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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF LERCANIDIPINE HYDROCHLORIDE AND ENALAPRIL MALEATE IN COMBINATION

Purvasha S. Khot*, Jaymin Ghanshyambhai Patel, Bhumi Rajdevbhai Patel
Sharda School of Pharmacy, Pethapur, Gandhinagar, Gujarat,
A-35 Ashok Tenament Opp. Cadila Lab Ghodasar Ahmedabad
purvashakhot@gmail.com,8460337525.

Abstract:
Stability indicating RP-HPLC method for simultaneous estimation of Lercanidipine Hydrochloride and Enalapril Maleate in their Combined Dosage Form has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Lercanidipine HCl and Enalapril Maleate in their combined dosage form. The separation was achieved by column C_{18} (250mm x 4.6 mm) Hypersil BDS and Buffer (pH 5.0): Methanol (30:70 % v/v) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 233 nm. Retention time of Lercanidipine HCl and Enalapril Maleate were found to be 4.057 min and 6.470 min respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Lercanidipine HCl 10-30 µg/ml and for Enalapril Maleate 20-60 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Lercanidipine HCl and Enalapril Maleate In Their Combined Dosage Form

Keywords: *Lercanidipine HCl, Enalapril Maleate, Stability indicating RP-HPLC Method, Validation Method.*

Corresponding author:
Purvasha S. Khot,
Sharda School of Pharmacy, Pethapur,
Gandhinagar, Gujarat,
A-35 Ashok Tenament Opp. Cadila Lab Ghodasar,
Ahmedabad.
purvashakhot@gmail.com,8460337525.

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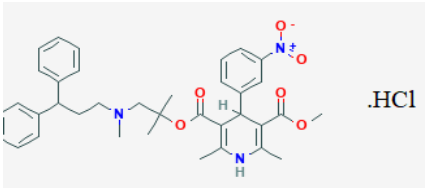
INTRODUCTION:

Lercanidipine Hydrochloride is 3-{1-[(3,3-diphenylpropyl)(methyl)amino]-2-methylpropan-2-yl}5-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5dicarboxylate hydrochloride is used in hypertension. It acts by deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum, Lercanidipine inhibits

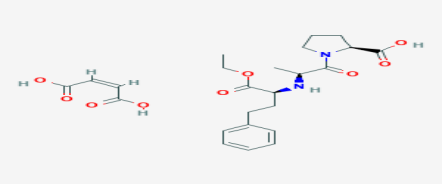


the influx of extracellular calcium across the myocardial and ascular smooth muscle cell membranes The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased afterload. Soluble in chloroform and methanol, but practically insoluble in water[1,2]. It is not official in any pharmacopoeia.[5,6,7]

Figure 1: Structure of Lercanidipine Hydrochloride

Enalapril Maleate is a 2S)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2yl]amino}propanoyl]pyrrolidine-2-carboxylic acid; (2Z)-but-2-enedioic acid. There are two isoforms of ACE: the somatic isoform, which exists as a glycoprotein comprised of a single polypeptide chain of 1277; and the testicular isoform, which has a lower molecular mass and is thought to play a role in sperm maturation and binding of sperm to the oviduct epithelium. Somatic ACE has two functionally active domains, N and C, which arise from tandem gene duplication. Although the two domains have high sequence similarity, they play distinct physiological roles. The C-domain is predominantly involved in blood pressure regulation while the N-domain plays a role in hematopoietic stem cell differentiation and proliferation. ACE inhibitors bind to and inhibit the activity of both domains, but have much greater affinity for and inhibitory activity against the C-domain. Enalaprilat, the principle active metabolite of Enalapril competes with ATI for binding to ACE and inhibits and enzymatic proteolysis of ATI to ATII. Decreasing ATII levels in the body decreases blood pressure by inhibiting the presser effects of ATII as described in the Pharmacology section above. Enalapril also causes an increase in plasma renin activity likely due to a loss of feedback inhibition mediated by ATII on the release of renin and/or stimulation of reflex mechanisms via baroreceptors. Enalaprilat's affinity for ACE is approximately 200,000 times greater than that of ATI and 300-1000 times greater than that enalapril. Freely soluble in methanol and in dimethylformamide; soluble in ethanol (95 per cent); sparingly soluble in water; slightly soluble in semi polar organic solvents; practically insoluble in nonpolar organic solvents[3,4]. It is official in IP-2014, BP-2009, USP30-NF25.[8]

Figure 2: Structure of Enalapril Maleate



MATERIALS AND METHODS:

Instrumentation:

The chromatography was performed on a Thermo separation product TSP UV 2000 instrument (LC-20AT) equipped with standard PDA 2000 UV Detector and Spinchrom software, BDS hypersil C18 column (250mm, 4.6mm and 5µm**)** thermo scientific was used as stationary phase Injector, 20µL fixed loop, Electronic analytical balance Corning volumetric flasks and pipettes were used in the study.

CHEMICALS AND SOLVENTS:-

Zeneril Tablet (Lercanidipine HCl 10mg and Enalapril Maleate 20mg) was produced by Recordati Pharmaceutical ltd. Lercanidipine Hydrochloride and Enalapril Maleate was procured as a gift samples from Torrent Pharmaceutical Ltd. HPLC grades Acetonitrile, Methanol, triple distilled water (Finar Chemicals Ltd., Mumbai, India) were used and AR grade Hydrochloric Acid, Sodium hydroxide, Ammonium Acetate, Potassium dihydrogen phosphate (Merck India Ltd. In Mumbai) were used. Whatman Filter paper no. 41 (Whatman International Ltd., England) was used in the study.

DOSAGE FORM INFORMATION

As this dosage form is not available in India. Tablet is prepared by Direct Compression Machine. Using the ingredients available and reference for the preparation is taken from Pharmaceutical Patented Documents is given in table: 1

Table 1: Dosage formulation

Excipients and Active Substances	Function	Mass(mg)
Enalapril Maleate	Active	20.0
NaHCO3	pH modifier	8.0
Lercanidipine HCl	Active	10.0
Microcrystalline cellulose	Filler	40.0
Povidone K30	Binder	8.0
Lactose monohydrate	Filler	92.0
Sodium starch glycolate	Disintegrant	20.0
Magnesium Stearate	Lubricant	2.0
Total tablet Core		200.0

Preparation of standard solutions:

Enalapril Maleate standard stock solution: (400µg/mL)

40 mg of Enalapril Maleate was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase.

Lercanidipine HCl standard stock solution: (200µg/mL)

A 20 mg of Lercanidipine HCl was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase

Preparation of standard solution of binary mixtures of Lercanidipine HCl (20 µg/mL) and Enalapril Maleate (40 µg/mL)

Take 1 mL from the Enalapril Maleate stock solution and 1mL from Lercanidipine HCl stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Analytical Method Development:-

To optimize the HPLC parameters, several mobile phase compositions were tried. Satisfactory results were obtained from given chromatographic condition for Lercanidipine Hydrochloride and Enalapril Maleate mentioned in Table no: 2

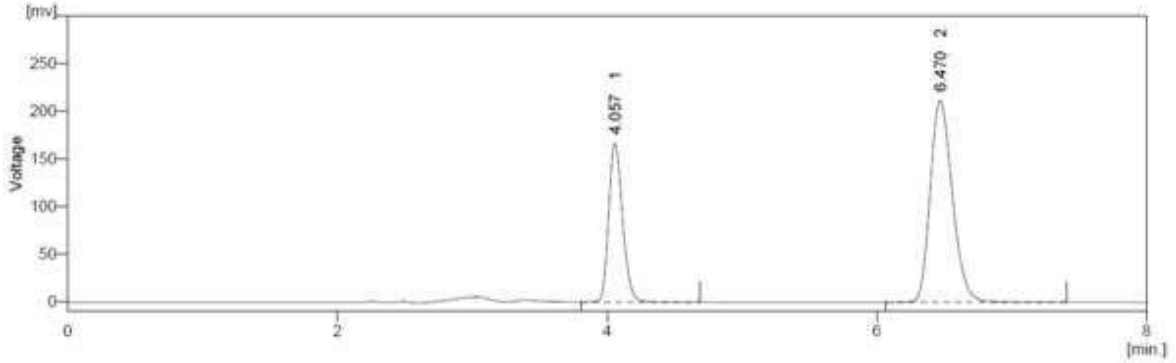


Figure 3: Chromatogram of HPLC Chromatogram of Enalapril Maleate 40 ppm & Lercanidipine HCl 20 ppm in Buffer (Adjusted pH 5.0, 0.05M): Methanol (30:70)

Table 2: Method Development Parameters

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	ssium Phosphate, pH 5.0) : Methanol (30:70)
Column	C18 (25cm x 0.46 cm) Hypersil BDS,5µ
Flow rate	1ml/min
Runtime	8 min
Injection volume	20 µL
Detection wavelength	233 nm

Analytical Method Validation:-

The developed chromatographic method was validated as per ICH guideline for following parameters.

System Suitability:-

As per USP-24, system suitability tests were carried out on freshly prepared standard stock solution of Lercanidipine Hydrochloride and Enalapril Maleate.of both drugs under optimized chromatographic condition and parameters were studied to evaluate the suitability of the system. Results are shown in Table:3

Table 3: System Suitability Studies

Parameters	Lercanidipine HCl	Enalapril Maleate
Retention Time	4.057	6.470
Theoretical Plates	7098	7158
Asymmetry	1.385	1.357
Resolution	9.682	

Linearity and Range:-

The linearity for Lercanidipine Hydrochloride and Enalapril Maleate were assessed by analysis of combined standard solution in range of 10-30 and 20-60 µg/ml respectively.

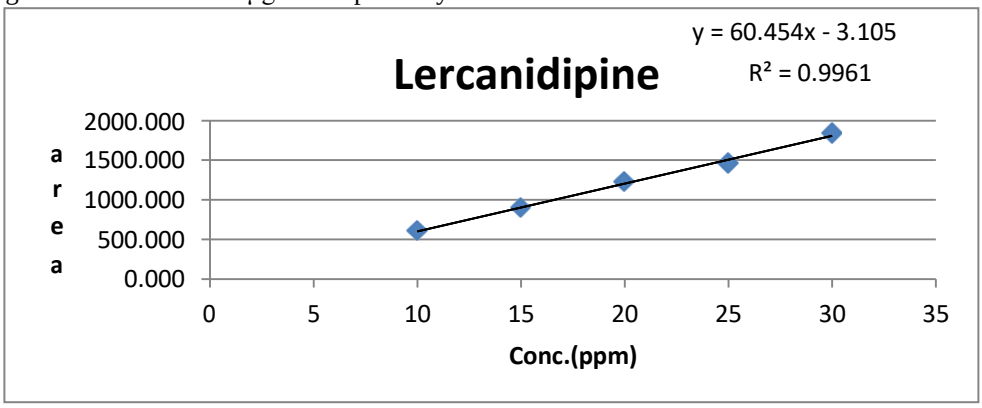


Figure 4: Calibration curve of Lercanidipine HCl

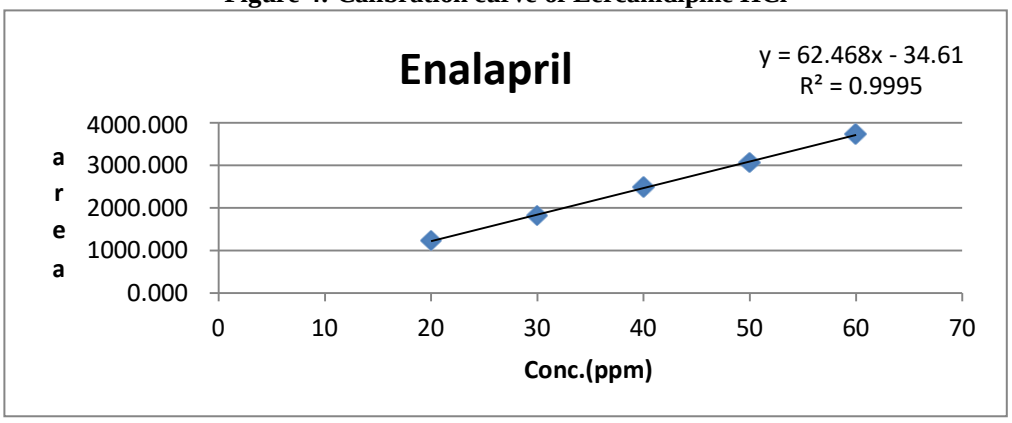


Figure 5: Calibration curve of Enalapril Maleate

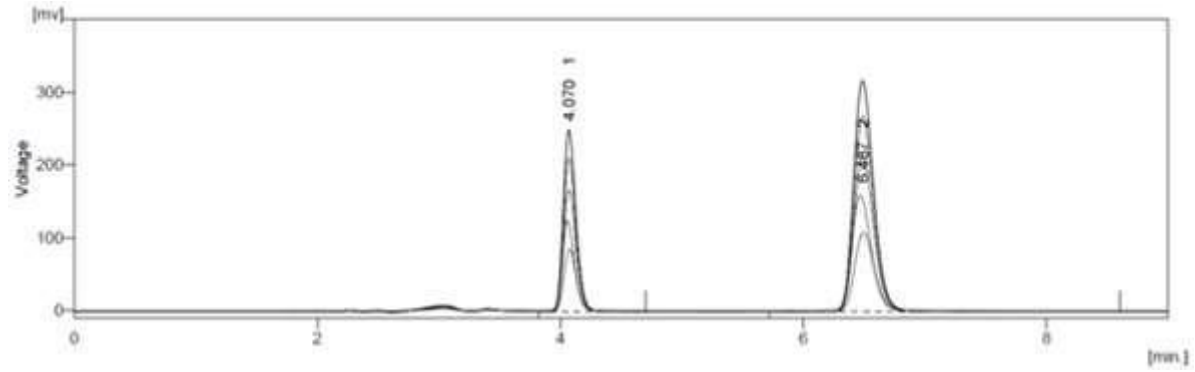


Figure 6: Overlay chromatogram of different concentrations of binary mixtures of Lercanidipine Hydrochloride and Enalapril Maleate

Accuracy:-

Good recoveries of Lercanidipine HCl and Enalapril Maleate were obtained at various added concentrations. By spiking standards like 80 %, 100 % and 120 %. Results are shown in Table 3.

Precision:-

The results of the repeatability, intra-day and inter-day precision experiments are shown respectively as given in Table 3. The developed method was found to be precise as the % RSD were < 2%.

Robustness:-

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the analytical procedure parameters [pH (±0.2), Flow rate (±0.2 ml) and proportion of mobile phase (±2.0 v/v]. The standard deviation of the peak is calculated for each parameter and the %RSD was found to be less than 2%. Results are shown in Table 3.

Degradation Study:

The drug content was employed for acidic, alkaline, and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature, 20µl were injected into the system and the chromatograms were recorded to assess the stability of sample. Specific degradation conditions described as following.

Acidic Degradation Condition:-

Acid decomposition studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and put for 3 hrs. at RT. Add 2ml of NaOH to neutralize the solution. Then the volume was adjusted with diluent to get 20µg/ml for Lercanidipine HCl and 40µg/ml for Enalapril maleate.

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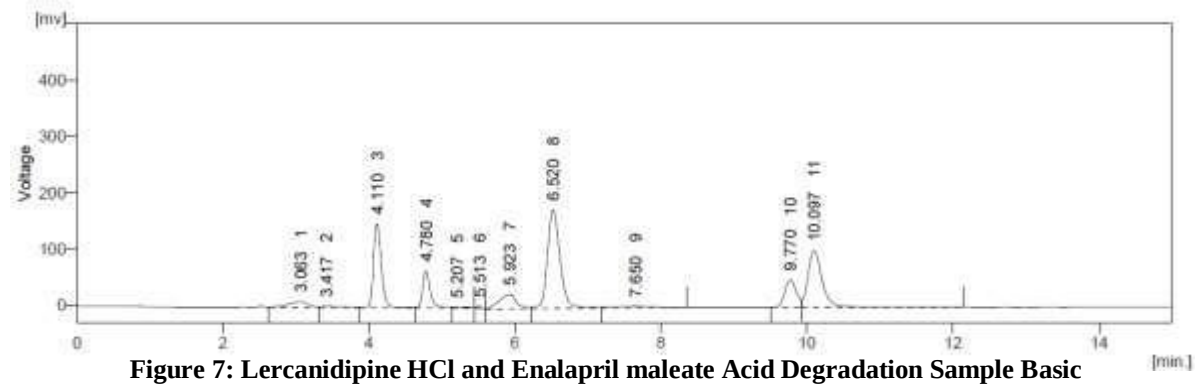

Figure 7: Lercanidipine HCl and Enalapril maleate Acid Degradation Sample Basic Degradation Condition:-

Base decomposition studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 5 hrs. at RT. Add 2ml of HCL to neutralize the solution. Then the volume was adjusted with diluent to get 20µg/ml for Lercanidipine HCl and 40µg/ml for Enalapril maleate.

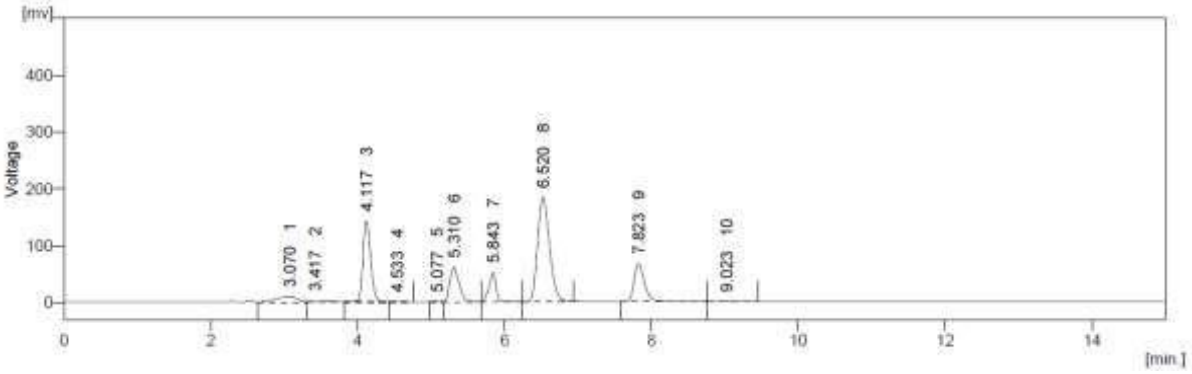

Figure 8: Lercanidipine HCl and Enalapril maleate Base Degradation Sample

Oxidative Degradation Condition:–

Oxidation decomposition studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and put for 3 hrs. at RT. Then the volume was adjusted with diluent to get 20µg/ml for Lercanidipine HCl and 40µg/ml for Enalapril maleate.

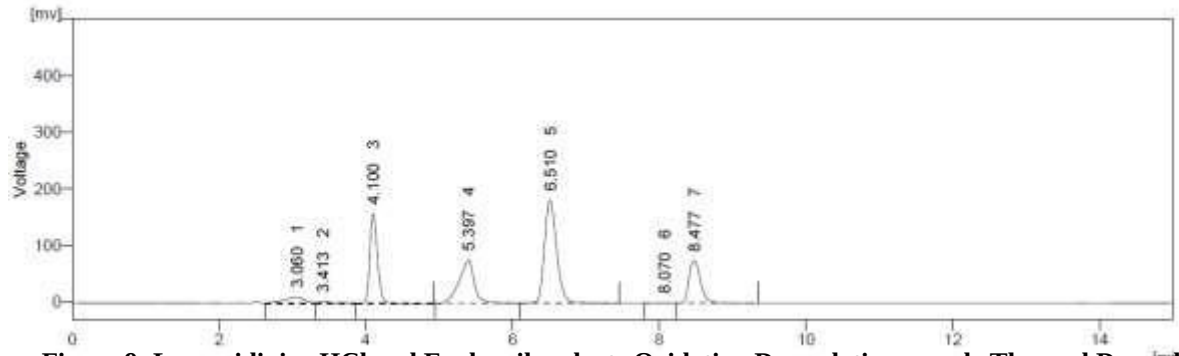

Figure 9: Lercanidipine HCl and Enalapril maleate Oxidation Degradation sample Thermal Degradation Condition: –

Thermal decomposition studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Then the flask was kept for 6 hrs in a oven at 100^0C temperature. Then the volume was adjusted with diluent to get 20µg/ml for Lercanidipine HCl and 40µg/ml for Enalapril maleate.

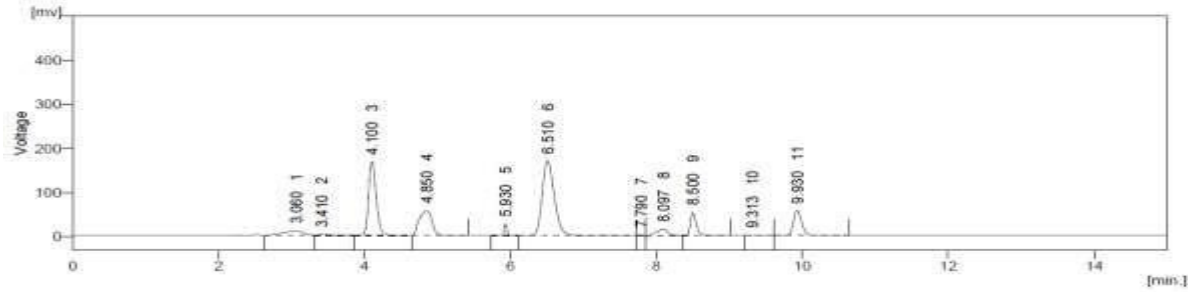

Figure 10: Lercanidipine HCl and Enalapril maleate Thermal Degradation sample

Photolytic Degradation Condition:-
Photo decomposition studies were performed by transferring 1 ml of stock solution to 10 ml of volumetric flask. Then the flask was kept for 12 hrs. under UV Light in UV chamber. Then the volume was adjusted with diluent to get 20µg/ml for Lercanidipine HCl and 40µg/ml for Enalapril maleate.

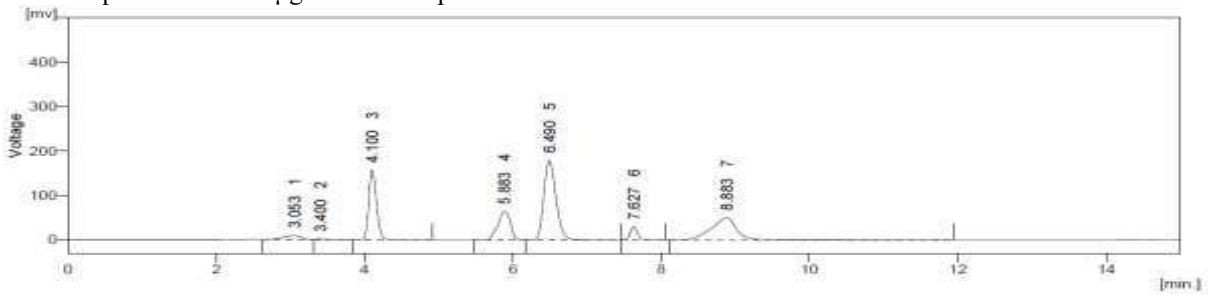

Figure 11: Lercanidipine Hydrochloride and Enalapril Maleate Photo Degradation sample

RESULTS AND DISCUSSION

Validation Parameters:-The method was validated in compliance with ICH guidelines[9].

Force Degradation Studies:-In the present investigation of the Lercanidipine Hydrochloride and Enalapril Maleate were subjected to its stability studies as per ICH guideline[9]. The results of the forced degradation study of Lercanidipine Hydrochloride and Enalapril Maleate are summarized in Table 4 & 5**.**

Table 3: Regression analysis data and summary of validation parameter

PARAMETERS		LERCANIDIPINE HYDROCHLORIDE	ENALAPEIL MALEATE
Linearity Range(n=3) (µg/ml)		10-30	20-60
Regression Equation(R^2)		y = 60.454x - 3.105	Y=62.468x - 34.61
Co-relation Coefficient		0.999	0.996
LOD(µg/ml)		1.875	1.353
LOQ(µg/ml)		5.683	4.100
Recovery%		99.69-100.10	99.49-99.51
Repeatability(% RSD NMT 2)		0.541	0.621
Intra-day (n=3) Precision (% RSD NMT 2)		0.640-0.715	0.490-0.871
Inter-day (n=3) Precision (% RSD NMT 2)		0.389-1.020	0.304-0.802
Robustness	pH (± 0.2)	(-)1.156,(+)1.603	(-)0.965,(+)1.042
	Flow rate (± 0.2 ml)	(-)0.583,(+)0.952	(-)0.696,(+)1.020
	Mobile phase Ratio (± 2 ml)	(-)0.734,(+)1.134	(-)0.882,(+)0.846
Assay		101.863±0.627	98.695±0.490

Table 4: Results of forced degradation study of Lercanidipine HCl

Lercanidipine HCl				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	1092.428	21.434	1114.276	19.862
Base	1062.015	23.621	1056.378	24.026
Oxidation	1170.711	15.804	1152.653	17.102
Photo	1115.121	19.802	1153.831	17.018
Thermal	1197.335	13.889	1224.238	11.954

Table 5: Results of forced degradation study of Enalapril maleate

Enalapril maleate				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	2066.794	14.633	2123.972	12.272
Base	2051.637	15.259	2087.833	13.764
Oxidation	2134.524	11.836	2095.985	13.428
Photo	2143.947	11.447	2053.485	15.183
Thermal	1998.711	17.445	1982.289	18.124

CONCLUSION:

The HPLC method developed for the analysis of Lercanidipine HCl and Enalapril Maleate in their pharmaceutical preparations is simple, rapid and economic with less run time. The method has been validated and it has been shown that it is reliable, linear, accurate and precise as well as robust with minor variations in chromatographic parameters. Therefore, it can be applied for both routine analytical and quality control assay and it could be a very powerful tool to investigate stability of Lercanidipine HCl and Enalapril Maleate.

ACKNOWLEGEMENT:

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