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Analytical Method Development and Validation using RP - HPLC for Simultaneous Estimation of Enalapril and Losartan in bulk samples and tablet dosage forms

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Abstract:

Objective: *A simple, Accurate, precise method was developed for the simultaneous estimation of the Enalapril and Losartan in pharmaceutical dosage form.*

Methods: *Chromatogram was run through Phenomene x C 18 column (150x4.6mm, 5µm). Mobile phase containing Buffer: ACN:Methanol (30:5:65) pH 3.5 was pumped through column at a flow rate of 1.2 ml/min. Buffer used at pH 3.5. Temperature was maintained at Ambient. Optimized wavelength for Enalapril and Losartan was 249 nm.*

Results: *Retention time of Enalapril and Losartan were found to be 5.04 min and 9.71 min. The % purity of Enalapril and Losartan was found to be 100.03 % and 99.75 % respectively. The system suitability parameters for Enalapril and Losartan such as theoretical plates and tailing factor were found to be 4836,0.97 and 3568, 1.42. the resolution was found to be 8.0. The linearity study for Enalapril and Losartan was found in concentration range of 5µg-15 µg and 200 µg-600 µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100.19 % and 100.84 %, %RSD for repeatability was 0.75 and 0.36 %. The precision study was precise, robust and repeatable. LOD value was 0.08 and 0.25, and LOQ value was 1.08 and 0.35 respectively*

Conclusion: *The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Enalapril and Losartan in pharmaceutical dosage form.*

Keywords: *Enalapril, Losartan, RP-HPLC, Simultaneous estimation.*

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INTRODUCTION:

Enalapril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor drug class that works on the renin-angiotensin-aldosterone system, which is responsible for the regulation of blood pressure and fluid and electrolyte homeostasis. Enalapril is an orally-active and long-acting nonsulphydryl antihypertensive agent that suppresses the renin-angiotensin-aldosterone system to lower blood pressure. It was developed from targeted research programmed using molecular modelling. [2] Being a prodrug, enalapril is rapidly biotransformed into its active metabolite, enalaprilat, which is responsible for the pharmacological actions of enalapril. The active metabolite of enalapril competitively inhibits the ACE to hinder the production of angiotensin II, a key component of the renin-angiotensin-aldosterone system that promotes vasoconstriction and renal reabsorption of sodium ions in the kidneys. Ultimately, enalaprilat works to reduce blood pressure and blood fluid volume. [1-4] IUPAC name (2S)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl]pyrrolidine-2-carboxylic acid; (2Z)-but-2-enedioic acid. Enalapril maleate is a white to off-white, crystalline powder with a molecular weight of 492.53. It is sparingly soluble in water, soluble in ethanol, and freely soluble in methanol.

Losartan is an angiotensin II receptor blocker (ARB) used to treat hypertension. Angiotensin-converting enzyme (ACE) inhibitors are used for a similar indication but are associated with a cough.[2] When patients with ACE inhibitor associated coughs are switched to ARBs like Losartan, they have an incidence of cough similar to placebo or Losartan. [3] Losartan is available as Losartan potassium oral tablets as well as a combination tablet of Losartan potassium and Losartan. [2,3] Patients taking Losartan should have their renal function and potassium levels monitored. IUPACname (2-butyl-4-chloro-1-{[2'-(2H-1,2,3,4-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl] methyl}-1H-imidazol-5-yl) methanol. Losartan potassium is a white to off-white free-flowing crystalline powder with a molecular weight of 461 g/mol. It is freely soluble in water and soluble in alcohols.

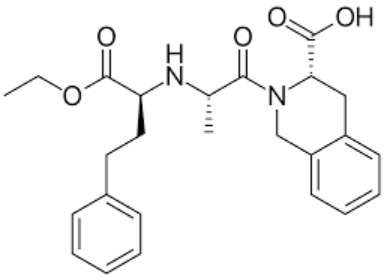

Figure 1: Structure of Enalapril

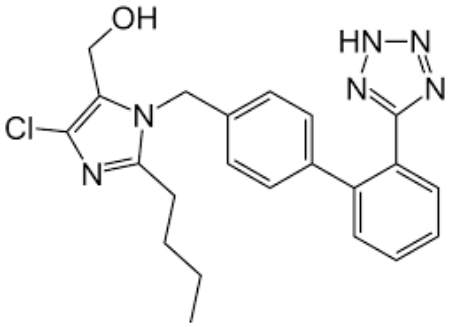

Figure 2: Structure of Losartan

The literature survey revealed that There are Various analytical methods were carried out for the estimation of Enalapril and Losartan as a single or combined with other drugs in pharmaceutical dosages Literature survey reveals that the retention time for the simultaneous estimation of Enalapril and Losartan is more. Hence the present study, we had made an attempt to develop simple, accurate, precise, less time consuming and with less retention time using RP-HPLC for the simultaneous estimation of Enalapril and Losartan in bulk and pharmaceutical dosage form by RP-HPLC. [4-8] To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

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MATERIALS AND METHODS:

Chemicals and Reagents:

Losartan and Enalapril were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions:

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 2 nm with Phenomene x C 18 column (150x4.6mm, 5µm). dimensions at 30 ^{0}C temperature. The optimized mobile phase consists of

Buffer: ACN:Methanol (30:5:65) pH 3.5. Flow rate was maintained at 1.2 ml/min.

Preparation of solutions:

Preparation of phosphate buffer solution:

.2568 gm of di-sodium hydrogen orthophosphate was gauged and adequate water (HPLC grade) was added to break up it. Then, at that point, sonicate for 10 min. Then, at that point, 1ml of tri ethanol amine was added, the last volume was made up to 1000ml with water and changed the pH to 3.5 with ortho phosphoric corrosive.

Preparation of mobile phase:

Methanol, Buffer and Acetonitrile were mixed in the ratio of 65:30:5 and sonicated for 20minutes, Filtered with 0.45 µ membrane filter.

Preparations of working standard solution:

500mg of Losartan and 12.5 mg of Enalapril were exactly checked and moved in to an alternate 50 ml volumetric cup and sufficient adaptable stage was added to separate the medicine. The last volume was made up to 50 ml with flexible stage (fundamental stock course of action). Pipette out 2ml from the above stock plan into a 50ml volumetric cup and the last volume was made adequate with the convenient stage

Preparation of Sample solution

20 tablets were checked and powdered, tablets powder similar to 500mg of Losartan and 12.5mg of Enalapril was moved in to a 50 ml volumetric cup, sufficient proportion of convenient stage was added and separated by 20 minutes ultrasonication. Then, made the volume adequate with the compact stage and isolated with 0.45 µ channel paper. Pipette out 2 ml from the above course of action and debilitated to 50ml with the adaptable stage.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters:

To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.2 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Phenomene x C 18 column (150x4.6mm, 5µm), the mobile phase of composition Buffer: ACN: Methanol (30:5:65) was allowed to flow through the column at a flow rate of 1.2 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation:

The proposed validated method was successfully applied to determine Losartan and Enalapril in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2

Validation of Analytical method:

Linearity: Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of Losartan and Enalapril are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 31.25ppm, 62.5ppm, 93.75ppm, 125ppm, 156.25ppm, 187.5ppm of Losartan and 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of Enalapril. The results are shown in figure 6 and 7.

Accuracy studies:

The accuracy was determined by help of recovery study. The recovery method carried out at three level 75%, 100%, 125% and 75%, 100%, 125 % Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Losartan and Enalapril and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies:

precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness:

To evaluate the intermediate precision of the method, Precision was performed on different day, different analyst, different instrument. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 5.

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 results are shown in table 6.

LOD and LOQ:

The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 7.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION:

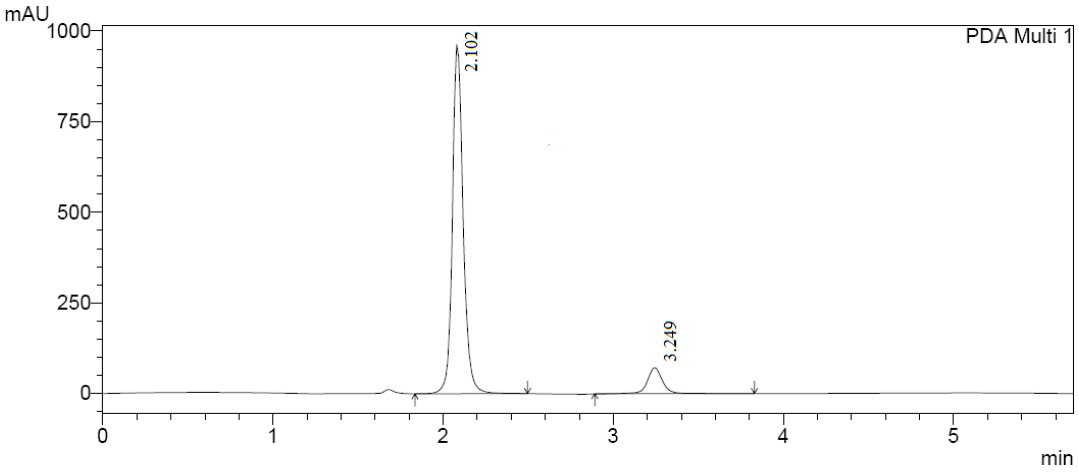

Figure 3: Standard chromatogram

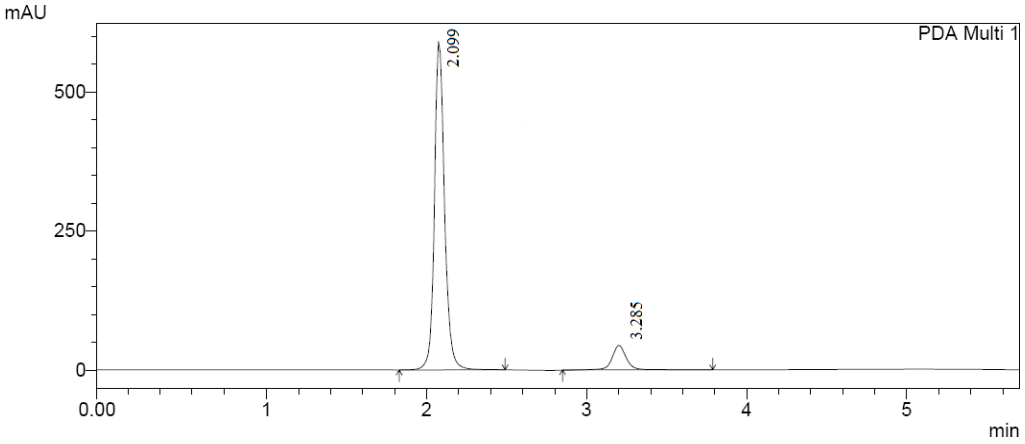

Figure 4: Sample chromatogram

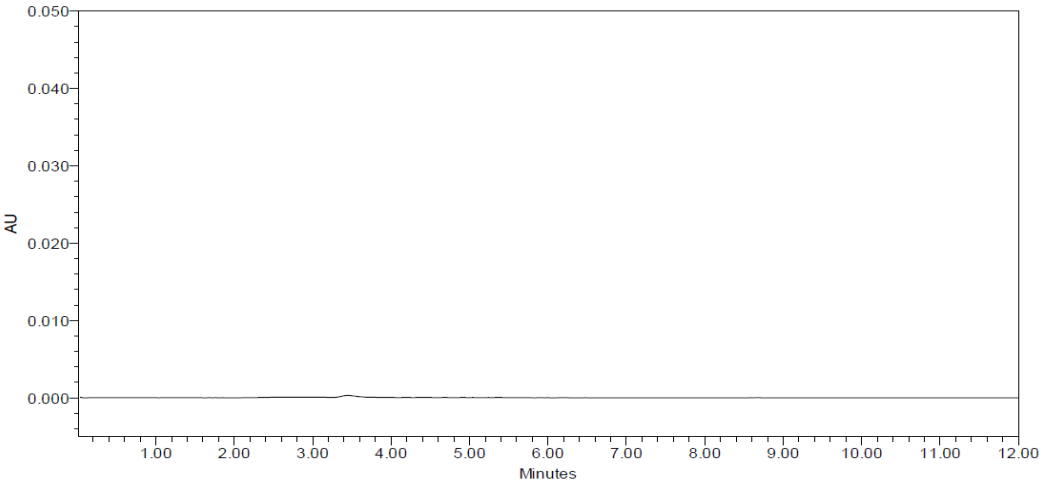

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Enalapril	Losartan
Retention time	2.085	3.219
USP Plate count	3568.306	4836.128
USP Tailing	1.5	1.8

Table 2: Assay results for Enalapril and Losartan

	Label Claim (mg)	% Assay
Enalapril	12.5	100.8
Losartan	500	100.4

Table 3: Linearity results of Losartan and Enalapril

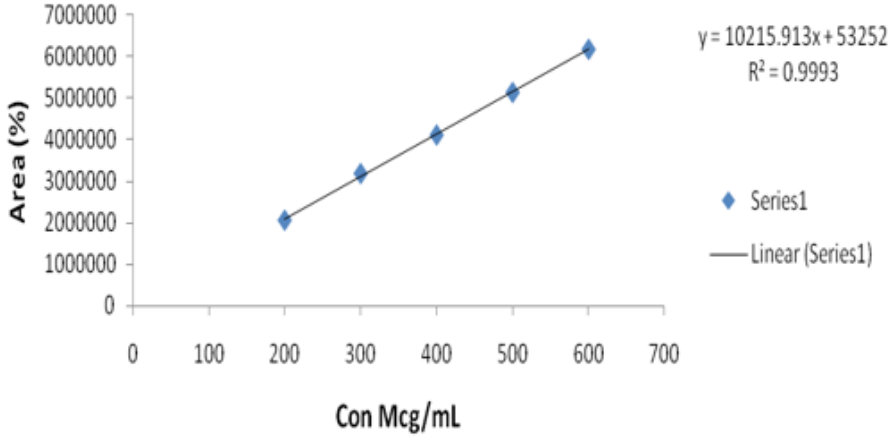

Figure 6: Linearity graph for Losartan

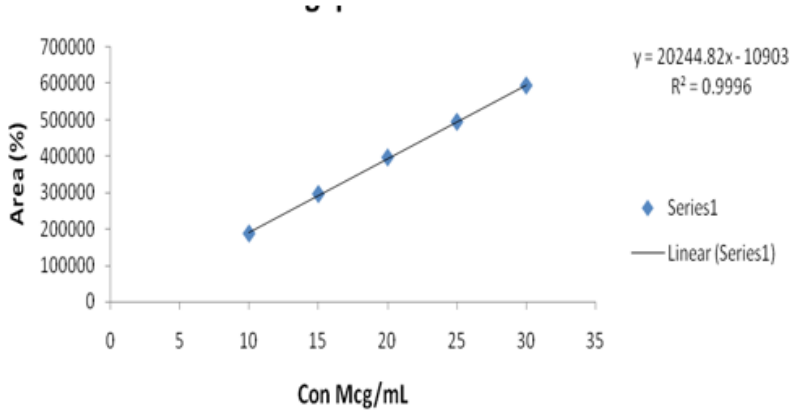

Figure 7: Linearity graph for Enalapril

Table 4: Showing accuracy results for Enalapril and Losartan

Concentration (%)	Added AMT amount (mg)		Amt recovered (mg)		Amt recovered (%)	
Losartan / ENALAPRIL	Losartan	Enalapril	Losartan	Enalapril	Losartan	Enalapril
75	376	9.385	374.66	9.395	99.91	100.30
100	501	12.6	495.18	12.659	99.04	101.29
125	622	15.725	621.84	15.497	99.59	99.12

Table 5: Precision and Intermediate precision results for Enalapril and Losartan

Parameters	Sampling time	Losartan			Enalapril		
		Amount present (mg)	Amount present (%)	RSD (%)	Amount present (mg)	Amount present (%)	RSD %
Repeatability	0 hrs	495.21	99.04	0.0921	12.63	100.92	1.4543
	8^{th} hrs	499.79	99.92	0.9448	12.38	100.62	0.5499
	16^{th} hrs	503.58	100.19	0.3634	12.61	100.84	0.7567
Intermediate precision	I st Day	504.53	100.02	0.4994	12.56	100.43	0.7713
	2^{nd} day	503.69	100.81	0.3198	12.64	101.07	0.6142
	3^{rd} day	497.63	99.51	0.1258	12.71	101.68	0.1256
	Analyst -1	502.36	100.55	0.1908	12.64	101.09	0.8082
	Analyst -2	504.45	100.97	0.1198	12.62	100.97	0.6499
	Instrument -1	501.10	100.30	0.7277	12.67	101.31	0.1558
	Instrument -2	504.96	100.98	0.1218	12.62	100.95	0.4288

Table 6: Robustness results for Enalapril and Losartan

		Losartan			Enalapril		
Parameters		**Amount present (mg)**	**Amount present (%)**	**RSD %**	**Amount present (mg)**	**Amount Present (%)**	RSD %
Wavelength (nm)	249	493.05	98.61	0.1149	12.65	101.17	0.0559
	251	505.58	101.12	0.1247	12.64	101.21	0.0514
Flow Rate (mL/min)	1.4	502.87	100.58	0.3726	12.62	100.95	0.4288
	1.2	502.91	100.59	0.7916	12.66	101.24	0.0163
Mobile phase (% of (Methanol)	68	502.98	100.69	0.3908	12.66	101.28	0.1760
	64	504.87	100.98	0.09943	12.59	100.67	0.3854
pH	3.65	498.77	99.76	1.1838	12.65	101.19	0.0644
	3.55	500.31	100.07	1.3818	12.66	101.09	0.0802

Table 7: LOD, LOQ of Enalapril and Losartan

Drug	LOD	LOQ
Enalapril	0.084	1.08
Losartan	0.359	0.25

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Losartan and Enalapril in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Losartan and Enalapril in pure and its pharmaceutical dosage forms.

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