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Research Article

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A RP-HPLC method development and validation for the estimation of aliskiren hemifumarate in bulk and pharmaceutical dosage forms

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Aliskiren Hemifumarate in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.0 mL min $^{-1}$ was employed on a Symmetry C_{18} column at ambient temperature. The mobile phase consisted of acetonitrile: phosphate buffer 60:40 (v/v) and the detection wavelength was at 234 nm. Linearity was observed in concentration range of 50-175 µg/mL. The retention time for Aliskiren was 2.28 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Aliskiren in pharmaceutical dosage forms.

Key Words: Estimation, Method development, Aliskiren Hemifumarate, RP-HPLC, Validation.

INTRODUCTION

Aliskiren Hemifumarate chemically described as (2(2*S*,4*S*,5*S*,7*S*)-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 [1] (Figure 1) is an orally active rennin inhibitor licensed for the treatment of essential hypertension and heart failure. Aliskiren metabolized slowly in the body resulting in stronger half lives which restrict it once a day dosing. The cytochrome P450 susceptibility is also less and a major proportion of the drug is eliminated unchanged via feces.

Literature survey reveals that few spectrophotometric methods [2] and HPLC methods [3-7] has been reported for the estimation of Aliskiren. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Aliskiren in pharmaceutical dosage form as per ICH guidelines [8].

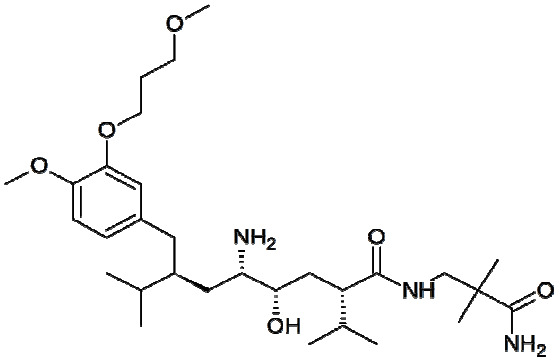

Figure 1: Chemical structure of Aliskiren

EXPERIMENTAL SECTION

Instrumental and analytical conditions

The HPLC analysis was carried out on Waters HPLC system (2695 module**)** equipped with 2487 dual lambda detector with auto Sampler and running on Waters Empower software. The column used is Symmetry C_{18} (150 × 4.6 mm, packed with 5 µm) and detection was performed at 234 nm. The injection volume of sample was 20 µL and the run time was 6 minutes. An isocratic mobile phase containing acetonitrile and 0.02 M phosphate buffer at 60: 40 (v/v) at the pH 3.5 was carried with the flow rate at 1.0mL min^{-1}. The mobile phase was filtered through 0.45µm membrane filter and degassed before use.

Reagents and chemicals

Aliskiren working standard was kindly gifted by pharmatrain, Hyderabad. Tablets were purchased from local pharmacy manufactured by Novartis (Rasilez). Ultra pure water was obtained from a millipore system. HPLC grade acetonitrile was obtained from Merck (India) limited. All other chemicals used were AR grade.

Preparation of mobile phase

Dissolved 2.72 g of Potassium Di hydrogen orthophosphate in 1000 mL of water and mixed, pH adjusted to 3.5 using ortho phosphoric acid, sonicated to degas the buffer. Transferred 600 volumes of acetonitrile and 400 volumes of buffer into a 1000 volumes mobile phase bottle and mixed. Then sonicated up to 15 minutes for degas the mobile phase and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of Standard Solution

Accurately weighed about 10 mg of Aliskiren and transferred into a 10mL volumetric flask and 7 mL of diluent was added and sonicate to dissolve it completely and the volume was adjusted with the mobile phase to get stock solution of 1000 µg/mL. Then 1 mL of stock solution is transferred into 10 ml volumetric flask and make up to volume with mobile phase and filter through 0.45 µm filters, which gives a solution of strength 100 µg/mL.

Preparation of sample solution

Weigh 20 Aliskiren tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 50 mg of Aliskiren into a 50 ml volumetric flask. Add about 25ml of diluent, sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

METHOD VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity

From the standard stock solution, the various dilutions of Aliskiren in the concentration of 50, 75, 100, 125, 150 and 175µg/mL were prepared. The solutions were injected using 20 µL injection volumes in to the chromatographic

system at the flow rate of 1.0 mLmin^{-1} and the effluents were monitored at 234 nm, chromatograms were recorded. Calibration curve of Aliskiren was obtained by plotting the peak area ratio versus the applied concentrations of Aliskiren, given in table 1. The linear correlation coefficient was found to be 1, shown in figure2.

Table 1: Linearity of Aliskiren

Concentration (µg/mL)	Average area
50	641426
75	969169
100	1286672
125	1607741
150	1932957
175	2257921

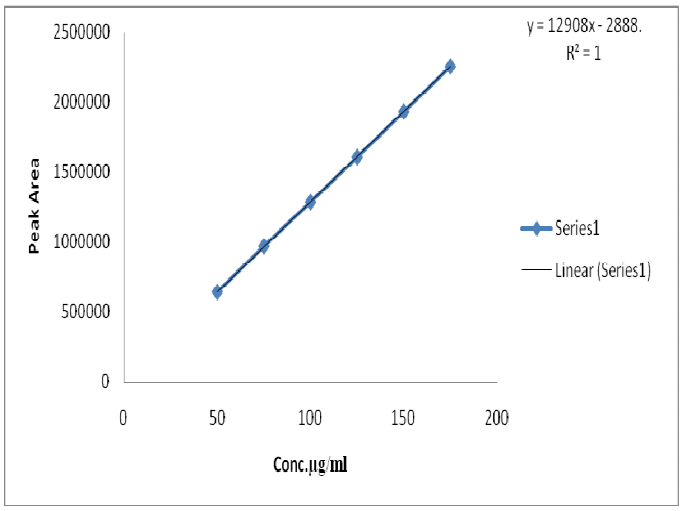

Figure 2: Linearity curve of Aliskiren

Precision

Repeatability of the method was checked by injecting replicate injections of 100 µg/mL of the solution for six times on the same day as intraday precision study of Aliskiren and the % RSD was found to be 0.15, given in table 2.

Table 2: Precision of Aliskiren

Injections	**Area**
1	1278827
2	1280763
3	1283837
4	1280931
5	1283027
6	1279532
Mean	1281153
SD	1947.051
% RSD	0.1517

Table 3: Accuracy of Aliskiren

%Conc	**Amount added (mg)**	**Amount found (mg)**	**% Recovery**	**Mean Recovery**
50%	5.0	4.96	99.2 %	99.17%
100%	10.0	9.96	99.6 %	
150%	15.0	14.8	98.7 %	

Accuracy
Aliskiren reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (50%, 100% and 150%). At each level, samples were prepared in triplicate and the recovery percentage was determined and presented in table 3.

Specificity
Spectral purities of Aliskiren chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks and no interference peaks were observed.

Robustness
To determine the robustness of the method, two parameters (flow rate, composition of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in table 4.

Table 4: Robustness of Aliskiren

Parameters	Adjusted to	Average Area	R_t	SD	% RSD
Flow rate as per method 1.0mL/min	0.8 mL/min	1295159	2.291	5918.5	0.45
	As it is	1289713	2.289	4993.7	0.39
	1.2ml/min	1297130	2.284	3887.0	0.30
Mobile phase composition Acetonitrile: Buffer (60:40)	Acetonitrile: Buffer (55:45)	1297717	2.279	3475.6	0.28
	As it is	1294644	2.282	4987.4	0.38
	Acetonitrile: Buffer (65:35)	1303208	2.284	5215.3	0.40

Ruggedness
Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Detection and quantitation limits
According to the determined signal-to-noise ratio, Aliskiren presented limits of detection of 0.06µg/mL and limits of quantitation of 0.8µg/mL, where the compounds proportion found in the sample solutions injected on to the chromatograph. However, the objective of the method is the quantitation of Aliskiren so that the values obtained should be considered as the limit of method sensitivity.

System Suitability
System suitability tests were carried out on freshly prepared standard stock solutions of Aliskiren and it was calculated by determining the standard deviation by injecting standards in six replicates at 6 minutes interval and the values were recorded and the system suitability parameters are shown in table 5.

Table 5: System Suitability for Aliskiren

Concentration	Injection	Area	R_t
100 µg/mL	Inj-1	1287115	2.285
	Inj-2	1283496	2.287
	Inj-3	1290171	2.288
	Inj-4	1289663	2.286
	Inj-5	1289921	2.289
	Inj-6	1290228	2.288
Statistical Analysis	Mean	1288432	2.287167
	SD	2686.291	0.001472
	% RSD	0.21	0.06
	Tailing Factor	1.6	
	Plate Count	2496.6	

Assay of Aliskiren tablet

Three different batches of Rasilez were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 50mg of Aliskiren was transferred to a 50 ml volumetric flask followed by the addition of 25 ml of mobile phase. The solution was sonicated for 3 minutes and volume adjusted with the mobile phase then filtered through 0.45 µm membrane filter. Further dilutions were made to get the final concentration equivalent to 100 µg/mL of Aliskiren. The mean peak area of the drug was calculated and the drug content in the tablets was quantified and the results were presented in table 6.

All the analyzed batches presented Aliskiren were very close to the labeled amount. The Aliskiren content in the tablets samples varied from 99.8 to 100.1%.

Table 6: Contents of Aliskiren in tablets (n=6)

Sample tablet	Batch	Label claim(mg)	Amount found (mg)±SD	%Amount found
Rasilez (150mg)	1	10	9.99±0.08	99.9
	2	10	9.98±0.12	99.8
	3	10	10.01±0.16	100.1

S.D=Standard Deviation

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Aliskiren was preferably analyzed by reverse phase chromatography and accordingly C_{18} column was selected. The elution of the compound from the column was influenced by polar mobile phase. The ratio of the acetonitrile to phosphate buffer was optimized to give symmetric peak with short run time. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of acetonitrile: phosphate buffer at the ratio of 60:40 (v/v).The retention time of Aliskiren was found to be 2.28 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 5. Developed chromatographic method was applied for the determination of Aliskiren in tablet formulation, given in table 7. A typical chromatogram showing the separation of Aliskiren is shown in figure 3.

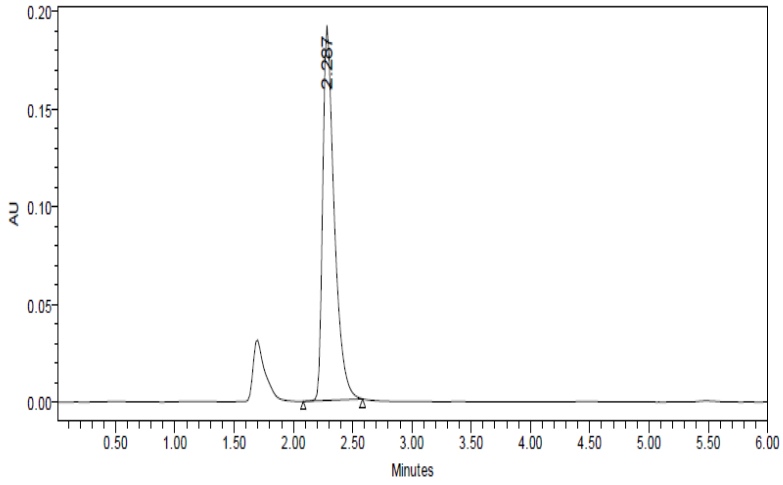

Figure 3: Standard Chromatogram of Aliskiren

Table7: Developed Chromatographic Conditions

Parameters	**Method**
Stationary phase (column)	Symmetry C_{18} (150 × 4.6 mm, packed with 5 µm)
Mobile Phase	60:40 (Acetonitrile : Phosphate Buffer)
pH	3.5 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	6.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	234
Drugs RT (min)	2.28

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Aliskiren in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it is suitable for the routine analysis of Aliskiren e in pharmaceutical dosage form.

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