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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LOSARTAN POTASSIUM AND ENALAPRIL MALEATE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Losartan potassium and Enalapril maleate in tablet dosage form. The using Agilent C8 (4.6 x 150mm, 3.5µm) column in Isocratic mode, in mobile phase containing Acetonitrile phosphate buffer (50:50) and adjusted Ph. 6.5with ortho phosphoric acid, the flow rate was 2ml/min. The detection was carried out at wavelength 256nm. The retention times of Losartan potassium and Enalapril maleate were found to be 3.537 and 2.109 min respectively. The linearity for Losartan potassium and Enalapril maleate were in the range of 25-125µg/ml and 5-25µg/ml respectively .The recoveries of Losartan potassium and Enalapril maleate were found to be 99.7%and99.2%,respectively. The LOD values for and LOQ values are found to be within acceptance criteria. The LOD values were found to be 2.97 and 2.95 for Losartan potassium and Enalapril maleate respectively and LOQ values were found to be 9.91 and 9.95 for Losartan potassium and Enalapril maleate respectively. The proposed method was validated and successfully applied for the estimation of Losartan potassium and Enalapril maleate in combined tablet dosage forms.

Keywords: Losartan Potassium, Enalapril Maleate, HPLC

INTRODUCTION

Losartan potassium is chemically nown as [2-butyl,4-chloro-1-[p-(o-1H-tetrazole-5-yl-phenyl) benzyl]-imidazole-5-methanol monopotassium salt. Losartan is a selective, competitive angiotensin II receptor type 1 (AT1) receptor antagonist, reducing the end organ responses to angiotensin II.Enalapril Maleate is chemically known as (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate salt. Enalapril Maleate isDiuretics, Antihypertensive An angiotensin converting enzyme (ACE) inhibitor. Different analytical methods have been reported in the literature forthe assay of Enalapril and Losartan in pharmaceuticals and include spectrophotometry, TLC, HPLC, HPTLC. The present investigation reports a simple RP- HPLC spectrophotometric method for the analysis in tablet dosage form. The developed method was validated as per ICH guidelines

EXPERIMENTAL

Reagents: Losartan potassium and Enalapril maleate were kindly supplied by Cadila Pharmaceutical Limited. Acetonitrile, Ortho phosphoric acid, Methanol, water(HPLCgrade,Merck) and all the other reagents of AR grade were purchased from MR Enterprisers. A Tablet of Envas RB 25 containing 5mgofEnalapril and 25 mg of Losartan.

Instrumentation: The RP-HPLC system consisted of a Waters model 515, PDA detector 2487 with 20µL sample loop. The output signals were monitored and integrated using Empower2software. The chromatographic column used Agilent C8 (4.6 x 150mm, 3.5µm) and analytical balance (SHIMADZU) used for weighing purpose.

Chromatographic conditions: The mobile phase was used a mixture of acetonitrile phosphate buffer at Pf 6.5 adjusted with ortho phosphoric acid (50 :50 v/v) filter through 0.45 membrane filter prior to use. The flow rate used was 1 ml/min detection was carried out at 256 nm at the ambient temperature. The total run time of 10 min was used. The injectionvolume of 20 µl was used.

Chemicals and reagents: Methanol, Acetonitrile (HPLC grade) was used. Buffer used was pH-6.5 (pH adjusted with orthophosphoric acid). (Reference standards Enalapril maleate and Losartan potassium were obtained from Sura laboratories. Envas RB 25 Tablets of EM (5mg) and LP(25mg) manufactured by Cadila pharmaceuticals Ltd., were procured from local market.

Preparation of Standard Solution: Accurately weighed and transferred 5 mg ofEM and 25 mg of LP workingstandard into a 100mL clean dry volumetric flask and added about 70mL ofdiluent. It was sonicated to dissolve completely and made volume up to the mark with thesame diluent.(Stock solution)(5, 25 µg/mL).From this, 3 ml of the solution was pipetted into another 10ml volumetric flask anddiluted up to the mark with diluent (15,75µg/mL).

Preparation of Sample Solution: Accurately weighed and transferred tablet powder equivalent to 5mg of EMand 25mg of LP into a 100mL clean dry volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made volume up to themark with the same diluent. (Stock solution)(5, 25 µg/mL)From this, 3 mL of the solution was pipetted into another 10ml volumetric flask and diluted up to the mark with diluents

Procedure:

20 µL of the standard and sample solutions were injected into the chromatographicsystem and areas for the EM and LPpeaks were measured.

Preparation of Buffer:

Accurately weighed 7.0 grams of KH_2PO_4 was taken in 1000mL of HPLC grade water. The pH was adjusted to 6.5 with orthophosphoricacid.

Preparation of Mobile Phase:

A mixture of above prepared buffer 500 mL (80%), and 500 mL of HPLC grade Acetonitrile (10%) were mixed and degassed inultrasonic water bath for 5 minutes. The mobile phase was filtered through 0.45 µ filter.under vacuum.

Diluent Preparation: Mobile phase is used as diluents.

Optimized chromatographic conditions:

Diluent **:** Buffer pH – 6.5(Phosphate buffer): Acetonitrile (50:50)

Mobile phase : Buffer p^{H} – 6.5(Phosphate buffer): Acetonitrile (50:50)

Flow rate : 1mL/min

Column : Aglient (C_8) (4.6mm x 150mm, 3.5µm)

Detector wavelength : 256nm

Injection volume : 20 µL

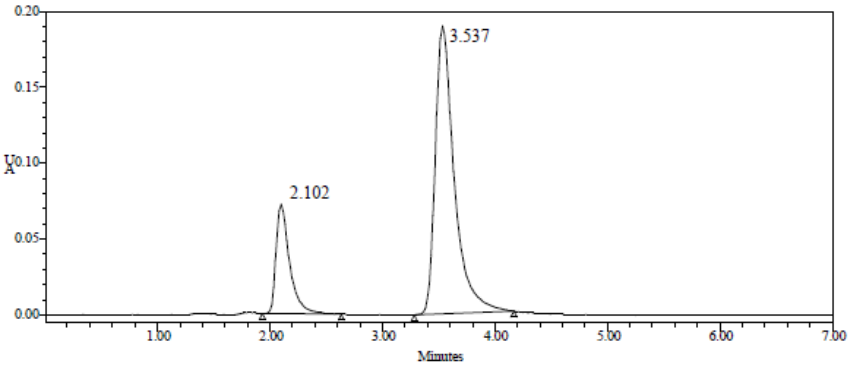

Fig 1: Standard chromatogram

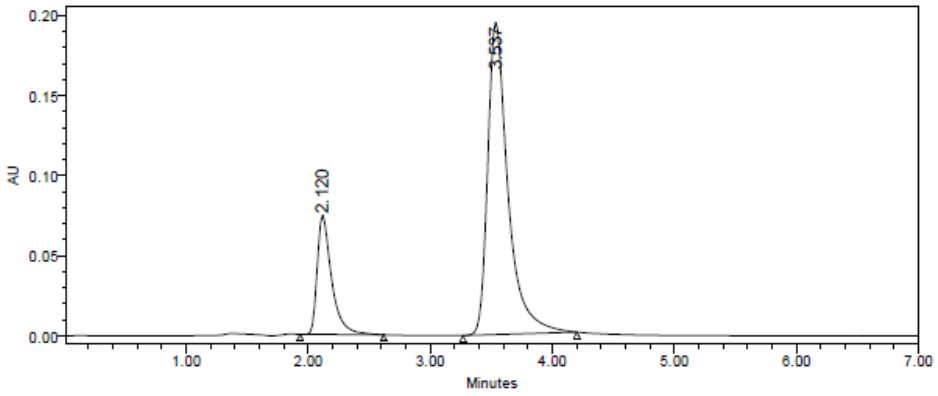

Fig 2: Sample chromatogram

METHOD VALIDATION

Linearity: Solutions were prepared containing 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml, concentrations of Enalapril maleate and 25µg/ml, 50 µg/ml, 75µg/ml, 100µg/ml, 125µg/ml, concentrations of Losartan potassium which corresponding to 50, 75, 100, 125 and 150% respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear- regression analysis.

Accuracy: Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected.

Precision: Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Robustness: The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), mobile phase composition.

Limit of detection (LOD) and Limit of quantification (LOQ): LOD and LOQ was calculated using S/N ratio

Specificity: Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both EM and LP from impurities.

RESULTS AND DISCUSSIONS

Several mobile phase compositions were tried to resolve the peak of EM and LP. The mobile phase containing buffer: Acetonitrile in proportion of 50:50v/v was found ideal to resolve the peak of EM and LP. Retention time of EM and LP were 2.1 and 3.5 min respectively . Result of assay is shown in Table-1. The proposed method was found to be linear in concentration range 5-25µg/ml for EM and 25-125 µg/ml for LP. The data was shown in Table-3 and Figure-3 & 4. System suitability parameters were evaluated and results shown in (Table-1), which were within acceptance criteria. The mean percentage recovery for EM and LP was found to be 99.2% and 99.9% respectively, which are well within the limit and hence the method was found to be accurate (Table-4). LOD and LOQ values were 2.95 and 2.97 and 9.95 and 9.91 for EM and for LP (Table-9,10). Results of intraday and interday precision were shown in the (Table-5 , 6). The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and mobile phase composition. The result obtained implies method is robust for routine qualitative analysis .

CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of Enalapril maleate and Losartan potassium using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

Table 1: Shown Assay Results

Formulation	Label Claim (mg)	% of Assay
Envas Rb25	Enalapril Maleate 5	100.3
	Losartan Potassium 25	100.75

Table2 : Results of System Suitability Parameters

S. No	Parameter	Enalapril maleate	Losatan Potassium
1	Retention time	2.109	3.537
2	Theoretical plates	2594.6	2389.9
3	Tailing factor	1.7	1.6
4	Area	609743	2245981
5	Resolution	-	4.832

Table 3 : Results of Linearity

Enalapril			Losartan		
Conc (µg/ml)	Rt (min)	Area (AU)	Conc (µg/ml)	Rt (min)	Area (AU)
5	2.104	214005	25	3.554	887881
10	2.104	391239	50	3.554	1628941
15	2.114	581128	75	3.551	233245
20	2.113	770162	100	3.571	3091811
25	2.106	949022	125	3.561	3859573

Table 4: Results of accuracy

S. NO	Enalapril					Losartan				
	Spiked level (%)	Area	Amount added (mg)	Amount found (mg)	% recover	Spiked level	Area	Amount added	Amount found	% recovery
1	50	287774	4.86	4.76	98	50	1104782	5	4.92	98.4
2	100	606495	10	10	100.4	100	2238655	10	9.97	99.7
3	150	898508	15	14.8	99.1	150	3577973	15.5	15.9	100.9
		Recovery			99.2		Recovery			99.7

Table 5: Results of intraday precision

S.NO	Enalapril			Losartan		
	Conc (µg/ml)	Rt (min)	Area (AU)	Conc (µg/ml)	Rt (min)	Area (AU)
1	5	2.108	602223	25	3.552	2220333
2	5	2.105	607748	25	3.550	2221573
3	5	2.113	607302	25	3.554	2215483
4	5	2.109	608674	25	3.564	2217379
5	5	2.109	607376	25	3.565	2211255
	Average		606665	Average		2217205
	Std. deviation		2542.3	Std. deviation		4100.75
	% RSD		0.42	% RSD		0.18

Table 6 : Results of interday precision

S.NO	Enalapril			Losartan		
	Conc (µg/ml)	Rt (min)	Area (AU)	Conc (µg/ml)	Rt (min)	Area (AU)
1	5	2.104	596608	25	3.552	2207732
2	5	2.105	598959	25	3.550	2202266
3	5	2.112	595728	25	3.554	2209375
4	5	2.107	594485	25	3.564	2204037
5	5	2.109	595267	25	3.563	2204466
Average			596209	Average		2205575
Std. deviation			1718.7	Std. deviation		2899.8
% RSD			0.29	% RSD		0.13

Table 7: Robustnessdatarelatingtochangeinflowrate

S.NO	Enalapril			Losartan		
	Flow Rate (ml/min)	System Suitability Results		Flow Rate (ml/min)	System Suitability Results	
1	0.75	2673.2	1.7	0.75	2522.7	1.7
2	1.0	2594.6	1.7	1.0	2389.9	1.6
3	1.2	2582.2	1.4	1.2	2452.3	1.3

Table 8 : Robustness data relating to Change in mobile phase composition

S.NO	Enalapril			Losartan		
	Change in organic composition of mobile phase	System Suitability Results		Change in organic composition of mobile phase	System Suitability Results	
1	10 % less	2642.0	1.4	10 % less	2522.7	1.2
2	Actual	2594.6	1.7	Actual	2389.9	1.6
3	10 % more	2599.4	1.4	10 % more	2299.0	1.6

Table -9. Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Enalapril	48	142	2.95
Lossartan	48	143	2.97

Table 10:Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Enalapril	48	478	9.95
Losartan	48	476	9.91

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