tablet)*	20	20.20	101.00	
	40	40.41	101.03	
	60	59.68	99.47	
	80	80.56	100.70	
	$\overline{X} \pm SD = 100.55 \pm 0.74$			100.15 ± 0.67
	t-value = 1.14 (2.57)			
	F-value = 1.22 (19.16)			
	LER			
	Proposed Method			Reference Method[27]
	20	19.85	99.25	
	40	40.26	100.65	
	60	60.60	101.00	
	80	79.44	99.30	
	$\overline{X} \pm SD = 100.05 \pm 0.91$			100.41 ± 0.76
	t-value = 0.77 (2.45)			
	F-value = 1.43 (9.28)			

*Composition of Tablet: ENM 10 mg, LER 10 mg, talc powder 5 mg, maize starch 20 mg and lactose (excipient) to 200 mg.

Each result is the average of three separate determinations.

Figures between brackets are the tabulated *t* and *F*-values at (P = 0.05).

Applications

Analysis of Synthetic Mixtures

The proposed method was successfully applied to the analysis of ENM and LER in synthetic mixtures containing 1:1 and 2:1 ratios ENM:LER. The average percent recoveries were based on the average of three replicate determinations. The results obtained were in good agreement with those obtained by the comparison methods[9,27] as shown in Table 3.

Analysis of Synthetic Co-formulated Tablets

The proposed HPLC method was applied to the determination of the ENM and LER in their synthetic co-formulated tablets. The results of the proposed method were compared with those obtained using the comparison method.[9,27] Statistical analysis of the results obtained using Student's *t*-test and variance ratio *F*-test (Table 5) revealed no significant difference between the performance of the compared methods regarding the accuracy and precision, respectively.

CONCLUSION

A simple, accurate, and rapid method was developed for the simultaneous determination of ENM and LER in a short chromatographic run (less than 5 minuets). The proposed method was applied for the determination of the two drugs either in pure forms or in synthetic co-formulated tablets. The good validation criteria of the proposed method allow its use in quality control laboratories using a simple chromatographic system. The detection limit of the proposed method was 0.80 and 0.93 $\mu g\,mL^{-1}$ and the quantitation limit was 2.42 and 2.80 $\mu g\,mL^{-1}$ for ENM and LER, respectively.

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