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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ALISKIREN AND ENALAPRIL IN BULK AND SYNTHETIC MIXTURE BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

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ABSTRACT

A simple, rapid, precise and accurate high performance liquid chromatography method was developed for simultaneous estimation of enalapril and aliskiren in synthetic mixture. The separation was obtained using a mobile phase consisting of acetonitrile and water in ratio of 80:20 and adjusting pH 4.0 with ortho phosphoric acid (10%) using Phenomenex-luna C18 (250 × 4.6 mm, 5µm) column. The flow rate 1.0 mL min^{-1} and UV detection at 210 nm was employed. The retention time for enalapril and aliskiren was 2.63 min and 7.25 min respectively. Linearity for enalapril and aliskiren was found to be in the range of 2-10 µg/mL and 15-75 µg/mL respectively. The method was validated as per the ICH guidelines and the results were within the acceptance criteria for precision, linearity, specificity, stability of solution and robustness.

Keywords: Method development, Validation, Enalapril, Aliskiren, RP-HPLC, Simultaneous estimation.

INTRODUCTION

The combination of enalapril with aliskiren is use in patients having hypertension with chronic heart failure was checked in a prospective trial (Identifier number NCT00853658) with conclusion results from this study suggest that the combination of aliskiren and enalapril in patients with chronic heart failure is tolerable.[1] Enalapril ((2S)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenyl-butan-2-yl] amino} propanoyl] pyrrolidine-2-carboxylic acid) having high angiotensin-converting enzyme (ACE) inhibitor activity. It is reported in pharmacopoeias such as BP[2], IP[3] and USP. Aliskiren [(2S, 4S, 5S, 7S)-5-amino-N-(2-carbamoyl-2,2-dimethylethyl)-4-hydroxy-7-{[4-methoxy-3-(3-methoxypropoxy)phenyl]methyl} -8-methyl-2-(propan-2-yl) nonanamide] is an rennin inhibitors. Several HPLC methods are reported in combination with other drugs for the determination of enalapril and aliskiren in the literature for its assay.[5-12] However, no method is reported for simultaneous estimation of enalapril and aliskiren by RP-HPLC in any literature. In the present investigation, a specific RP-HPLC method is described for the simultaneous estimation of these two drugs.

MATERIAL AND METHODS

Instrumentation

The HPLC system used was isocratic HPLC Shimadzu LC-20AD, series equipped with a 20 µL sample loop, and SPD-20A detector. The output signal was monitored and integrated using Spinchrom version 2.4.1.93 software. Phenomenex-luna C18 (250 × 4.6 mm, 5µm) column was used for the separation.

Materials

The drug sample of enalapril and aliskiren was obtained from Astron laboratory (Ahmedabad), India and Life Science Ltd. (Mumbai), India respectively. Acetonitrile HPLC Grade (Rankem chemicals), HPLC Grade water (S D Fine chemicals), HPLC Grade glacial acetic acid (Rankem chemicals) are used in the study.

Chromatographic Conditions

The analysis was carried out on an isocratic HPLC system using a Phenomenex-luna C18 (250 × 4.6 mm, 5µm) column as a stationary phase with UV detection at 210 nm at ambient room temperatures using a 20µL injection volume.

Mobile Phase

A mixture of acetonitrile and water in ratio of (80:20) was prepared and adjusted to pH 4.0 using ortho- phosphoric acid (10%), filtered, degassed and used. Ortho phosphoric acid (10%) was prepared by diluting 1 mL of concentrated ortho phosphoric acid in to 10 mL of HPLC grade water.

Standard Stock Solution

Stock solution of enalapril was prepared by dissolving 25 mg in 25 mL volumetric flask containing 15 mL of mobile phase, sonicated for about 10 min and the made up to volume with same mobile phase. Stock solution of aliskiren was prepared by dissolving 100 mg in 100 mL volumetric flask containing 70 mL of mobile phase, sonicated for about 10 min and the made up to volume with same mobile phase. Daily working standard solution of both enalapril and aliskiren were prepared by suitable dilution of the stock solution with appropriate mobile phase.

Sample Preparation

About 5.0 mg of Synthetic mixture was weighed accurately and transferred into a 50 mL volumetric flask. Dilute with the prepared solution 10 mL of mobile phase (Acetonitrile and Water) and sonicated volume was adjusted by diluent. The solution was thoroughly mixed and filtered through whatmann filter paper. The resulting solution (2 µg/mL of enalapril and 30 µg/ml of aliskiren) was filtered through 0.45 µm nylon membrane and injected into HPLC system.

Method Development

The mobile phase consisting of acetonitrile and water in varying proportions and change in pH was tried and finally ratio of 80:20 (pH-4.0 adjusted with diluted Ortho phosphoric acid) was selected because it was found to give good separation for the peaks of enalapril (R_t-2.63 min) and aliskiren (R_t-7.24 min), respectively as shown in the figure 1. In addition to this, UV spectra of individual drugs were recorded at the wavelength range from 200 to 400 nm and the response for optimization was compared. The choice of wavelength 210 nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity.

Method Validation

The method was validated in accordance with ICH guidelines.[14]

System Suitability

System performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as retention time (R_t), number of theoretical plates (N), asymmetry factor, resolution factor were determined. The results are shown in table I.

Linearity

Mixed standard solution of Enalapril and Aliskiren were prepared with mobile phase in such a way that the final concentration of Enalapril and Aliskiren is in the range of 2-10 µg/mL and 15-75 µg/mL respectively. The peak area was recorded for all the peaks as shown in table II and table III for Enalapril and Aliskiren respectively. The plots of peak area versus the respective concentration were found to be linear with regression coefficient (r^2=0.997) for Enalapril propionate and (r^2=0.997) for Aliskiren as shown in figure 2 and figure 3.

Accuracy

For accuracy study data from nine determinations over three concentrations at 80%, 100% and 120% of expected sample concentration covering the specified range was determined & expressed as recovery values. The results were shown in table IV.

Precision

The method Precision was established by carrying out the analysis of two drugs using proposed

analytical method in six replicates. It indicates the sample repeatability of the method. The results were shown in table V.

Robustness

The robustness of method was determined to check the reliability of an analysis with respect to deliberate variation in method parameters.

The typical variations are given below:

Variation in wavelength by ± 2 nm.

Variation in mobile phase composition by ± 2 volume of solvent.

The robustness data are shown in table VI & VII.

Assay

The validated HPLC method was applied to simultaneous determination of Enalapril and Aliskiren in Synthetic mixture About 5.0 mg of Synthetic mixture was weighed accurately and transferred into a 50 mL volumetric flask. Dilute with the prepared solution 10 mL of mobile phase (Acetonitrile and Water) and sonicated volume was adjusted by diluent. The solution was thoroughly mixed and filtered through whatmann filter paper. The resulting solution (2 µg/mL of Enalapril and 30 µg/mL of Aliskiren) was filtered through 0.45 µm nylon membrane and injected into HPLC system are shown in table VIII.

RESULTS AND DISCUSSION

System Suitability

System suitability parameter such as retention time (R_t), number of theoretical plates (N), asymmetry factor, resolution factor were shown in table I.

Linearity

The linearity study was carried out for both drugs at five different concentration levels. The linearity of Enalapril and Aliskiren was in the range of 2-10 µg/mL and 15-75 µg/mL respectively shown in the table II & calibration curve shown in figure 2 & 3.

Accuracy

Accuracy of the method was confirmed by recovery study from Synthetic mixture at three levels. The results were shown in table III.

Precision

The data for the repeatability of the peak area measurement for the Enalapril and Aliskiren, based on six measurements of same solution of Enalapril and Aliskiren were shown in table IV.

Robustness

The robustness parameters for the method were shown in table V & VI.

Assay

Applicability of the proposed method was tested by analysing the Synthetic mixture. The results were shown in table VII.

CONCLUSION

The proposed HPLC method provides a rapid, accurate, precise and rugged assay with stability indicating potential for these two drugs in synthetic mixture. In conclusion, the developed method is strongly recommended for the assay of two drugs in synthetic mixture.

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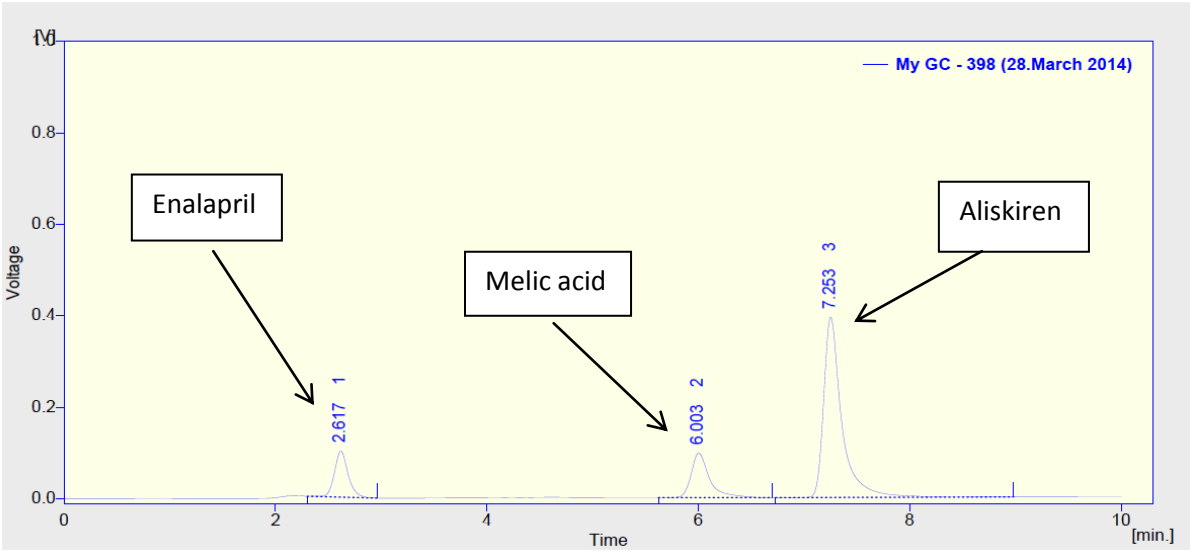

Figure 1: A typical chromatogram of mixture of Enalapril (6 µg/mL) and Aliskiren (45 µg/mL)

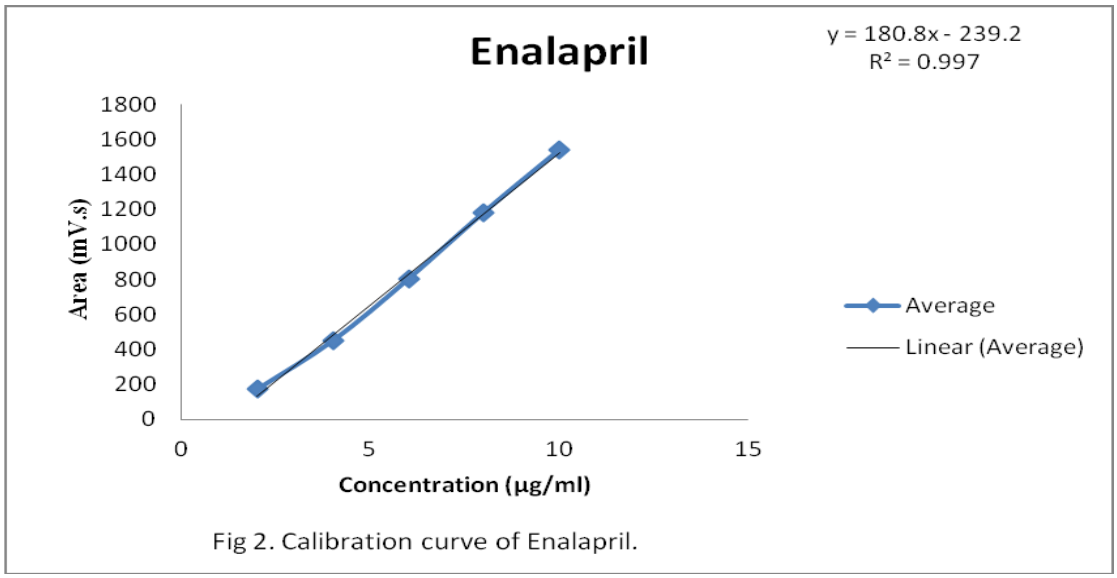

Figure 2: Calibration curve of Enalapril

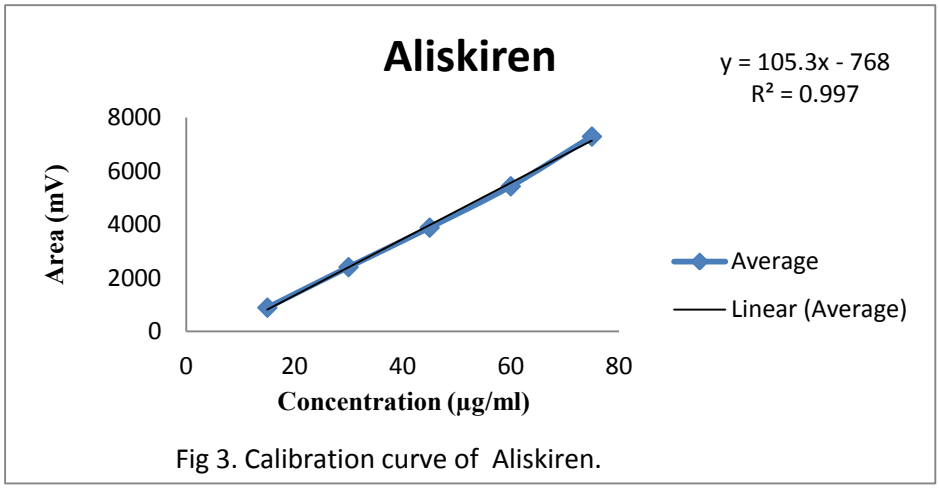

Figure 3: Calibration curve of Aliskiren

Table I: System Suitability Parameters

Parameters	Enalapril	Aliskiren
Retention time (R_t)	2.65 min	7.23 min
Asymmetry factor	1.30	1.86
Theoretical plates	2134	10925
Resolution factor	-	4.41

Table II: Linearity of Enalapril and Aliskiren

Enalapril			Aliskiren		
Concentration (µg/mL)	Peak area ± SD (n=5)	% RSD	Concentration (µg/mL)	Peak area ± SD (n=5)	% RSD
2	176.77±1.23	0.386	15	892.84±2.21	0.248
4	447.73±1.68	0.376	30	2405.79±3.58	0.148
6	802.89±1.92	0.239	45	3882.56±5.39	0.138
8	1184.41±3.01	0.254	60	5427.73±5.53	0.124
10	1616.59±2.15	0.133	75	7297.32±9.65	0.132

Table III: Accuracy study parameter

Name of sample	Amount taken (µg/mL)	Amount added (µg/mL)	Recovered Concentration (µg/mL)	%Recovery ± SD (n=3)
Enalapril	2	1.6	3.57	99.42±0.19
	2	2	3.92	99.86±1.01
	2	2.4	4.35	99.09±1.10
Aliskiren	30	24	54.02	100.04±0.28
	30	30	59.87	99.78±1.02
	30	36	65.93	99.89±1.3

Table IV: Precision study parameter

Enalapril			Aliskiren		
Concentration (µg/mL)	Peak area ± SD (n=6)	% RSD	Concentration (µg/mL)	Peak area ± SD (n=6)	% RSD
6	803.18±1.43	0.17	45	3879.78±6.41	0.386

Table V: Robustness parameter by changing wavelength

Change in wavelength (nm)	Enalapril			Aliskiren		
	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay ±SD(n=3)	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay ±SD(n=3)
208	4	3.93	98.25 ±0.96	30	29.76	99.2 ±1.26
210	4	3.99	99.75 ±1.60	30	30.05	100.16 ±1.81
212	4	3.97	99.25 ±1.12	30	29.91	99.7 ±2.14

Table VI: Robustness parameter by changing mobile phase composition

Change in mobile phase composition (Acetonitrile: Water)	Enalapril			Aliskiren		
	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay±SD (n=3)	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay±SD (n=3)
78:22	4	3.95	98.75 ±0.80	30	29.9	99.66 ±1.38
80:20	4	3.99	99.75 ±1.39	30	30.01	100.03 ±2.01
82:18	4	3.91	97.75 ±0.66	30	29.92	99.73 ±1.94

Table VII: Assay study parameter

Enalapril			Aliskiren		
Concentration (µg/mL)	Amount found (µg/mL)	% Assay ±SD (n=3)	Concentration (µg/mL)	Amount found (µg/mL)	% Assay ±SD (n=3)
2	2.03	100.52±0.84	30	30.15	100.65±1.35

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