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Research Article

HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ALISKIREN AND AMLODIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of aliskiren and amlodipine in bulk and capsule dosage form. A column of Altima (150mm 4.6mm; i.d and 5µ particle size) was used. The mobile phase comprises of 0.02M di potassium hydrogen orthophosphate buffer (pH adjusted to 3.1) and acetonitrile in the ratio of 15: 85 (v/v). The flow rate was 1.0 ml/min and the effluents were monitored at 250 nm. The retention time for aliskiren was 2.578min and amlodipine was 3.187min.The detection concentration was linear over 45-270ppm for aliskiren and 3-18ppm for amlodipine. Regression equation of aliskiren and amlodipine were found to be y = 19702x + 4807 and y = 86417x + 2088 respectively with regression co-efficient 0.999. The developed method was successfully validated in accordance to ICH guidelines. Hence, this method can be conveniently adopted for the routine analysis in quality control laboratories.

INTRODUCTION

Aliskiren is a direct renin inhibitor, decreasing plasma renin activity and inhibiting conversion of angiotensinogen to angiotensin I.Aliskiren blocks the clinical effect of increased renin levels. Its current licensed indication is essential (primary) hypertension. While used for high blood pressure other better studied medications are typically recommended due to concerns of higher side effects and less evidence of benefit. [1,2]

Chemically aliskiren is (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 [3] with chemical formula $C_{30}H_{53}N_3O_6$•HCl and molecular weight 588.22 g/mol.

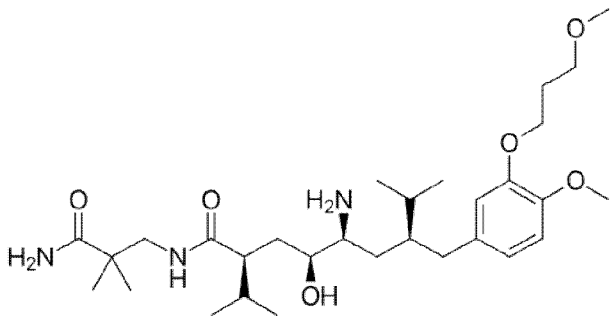

Figure 1 Structure of aliskiren

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Amlodipine belongs to a class of medications called calcium channel blockers (CCBs). These medications block the transport of calcium into the smooth muscle cells lining the arteries of the heart (coronary arteries) and other arteries of the body. Since the action of calcium is important for muscle contraction, blocking calcium transport relaxes arterial muscles and expands (dilates) coronary arteries and other arteries of the body. By dilating coronary arteries, amlodipine increases the flow of blood to the heart and is useful in preventing heart pain (angina) resulting from reduced flow of blood to the heart caused by coronary artery spasm (contraction). [4,5]

Amlodipine is chemically (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate with molecular formula $C_{20}H_{25}ClN_2O_5$ and molar mass 408.879 g/mol.

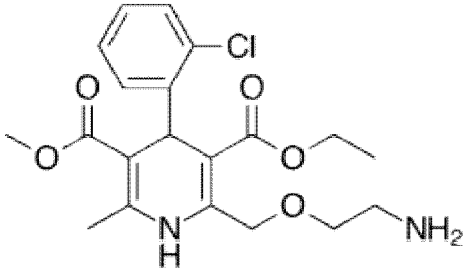

Figure 2 Structure of amlodipine

Few HPLC methods were developed for estimation of aliskiren and amlodipine were reported [7-14]. The developed

method was successfully validated in accordance to ICH guidelines [6]. The results of the study showed that the proposed RP-HPLC method is useful for the routine determination of aliskiren and amlodipine in bulk drug and in its pharmaceutical dosage form.

MATERIALS AND METHODS

Aliskiren and amlodipine were obtained as a gift sample from Hetero Drugs Ltd. Hyderabad. Acetonitrile, methanol, potassium dihydrogen phosphate and ortho-phosphoric acid used were of analytical grade. Commercially available aliskirine capsules (Rasilez ®-150 mg) and amlodipine (Norvasc ®-5mg) were procured from local market.

Instruments

Quantitative HPLC was performed on Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing powered with Empower-2 Software. An Altima column of 150mm 4.6mm: i.d and 5µ particle size was used. PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for UV measurements.

Preparation of standard solution

Accurately weighed 18mg of aliskiren and 3mg of Amlodipine were taken into 10ml and 25ml clean dry volumetric flasks, diluted with 3/4 ml of diluents. The contents sonicated for 5 minutes and made up to the final volume with diluents. The concentrations of Aliskiren and Amlodipine obtained were 1800µg\ml and 120 µg\ml respectively.

1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml with diluents. The final concentration of aliskiren and amlodipine were 180 µg\ml and 12 µg\ml respectively.

Preparation of working standard

From the standard solutions 0.2ml each were pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Preparation of mobile phase

A mixture of 35% buffer and 65% of acetonitrile prepared, degased in ultrasonic water bath for 5 minutes. Contents filtered through 0.45 µ filter under vacuum filtration.

Preparation of Buffer (0.01 KH_2PO_4)

Accurately weighed 2.72gm of Potassium di-hydrogen phosphate (KH_2PO_4) taken a 1000ml volumetric flask, added about 900ml of milli-Q water, degassed and finally made upto the volume with water. The pH adjusted to 3.1 with dilute ortho phosphoric acid.

Experimentation

System Suitability

The system suitability studies were evaluated by comparing with standard chromatogram and by obtaining the parameters retention time, column efficiency and tailing factor. All the system suitability parameters are within range and satisfactory as per ICH guidelines [6].

Table 1 System suitability data of Aliskiren and Amlodipine

Property	Aliskiren	Amlodipine
Retention time	2.5± 0.3 min	3.1±0.3min
Theoritical plates	4740 ± 163.48	6245± 163.48
Tailing factor	1.41± 0.117	1.25± 0.117

Linearity

Aliquots of standard drug stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with the diluent such that the final concentrations of aliskiren was in the range of 0-270 µg/ml and for amlodipine was 0-18µg/ml. Each of these drug solutions (20 µL) was injected three times into the column, the peak area and retention time were recorded. Evaluation was performed with PDA detector at 250 nm. Six linear concentrations of aliskiren (45-270ppm) and amlodipine (3ppm -18ppm) were prepared and injected [6]. Regression equation of aliskiren and amlodipine were found to be y = 19702x + 4807 (Figure 3) and y = 86417x + 2088(Figure 4). The regression co-efficient was 0.999. (Table 6)

Table 2 Calibration data of Aliskiren and Amlodipine

S.no	Concentration Aliskiren (µg/ml)	Response	Concentration Amlodipine (µg/ml)	Response
1	0	0	0	0
2	45	901306	3	270487
3	90	1778308	6	529287
4	135	2662859	9	776821
5	180	3556267	12	1014269
6	225	4410829	15	1289069
7	270	5342532	18	1578966

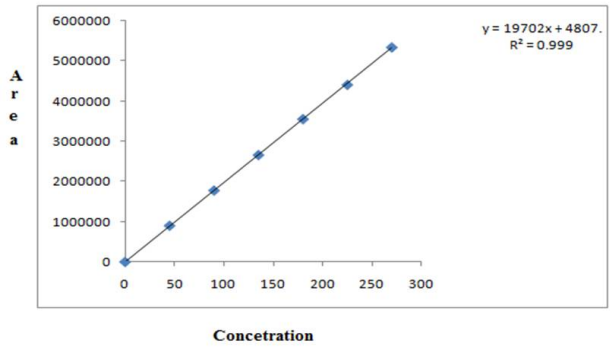

Figure 3 Calibration Curve of Aliskirene

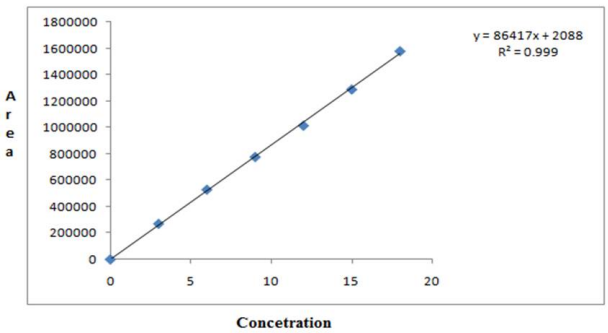

Figure 4 Calibration Curve of Amlodipine

Assay studies

Standard preparations were prepared from the standards and sample solutionsof formulation [6]. Six homogeneous samples of both sample and standard were injected.

Percentage assay of the drug in the formulation was estimated. The average % assay was calculated and found to be 100.15% and 100.05% for aliskiren and amlodipine respectively. The assay data was tabulated in Table3.

Table 3 Assay data Aliskiren and Amlodipine

S. No.	Aliskiren %Assay	Amlodipine%Assay
1	100.46	101.48
2	99.29	99.34
3	99.40	99.64
4	100.82	100.67
5	100.47	100.48
6	100.47	98.68
AVG	100.15	100.05
SD	0.6400	1.019
%RSD	0.64	1.02

mean ($\overline{x}$) - $\frac{\text{Sum of six observations (X)}}{6\ (N)}$

Standard deviation (S) - $\sqrt{(\Sigma(x-\overline{x})^2/N-1)}$ where x is absorbance

% RSD - $S*100/\overline{x}$

Precision

Intraday precision was performed and % RSD for aliskiren and amlodipine were found to be 0.64% and 1.02% respectively.(Table 4)

Table 4 Intraday precision studies

S. No.	Aliskiren	Amlodipine
1	3595695	1111223
2	3553898	1087754
3	3557781	1091036
4	3608685	1102346
5	3595972	1100184
6	3595970	1080463
Mean	3584667	1095501
SD	22906.9	11154.0
%RSD	0.64	1.02

Interday precision was performed with 24 hrs time lag and the %RSD obtained for aliskiren and amlodipine were 0.13% and 0.12%.(Table 5)

Table 5 Inter day precision studies

S. No.	Aliskiren	Amlodipine
1	3461009	1020292
2	3451205	1021233
3	3458388	1020411
4	3462553	1022681
5	3458635	1022702
Mean	3458358	1021464
SD	4355.1	1177.87
%RSD	0.13	0.12

mean ($\overline{x}$) - $\frac{\text{Sum of six observations (X)}}{6\ (N)}$

Standard deviation (S) - $\sqrt{(\Sigma(x-\overline{x})^2/N-1)}$ where x is absorbance

% RSD - $S*100/\overline{x}$

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD* and LOQ** of the drug were calculated using the following equations designated by International Conference on Harmonization (ICH) guideline [6].

* LOD = 3.3 × /S

**LOQ = 10 × κS

Where p= the standard deviation of the response *

S = Slope of calibration curve

LOD for aliskiren and amlodipine were found to be 0.81ppm and 0.08ppm respectively. LOQ for aliskiren and amlodipine were found to be 2.44 and 0.24 respectively. (Figure 5 and Figure 6).

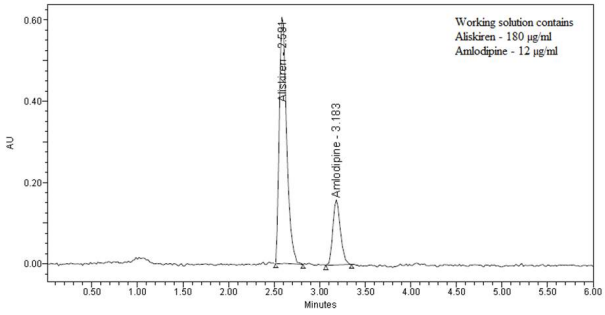

Figure 5 LOD Chromatogram of Aliskiren and Amlodipine

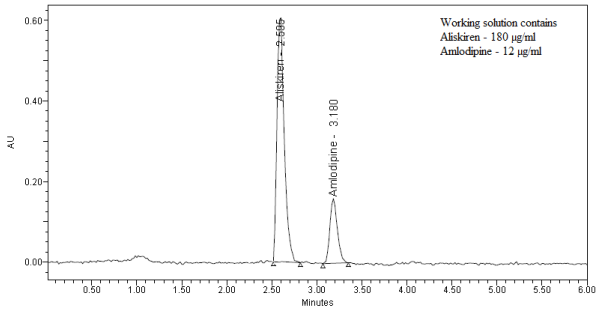

Figure 6 LOD Chromatogram of Aliskiren and Amlodipine

Table 6 Detection characteristics of alisliren and amlodipine

Parameters	Aliskiren	Amlodipine
Calibration range (µg / ml)	45-270ppm	3-18ppm
Optimized wavelength	240nm	240nm
Retention time	2.5min	3.1min
Regression equation (Y)	y = 19702x + 4807	y = 86417x + 2088.
Correlation coefficient(r^2)	0.999	0.999
Precision (% RSD)	0.64	1.02
% Recovery	100.22%	100.11%
Limit of Detection (µg / ml)	0.81ppm	0.08ppm
Limit of Quantitation (µg / ml)	2.44ppm	0.24ppm

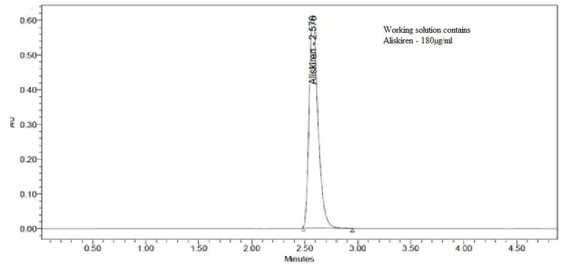

Figure 7 Chromatogram of Aliskiren

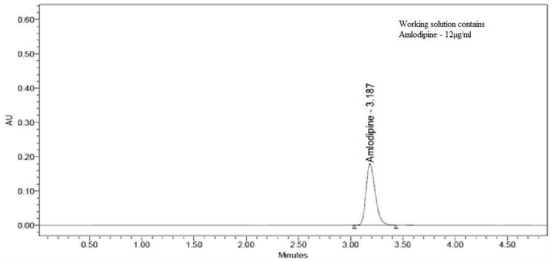

Figure 8 Chromatogram of Amlodipine

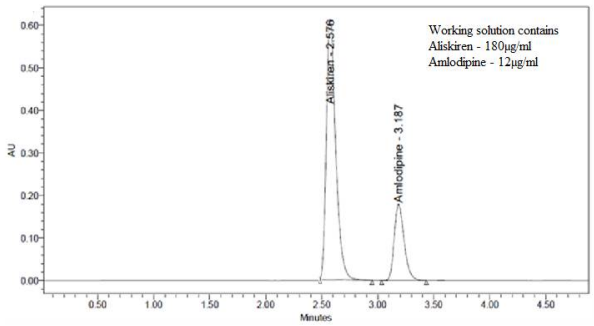

Figure 9 Typical chromatogram of aliskiren and amlodipine

RESULTS AND DISCUSSION

From the typical chromatogram of drugs as shown in Figure 9, the retention time were found to bealiskiren is 2.578min and amlodipine is 3.187min. The mobile phase used was 0.02M di potassium hydrogen orthophosphate buffer (pH adjusted to 3.1) and acetonitrile in the ratio of 15: 85 (v/v) over 1.0 ml/min minutes in gradient mode of separationwhich was found to be most suitable to obtain a peak well defined and free from tailing.

In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r=0.9999) was observed between the concentration range of 45-270 µg/ml for aliskiren and 3-18µg/ml for amlodipine. Low values of standard deviation are indicative of the high precision of the method. (Table 6).

The assay of aliskiren was 100.32% and amlodipine was 101.48% (Table 3). The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the formulation. The limit of detection (LOD) and limit of quantification (LOQ) for aliskiren were found to be 0.81 and 2.44 ppm; for amlodipine were 0.08 and 0.24ppm respectively.

This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and Tablets dosage form of the drugs within a short analysis time.

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