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A VALIDATED RP-HPLC METHOD FOR DETERMINATION OF ALISKIREN AND AMLODIPINE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, rapid, precise, specific and cost effective reverse phase high performance liquid chromatography (RP-HPLC) method have been developed and subsequently validated for simultaneous estimation of Aliskiren and Amlodipine in pharmaceutical dosage forms. Chromatography is carried out isocratically at 30°C ± 0.5°C on an Water's X-bridge C-18 column (4.6 x 150mm, 5µ particle size) with a mobile phase composed of acetonitrile - phosphate buffer (pH-2.5) (40:60, v/v) at a flow rate of 1.0 mL/min. Detection was carried out using a PDA detector at 230 nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the International Conference on Harmonization guidelines. The retention times for Aliskiren and Amlodipine are 3.8 min and 5.1 min respectively. The linearity range for Aliskiren and Amlodipine are 18.75-187.5µg/ml and 1.25-12.5 µg/ml respectively. The correlation coefficients for both components are close to 1. The relative standard deviations for six replicate measurements of samples in tablets are always less than 2%.

Keywords: RP-HPLC, Aliskiren, Amlodipine, Simultaneous estimation

INTRODUCTION

Aliskiren (ALI) is chemically named as (2*S*,4*S*,5*S*,7*S*)-5-amino-*N*-(2-carbamoyl-2,2-dimethyl ethyl)-4-hydroxy-7-{[4-methoxy-3-(3-methoxyprop-oxy)phenyl]methyl}-8-methyl-2-(propan-2-yl)nonan amide (Figure 1). It is a first in a class of drugs called direct renin inhibitors[1-2]. Amlodipine (AML) is chemically named as (*RS*)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate[2] (Figure 2). It is a long-acting calcium channel blocker used as an anti-hypertensive and in the treatment of angina[3]. Various UV and HPLC assay methods are reported in the literature for the estimation of Aliskiren[4-6] and Amlodipine[7-8] individually and in combination with other drugs[9-12]. According to literature survey there is no method reported for the simultaneous estimation of Aliskiren and Amlodipine by RP HPLC in combined tablet dosage forms. Hence, an attempt has been made to develop and validate new method in accordance with ICH guidelines[13-15].

MATERIALS AND METHODS

Instrumentation: Chromatography was performed with Water's 2695 HPLC system provided with Hamilton Syringe, autosampler and Photodiode array detector. Detector is connected to software Empower for controlling the instrumentation as well as processing the data generated was used. The column used was a Water's X-Bridge C-18 Column 5 µm 4.6 × 150 mm. The mobile phase was prepared daily, filtered through a 0.45 µm membrane filter.

Reagents and chemicals: Pharmaceutically pure sample of Aliskiren and Amlodipine were obtained

from Dr. Reddy's Laboratories, Hyderabad as gift samples along with their analytical reports. Acetonitrile of HPLC grade was obtained from Merck chemical division, Mumbai and Commercial tablet of Aliskiren (150mg), and Amlodipine(10mg), TEKAMLO were procured from the local drug market

Chromatographic condition: The isocratic mobile phase consisted of acetonitrile: phosphate buffer (pH-2.5) in the ratio of 40:60v/v at a flow rate of 1.0 ml min-1. A Water's X-bridge C-18 column (4.6 x150mm, 5µ particle size) was used as the stationary phase. Although the ALK and AML have different λmax, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs; 230 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution: Standard stock solutions were prepared by dissolving 75 mg of Aliskiren drug and 5mg of Amlodipine into a clean and dry 50 ml volumetric flask add 30 ml of diluent, then volume was made up to 50 ml with diluent to get a concentration of 1500 µg/ml of Aliskiren and 100 µg/ml of Amlodipine(Stock Solution).

Preparation of Working Standard Solutions: Aliquot of 0.125ml, 0.25ml, 0.375ml, 0.5ml 0.75ml and 1.25ml were pipette out from stock-A into 10 ml volumetric flask separately and volume was made up to 10ml with diluent. This gives the solutions of 18.75µg/ml, 37.5µg/ml, 56.25µg/ml, 75µg/ml 112.5µg/ml and 187.5µg/ml respectively for Aliskiren, and 1.25µg/ml, 2.5µg/ml, 3.75µg/ml, 5µg/ml 7.5µg/ml and 12.5µg/ml respectively for Amlodipine.

Sample preparation: Twenty tablets of TEKAMLO containing ALK and AML 150mg:10mg, respectively were weighed and crushed to fine powder. Powder equivalent to 750mg Aliskiren and 50mg of Amlodipine was weighed and dissolved in 500 ml diluent, sonicated for 25 min and filtered through PVDF 0.45µ filter. From the filterate 0.5 ml was pipeted and transferred into a 10ml volumetric flask and the solution was made up to the volume with diluents.

Method validation: Validation parameters like system suitability, specificity, linearity, precision, accuracy, LOD, LOQ and solution stability were performed as per ICH guidelines.

RESULTS AND DISCUSSION

Chromatography: Initially reverse phase LC separation was tried to develop using Acetonitrile and 0.1%OPA buffer (50:50) as mobile phase, in which both the drugs responded properly, but the resolution was poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH 2.5 both drugs eluted with better separation. Thereafter, acetonitrile-phosphate buffer (pH-2.5) (40:60 v/v) was selected to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 210nm to 310nm. The wavelength at which both Aliskiren and Amlodipine showed maximum absorption (230nm) was selected for our proposed method. The chromatogram obtained was shown in the figure 3.

Validation of the proposed method

System suitability: System suitability parameters like number of theoretical plates and peak tailing were determined. The values for the parameters were shown in the table-1.

Specificity: A study conducted to establish specificity of the proposed method involved injecting blank and placebo using the chromatographic conditions defined for the proposed method. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample.

Linearity: Linearity was performed by preparing mixed standard solutions of Aliskiren and Amlodipine at different concentration levels including working concentration mentioned in experimental condition i.e., respectively. Ten microlitres of each concentration was injected in triplicate into the HPLC system. The response was read at 230 nm and the corresponding chromatograms were recorded and tabulated. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. The linearity response for both drugs Aliskiren and Amlodipine was between 18.75-187.5µg/ml (Figure 4) and 1.25-12.5 µg/ml (Figure 5).

Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at three concentration levels of 50%, 100% and 150%. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD at each level was calculated and results are presented in Table 2. Satisfactory recoveries ranging from 100.39% for Aliskiren and 101.19% for Amlodipine respectively were obtained by the proposed method. This indicates that the proposed method was accurate.

Precision:

Repeatability: Six replicates of same concentrations were analyzed in same day for repeatability and results were found to be within acceptable limits (RSD <2) as shown in table 3**.**

Intermediate Precision: Six replicates of same concentrations were analyzed on two different days and two analysts for day to day and analyst to analyst variation and results were found to be within acceptable limits(RSD<2) as shown in table 3.

Robustness: The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. The samples of Aliskiren and Amlodipine were analyzed under these changed experimental conditions. The change was made in the ratio of mobile phase by ±5%, column temperature $\pm 5^0$c and the flow rate ±0.1mL. There were no significant changes in the chromatography pattern when the above modifications were made in the experimental conditions, showing that the method is robust.

Stability of sample solution: the sample solution injected after 24hr did not show any appreciable change. Results are shown in table 4.

LOD and LOQ: LOD and LOQ were determined by calibration curve method. ALI and AML solutions were prepared in the concentration range of 18.75-187.5µg/ml for and 1.25-12.5 µg/ml, respectively and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations.

$LOD= (3.3\times Syx)/b$, $LOQ= (10.0\times Syx)/b$

Where Syx is residual variance due to regression; b is slope. LOD and LOQ for ALI were 0.473704 and 1.435468 µg/ml respectively and for AML were 1.496712 and 4.535492 µg/ml respectively.

Tablet analysis: Twenty tablets were weighed and calculated the average weight of each tablet. Then the tablets were powdered and weight equivalent to 5 tablets were transferred into a 100 mL volumetric flask, 80mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.1ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent. Table 5 shows results obtained by the method for amount of ALI and AML present in the tablets. The low values of RSD indicate that the method is precise and accurate.

CONCLUSION

Simultaneous estimation of Aliskiren and Amlodipine in tablet dosage form by RP-HPLC method was developed and validated. For both the drugs Aliskiren and Amlodipine, the regression value was found to be 0.999, which shows the linear response from 18.75-187.5µg/ml for Aliskiren and 1.25-12.5 µg/ml for Amlodipine. Selectivity experiment showed that there is no interference or overlapping of peaks either due to excipients or diluents with the main peak of Aliskiren and Amlodipine. The % RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 8 min for eluting both the drugs. So, this method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

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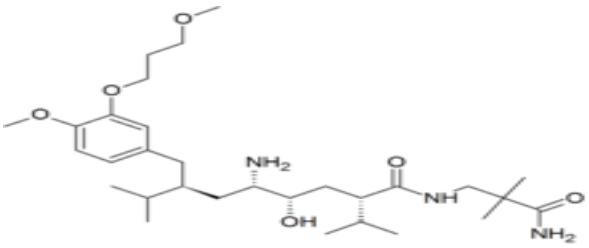

Figure1: Structure of Aliskiren

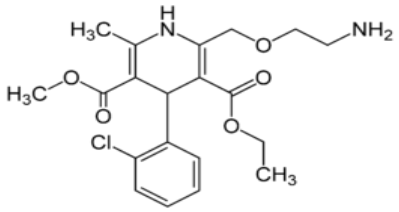

Figure 2: Structure of Amlodipine

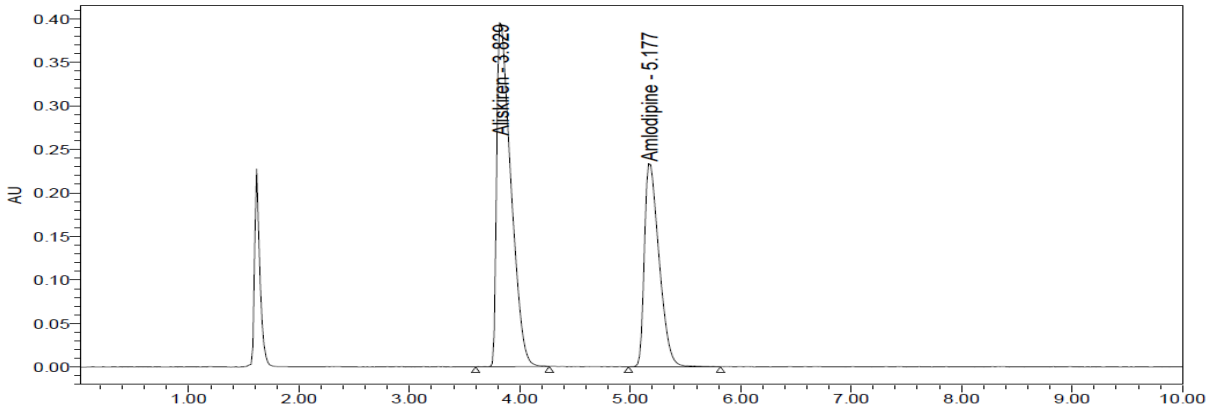

Figure 3: Chromatogram of Aliskiren and Amlodipine

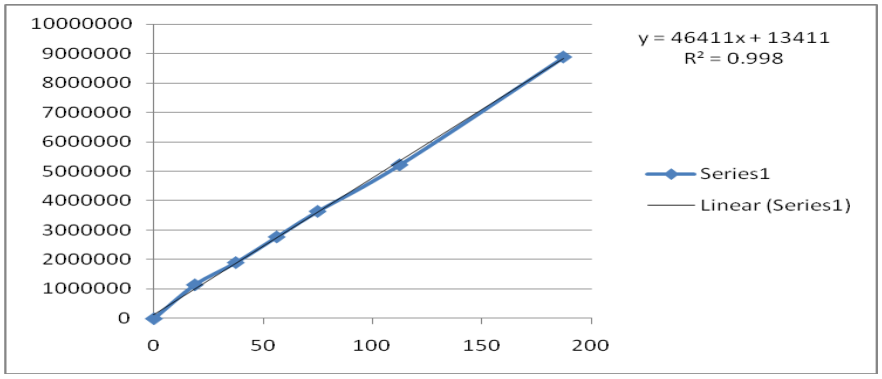

Figure 4: Calibration curve for Aliskiren

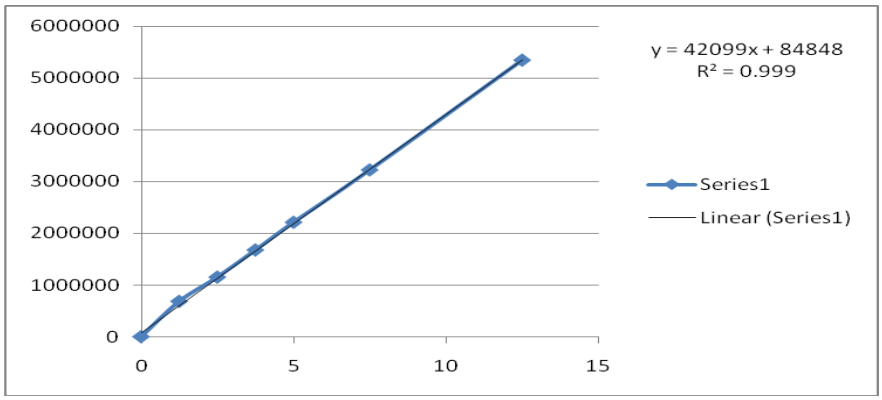

Figure 5: Calibration curve for Amlodipine

Table 1: System suitability of Aliskiren and Amlodipine

PARAMETERS	ALI	AML
No. of theoretical plates	7156	9358
Tailing factor	1.26	1.08
Area mean	3525629	2133241
SD	16496.06	10000.71
%RSD	0.467	0.468
Resolution	-	6.4

Table 2: Results of Accuracy of Aliskiren and Amlodipine

	Spiked amount (ppm)		% Recovered	
	ALI	**AML**	**ALI**	**AML**
50%	37.5	2.5	101.98	98.12
	37.5	2.5	99.70	102.15
	37.5	2.5	101.36	103.00
100%	75	5	100.98	101.65
	75	5	100.53	100.93
	75	5	100.25	100.55
150%	112.5	7.5	99.36	99.34
	112.5	7.5	99.16	102.97
	112.5	7.5	100.20	102.02
		MEAN	100.39	101.19
		SD	0.930	1.641
		%RSD	0.92	1.62

Table 3: Results of Precision of Aliskiren and Amlodipine

Sample	Repeatability		Day to Day	
	ALI	**AML**	**ALI**	**AML**
1	3476457	2113253	3471163	2115068
2	3503968	2129370	3475762	2103860
3	3476268	2107541	3478116	2113735
4	3480761	2097923	3469594	2116223
5	3492564	2128393	3457320	2120928
6	3498244	2102499	3460509	2107514
%Mean	3488044	2113163	3468744	2112888
SD	11866.08	13207.67	8270.233	6190.611
%RSD	0.34	0.62	0.23	0.29

Table 4: Stability data of Aliskiren and Amlodipine

Drug	% Assay at 0 hr	% Assay at 24 hr
ALI	98.43	98.75
AML	100.22	99.78

Table 5: Results for HPLC Analysis of Tablets

Sample No.	% Assay	
	ALI	**AML**
1	98.11	98.76
2	98.88	99.51
3	98.1	98.49
4	98.23	98.04
5	98.56	99.47
6	98.72	98.26
AVG	98.43	98.75
SD	0.333	0.617
%RSD	0.33	0.62

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