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Newer method Development – Validation of Simultaneously Estimation of Lercanidipine HCl and Enalapril Maleate by RP-HPLC in Bulk and Tablet form

Pedababu Tadigadapa [1], Sujana Kamepalli[2], Surendra Babu Lagu[3*]

[1,3]Adikavi Nannaya University College of Pharmaceutical Sciences, Tadepalligudem-534101, AP, India.
[1,2]Acharya Nagarjuna Unversity College of Pharmaceutical Sciences, Nagarjuna Nagar, Guntur-522510, AP, India.

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ABSTRACT: Lercanidipine HCl tablets are utilized in the treatment of hypertension, functioning as a calcium channel blocker to manage elevated blood pressure. Enalapril Maleate is administered independently to individuals whose hypertension has not been successfully managed with alternative medications. When both drugs, at a dosage of 10 mg, are employed, they exert an impact on smooth muscles, contributing to an efficacious therapeutic outcome. **Aim:** Create an easy, quick, and cheap way to compute the Lercanidipine HCl and Enalapril Maleates together using RP-HPLC. **Method:** A stability indicating RP-HPLC method has been meticulously developed and validated for the estimation of Lercanidipine HCl and Enalapril Maleate in tablet forms. The method was developed using a EZchrome Agilent-1200 by using Kromasil 2 µ ODS C18 (250x4.6 mm, 5 µ) column and the mobile phase containing Acetonitrile: 0.1% TEA PH-2.5/OPA in the ratio of 60:40 v/v. The flow rate was adjusted at 1.0 ml/min. The display wavelength was set at 220 nm. Retention time of Lercanidipine HCl and Enalapril Maleate is revealed as 2.447 min and 4.453 min. **Results:** The ICH regulation Q2-R1 was fulfilled the developed method and validated, values are within range (accuracy, precision and other statistical analysis) **Conclusion:** The proposed method was successfully applied to the tablet form consisting of Lercanidipine HCl and Enalapril Maleate for analysis.

Corresponding author:
Dr. Surendra Babu Lagu
Assistant professor,
AKNU College of Pharmaceutical Sciences,
Tadepalligudem, West Godavari-534101, AP India.
Tel: +91-8341405043
E. Mail ID: ysbabu033@gmail.com

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INTRODUCTION:

Hypertension is alteration of pressure excite, flow of blood within blood vessel, if it serious when is not treated. Premature death is major in middle income countries, inspite of congested life style [1]. It impacts on above 30-year age groups older is near 1,280 × 10^6 million people was displayed by WHO[2, 3]. Lercanidipine HCl injection is used to treat is a calcium channel blocker of the dihydropyridine class. It is sold under various commercial names including Zanidip. Its molecular weight is 648.2 g/mol with an empirical formula $C_{36}H_{42}ClN_3O_6$. Enalapril Maleates belongs to a family of drugs known as angiotensin-converting

enzyme inhibitors. It inhibits the development of sodium ions causing them to be eliminated. Its molecular weight is 376.4467 g/mol with an empirical formula $C_{20}H_{28}N_2O_5$. It is a prodrug of an ACE inhibitor used to treat hypertension and congestive heart failure [4-8]. As of now literature review displayed the several methods are available for the estimation of Lercanidipine HCl and Enalapril Maleate separately as well as in combination with other drugs with Spectrophotometric and HPLC method [9-17]. The objective of the study is to develop a simple, rapid and precise method for the simultaneous estimation of Lercanidipine HCl and Enalapril Maleate by using RP-HPLC for better treatment with less complication.

MATERIAL AND METHODS:

Chemicals and reagents:

Lercanidipine HCl and Enalapril Maleate were procured from Chandra laboratory, Hyderabad. HPLC grade Acetonitrile and Methanol were procured from Merck Ltd. All other chemical reagents were of analytical grade.

Preparation of 0.1% TEA Buffer:

About 1 ml of Triethylamine was dissolved in 1 l of HPLC water and the pH was adjusted to 2.5 with OPA and filtered through 0.45 µ membrane filter paper.

Preparation of Mobile Phase:

Mobile phase was prepared by mixing Acetonitrile and 0.1 % TEA pH-2.5/OPA, which were taken in the ratio 60:40. It was filtered through a 0.45 µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of Standard Solution:

Accurately weighed and transferred 25 mg of Lercanidipine HCl, 25 mg of Enalapril Maleate working standard into a 50 ml clean dry volumetric flask. The diluent was added and sonicated to dissolve it completely and the volume was made up the mark with the same solvent (Stock solution).

Further 1 ml of the above stock solutions were pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent. The solution was passed through a 0.45µm filter.

Preparation of Sample Solution:

Accurately weighed 20 tablets and transferred 25 mg of Lercanidipine HCl and 25mg of Enalapril Maleate sample into a 50 ml clean dry volumetric flask. The diluent was added and sonicated up to 30 min, and centrifuged for 30 min, it was dissolved completely and the volume was made up to the mark with the same solvent. Then it is filtered through a 0.45 µ Injection filter (Stock solution). Further pipetted out 1 ml of the above stock solutions into 10 ml volumetric flask and diluted up to the mark with diluents.

Chromatographic study:

Lercanidipine HCl and Enalapril Maleate in all solutions were determined by HPLC by using the chromatographic conditions as mentioned in Table 1. The Chromatographic data were analysed and Specificity, Linearity and range, Robustness, precision, and accuracy were determined.

Table 1. Chromatographic conditions for analytical study.

Specifications	Result
Flow Rate	1 ml/min
Column Temperature	Ambient (40 ºC)
Column	TC C18 (250 × 4.6 mm, 5 µ)
Detection Wave Length	234 nm
Injection volume	20 µL
Instrument used	Agilent 1200 HPLC with UV detector.
Mobile Phase	Acetonitrile: 0.1 % TEA pH-2.5/OPA (60:40)
Mode of separation	Isocratic mode
Run time	10 min

RESULTS AND DISCUSSION:

The developed method for determination of Lercanidipine HCl and Enalapril Maleate were validated by using the following parameters.

System suitability:

For establishing the system suitability, the procedure described in the methodology was followed before starting the analysis. System suitability data has been presented in Table 1 and 2.

Tailing factor for the peaks due to Lercanidipine HCl and Enalapril Maleate in standard solution should not be more than 2.0. The theoretical plates for the Lercanidipine HCl Enalapril Maleate in standard solution should not be less than 2000. ` Resolution for the Lercanidipine HCl and Enalapril Maleate peaks in standard solution should not be less than 2.

Table 2. System suitability data of Lercanidipine HCl and Enalapril Maleate.

Parameter	Lercanidipine HCl	Enalapril Maleate
Retention time	2.447	4.453
Plate count	2585	4673
Tailing factor	1.27	1.19
Resolution	8.856	

Specificity:

There were no interfering peaks at the retention time of Lercanidipine HCl and Enalapril Maleatein the presence of excipients. Further, to demonstrate the specificity of the method, the sample was subjected to acid, base, oxidation, thermal, and photolytic degradation. This was evaluated by using a Photo Diode Array detector (PDA). Retention times of Lercanidipine HCl and Enalapril Maleate were 2.447 min and 4.453 min respectively. We did not find any interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity:

Standard solutions containing Lercanidipine HCl and Enalapril Maleate were prepared. Linearity levels of both drugs at five different concentrations of 24, 32, 40, 48, and 56. The average peak areas were plotted against concentration. Then, linearity was evaluated using the calibration curve to calculate coefficient of correlation, slope and intercept. In general, a value of correlation coefficient (r) > 0.9999 is considered as the evidence of an acceptable fit for the data to the regression line. The results obtained are presented in Table 3 which demonstrates that the current method was linear for the two analytes in the range specified above with a correlation coefficient better than 0.9993.

Table 3. Linearity data of Lercanidipine HCl and Enalapril Maleate.

Lercandipine		Enalapril Maleate	
Conc. (µg/ml)	Peak Area	Conc. (µg/ml)	Peak Area
24	1505	24	982
32	1952	32	1296
40	2422	40	1611
48	2812	48	1882
56	3247	56	2184
slop	54.337	slop	37.357
Intercept	214.36	Intercept	97.28
CC (r^2)	0.999	CC (r^2)	0.9993

CC – Correlation Coefficient.

Degradation Studies:

Forced degradation studies of Lercanidipine HCl and Enalapril Maleate were observed in various conditions such as acidic, basic, peroxide, reduction, thermal, photolytic, and UV conditions. The Lercanidipine HCl and Enalapril Maleate were stable under, reduction, thermal, photolytic and hydrolytic conditions. The drug showed significant degradation in acidic, basic and peroxide conditions represented in Fig 1 to 5. The results of forced degradation studies are presented in Table 4.

Table 4. Forced Degradation study summary of Lercanidipine HCl and Enalapril Maleate.

	Retention time		Area	
	LCD HCl	EM	LCD HCl	EM
Acid	2.47	4.497	2427.357	1609.365
Alkali	2.44	4.437	2417.427	16.6.201
Peroxide	2.47	4.497	2442.551	1618.918
Heat	2.477	4.5	2380.434	1649.851
UV	2.473	4.503	2574.539	1645.85

LCD – Lercanidipine, EM - Enalapril Maleate.

Precision:

For Assay, precision was determined by preparing the standard and sample as per the methodology. The sample was prepared in five replicates and injected into the chromatograph. The % Assay value of each preparation was calculated and finally the % RSD of the five replicate preparations was deduced. The data obtained for five replicate standard injections and the six sample preparations have been presented in Table 5.

Table 5. System precision table of Lercanidipine HCl and Enalapril Maleate

Type	Lercandipine	Enalapril maleate
System precision (%RSD)	1.03	0.73
Method precision (%RSD)	0.80	0.45
Intermediate precision (%RSD)	0.14	0.36

System Precision:

System precision is checked by using standard chemical substances to ensure that the analytical system is working properly. In this peak ar ea and % of drug of 5 determinations is measured and % RSD should be calculated.

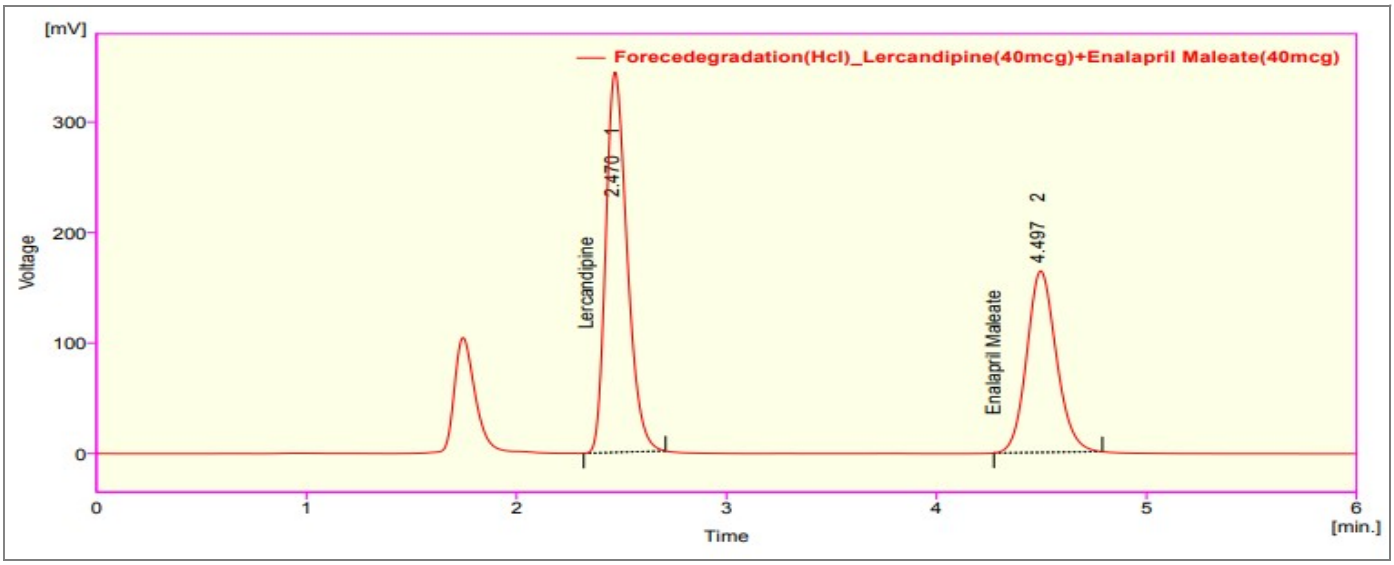

Fig 1. The chromatogram of Lercanidipine HCl and Enalapril Maleate obtained in acid.

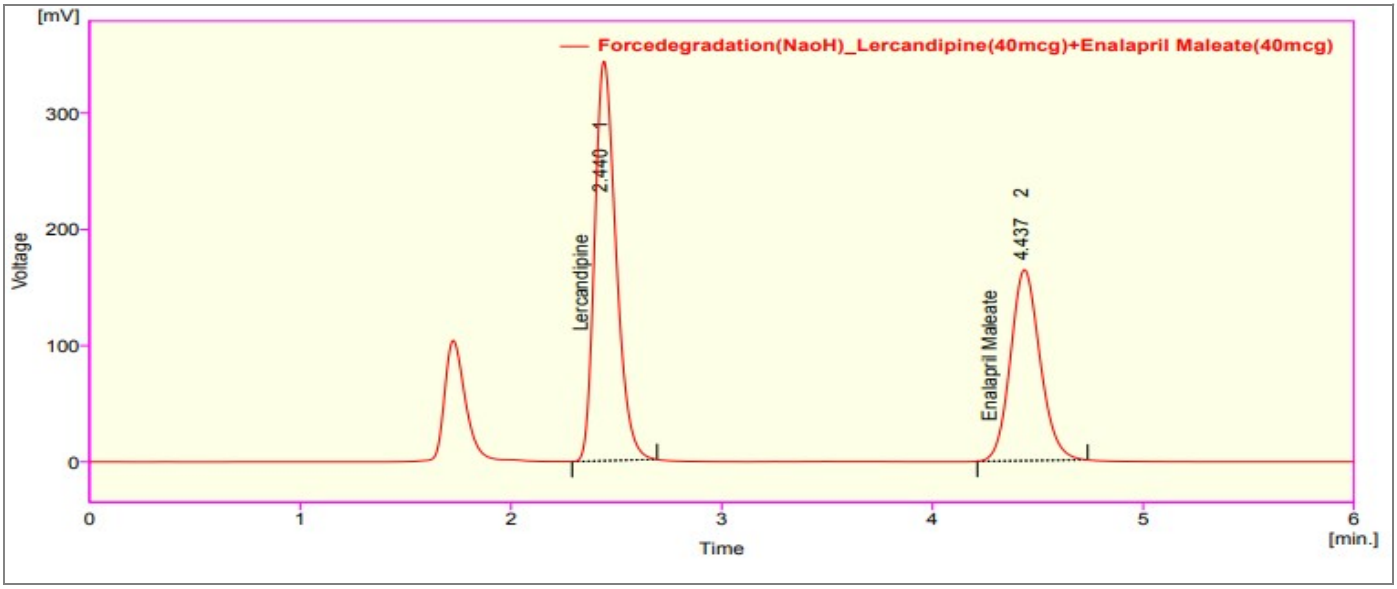

Fig 2. The chromatogram of Lercanidipine HCl and Enalapril Maleate obtained in alkali.

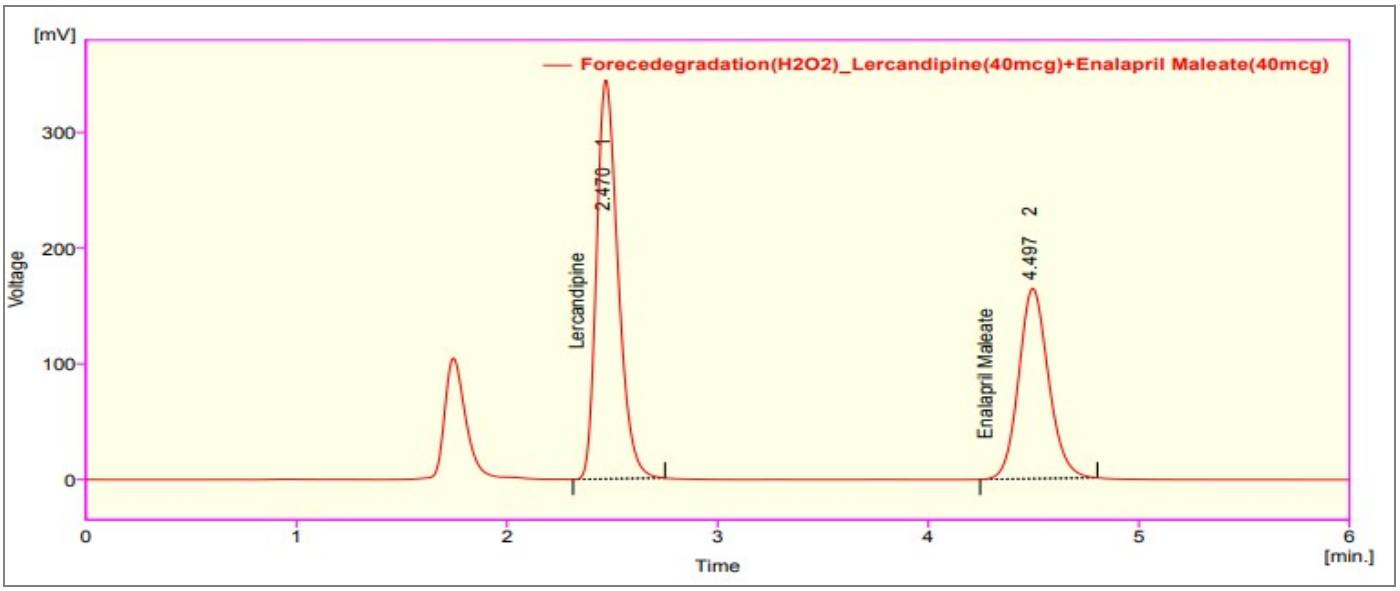

Fig 3. The chromatogram of Lercanidipine HCl and Enalapril Maleate obtained in peroxide.

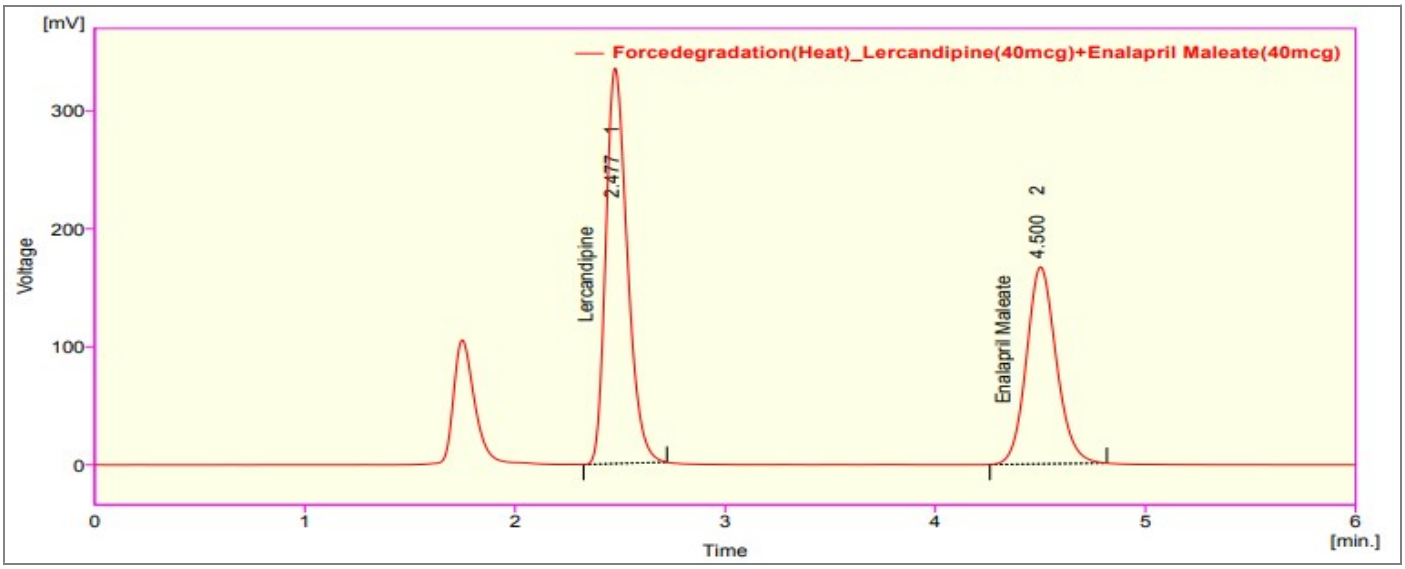

Fig 4. The chromatogram of Lercanidipine HCl and Enalapril Maleate obtained in Heat.

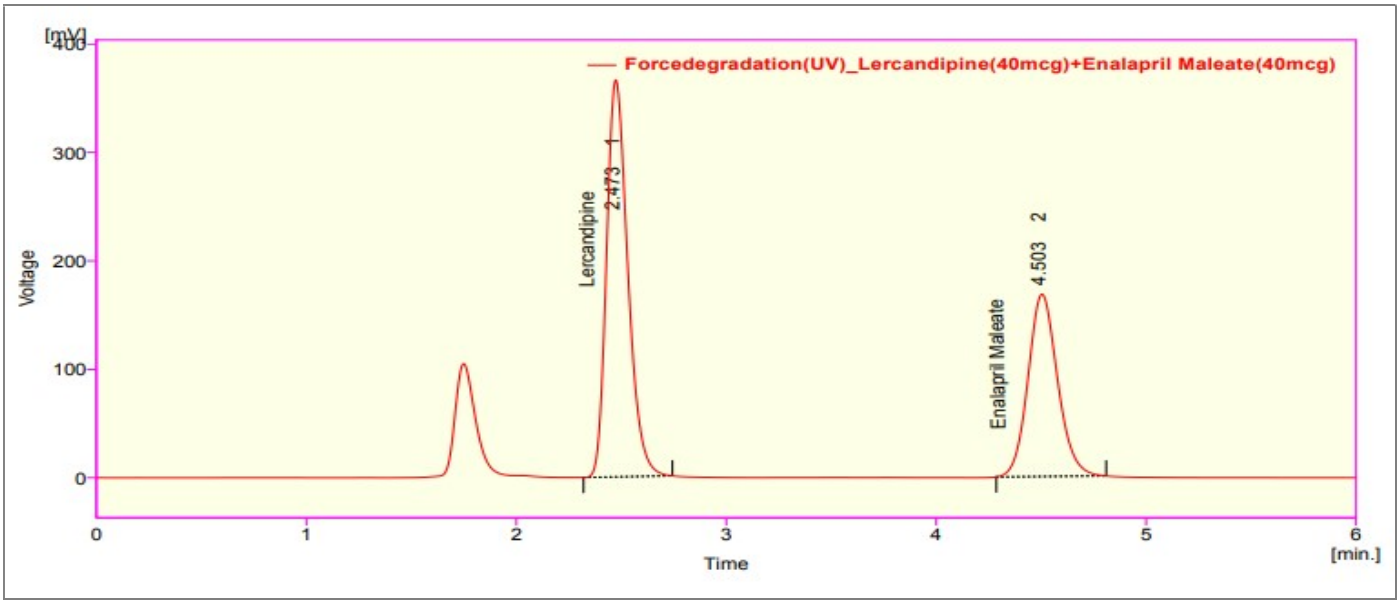

Fig 5. The chromatogram of Lercanidipine HCl and Enalapril Maleate obtained in UV degradation.

Table 8: Robustness results of lercandipine HCl and enalapril maleate by RP-HPLC

Drug	Variation in flow rate				% RSD	Change in PH				% RSD
	0.8 ml/min		1.2 ml/min			PH 2		PH 3		
	RT (min)	Plate count	RT (min)	Plate count		RT (min)	Plate count	RT (min)	Plate count	
LCD HCl	3.433	2902	1.940	2574	1.3	2.48	2646	2.47	2639	0.66
EM	6.227	5195	3.557	4368	1.36	4.5	4772	4.50	4779	0.63

LCD – Lercanidipine, EM - Enalapril Maleate.

Intermediate precision:

In method precision, a homogenous sample of a single batch should be analyzed 5 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD.

Accuracy:

For Assay, the accuracy of the proposed method had been demonstrated by the recovery study performed by the standard addition method at levels 80, 100, and 120 % of the target concentration. The data obtained had been presented in Table 8. was calculated and finally the

% RSD of the three replicate preparations was deduced. Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 99.29 and 99.86 % for Lercanidipine HCl and Enalapril Maleate respectively can be seen in Table 6 and 7.

Table 6. Accuracy results of Lercanidipine HCl by RP-HPLC method.

% Conc. (ASL)	Amount (µg)		Area at 220 nm	Amt. Reco-very	% Reco-very	Mean Reco-very	% R.S.D
	Pure drug	Tab-let					
80	32	40	2399	71.63	99.07	99.29	1.056
			2431				
			2422				
100	40	40	2871	78.44	98.82		
			2933				
			2812				
120	48	40	3270	88.01	100.2		
			3330				
			3244				

ASL - at specification Level.

Table 7. The Accuracy results for Enalapril Maleate by RP-HPLC method.

% Conc. (ASL)	Amount (µg)		Area at 220 nm	Amt. Reco-very	% Reco-very	Mean Reco-very	% R.S.D
	Pure drug	Tab let					
80%	32	40	1603	71.59	99.96	99.86	1.057
			1594				
			1614				
100%	40	40	1922	79.48	98.82		
			1943				
			1873				
120%	48	40	2192	48.07	100.13		
			2223				
			2179				

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition,Temperature Variation was made to evaluate the impact on the method. The data display in Table 9

Table 9. Ruggedness results of lercandipine HCl and enalapril maleate by RP-HPLC.

Statistical Parameter	LCD	EM	Limit
% R.S.D	0.7143	0.6832	NMT 2.0%

LCD – Lercanidipine, EM - Enalapril Maleate.

Ruggedness:

Ruggedness of method was demonstrated by preparing the standard and sample as per the methodology by a different analyst on a different day, using a different column lot and using a different HPLC system. The sample was prepared in six replicates and injected into the chromatograph. The % Assay value of each preparation was calculated and finally the % RSD of the six replicate preparations was deduced. The cumulative data have been presented in Table 10.

Table 10. Sensitivity parameters (LOD and LOQ) by RP-HPLC.

Para-meter	Lercanidipine HCl		Enalapril Maleate	
	(µg/ml)	Area	(µg/ml)	Area
LOD (µg/ml)	0.77	41.77	3.03	113.36
LOQ (µg/ml)	2.3	126.56	9.19	343.53

Limit of detection and Limit of quantification:

The limit of detection (LOD) limit of quantification (LO Q) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma / S$ …(1)

$LOQ = 10 \times \sigma / S$ … (2)

LOD for Lercanidipine HCl, was found to be 2.45 µg/ml and LOQ for Lercani dipine HCl, was found to be 7.44 µg/ml, LOD for Enalapril Maleate was found to be 3.03 µg/ml and LOQ for Enalapril Maleate was found to be 9.19 µg/ml.

Assay:

The assay and % purity were calculated. The observed value was compared with that of standard value without interference from the recipients used in the formulation as computed in Table 11.

CONCLUSION:

This intended study concludes that the proposed method is economical, simple, sensitive and reliable. In addition, it is found to be accurate, precise, specific, stability indicating, rugged and for degradation studies. Hence, it can be employed for the routine estimation of Lercanidipine HCl and Enalapril Maleate.

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Table 11. Assay of Lercanidipine HCl and Enalapril Maleate.

Drug	Avg Standard area (n=5)	Avg sample area (n=5)	Std. weight (mg)	Sample weight. (mg)	Label amount(mg)	Std purity	Amount found (mg)	% assay
Lercandipine HCl	2430.557	2421.361	20	300.12	10	99.8	9.95	99.52
Enalapril Maleate	1607.62	1611.15	20	300.12	10	98.7	9.90	99.01

REFERENCES:

1. Martinez-Quinones P, McCarthy CG, Watts SW, Klee NS, Komic A, Calmasini FB, *et al*. Hypertension induced morphological and physiological changes in cells of the arterial wall. Am J hypertens, 2018; 31:1067-1078.
2. World Health organisaton. Specialized Information Services. https://www.who.int/news-room/fact (Accessed March 06, 2023)
3. Vigneswaran R, Ramasubramanian M, Vigneshwaran G, Abdulla A. A review on thyroid hormones associated with cardiovascular disease. J Pharm Adv Res, 2023; 6: 1927-1931.
4. El-Wasseef DR, El-Sherbiny DT, Abu El-Enin MA, El-Ashry SM. Simultaneous in vitro HPLC determination of enalapril maleate and lercanidipine HCl. J Liq Chromatogr Relat Technol, 2010; 34: 48-60.
5. Dave VM, Maheshwari DG. RP-HPLC method for simultaneous estimation of enalapril maleate and chlorthalidone in synthetic mixture. Int J Pharm Sci Rev Res, 2015; 6: 666-673.
6. Dyade GK, Sawant RL, Joshi HA, Shinde AD, Bandal RS, Gadhingleskar SV. A Novel Approach: Effect of polarity Index of mobile phase on Retention Time of Antihyperlipidemic Antihypertensive and Angiotensin inhibiting Drugs in RP-HPLC Method. Res J Pharmacy Technol, 2020; 13: 3065-3071.
7. Cizmáriková R, Valentová J, Némethy A, Pechová I. Antihypertensive drugs as chiral agents. Int J Med Biol Front, 2015; 21: 219.
8. Lagu SB, Lalam R, Rani BS. New method development and validation for the simultaneous estimation of Avelimab and Axitinib by using RP-HPLC. J Pharm Adv Res, 2023; 6: 2033-38
9. Sujatha N, Pavani KH. Analytical method development and validation of amitriptyline hydrochloride and chlordiazepoxide in tablet by RP-HPLC. Indian J Res Pharmacy Biotechnol, 2013; 1: 655.
10.Kumar VK, Sudhakar M, Reddy PY, Swapna A, Sekhar RV. Method development and validation for simultaneous estimation of pioglitazone and glimepiride in tablet dosage form by RP-HPLC and UV-spectrophotometric method. J Curr Pharma Res, 2011; 2: 404.
11. Rani JS, Devanna N. A new RP-HPLC method development and validation for simultaneous estimation of sofosbuvir and velpatasvir in pharmaceutical dosage form. Int J Eng Technol Sci Res, 2017; 4(11): 145-152.
12. Sahoo NK, Sahu M, Rao PS, Rani NS, Devi JI, Ghosh G. Validation of assay indicating method development of meloxicam in bulk and some of its tablet dosage forms by RP-HPLC. Springer Plus, 2014; 95: 1-6.
13. Prava R, Seru G, Pujala VK, Lagu SB. RP-HPLC method development and validation for the simultaneous determination of lamivudine, abacavir and dolutegravir in pharmaceutical dosage forms. World J Pharm Sci, 2017; 5(5): 168-181.
14. Elkady EF, Fouad MA, Faquih AA. A versatile stability-indicating liquid chromatographic method for the simultaneous determination of atenolol, hydrochlorothiazide and chlorthalidone. Curr Pharm Anal, 2020; 16:1037-51.
15. Prava R, Seru G, Krishna SR, Babu S. Design, characterization and impurity profiling of celecoxib by RP-HPLC. World J Pharmacy Pharm Sci, 2018; 6: 1829-1851
16. Kardani K, Gurav N, Solanki B, Patel P, Patel B. RP-HPLC method development and validation of gallic acid in polyherbal tablet formulation. J Appl Pharm Sci, 2013; 3: 37-42.
17. Cione AP, Liberale MJ, Silva PM. Development and validation of an HPLC method for stability evaluation of nystatin. Braz J Pharm Sci, 2010; 46: 305-310.

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