ICH GUIDELINE PRACTICE: A VALIDATED STABILITY INDICATING RP-UPLC METHOD DEVELOPMENT AND ITS APPLICATION FOR DETERMINATION OF ALISKIREN AND AMLODIPINE IN BULK AND FORMULATION

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ABSTRACT

A novel, accurate, specific and robust reverse phase liquid chromatographic method was developed and validated for the simultaneous estimation of Aliskiren and Amlodipine besylate in bulk and tablet dosage form. A reverse phase stability indicating Ultra High-Performance Liquid Chromatography (UHPLC) method was developed on an SD 3*100 mm x 1.8 μ column using isocratic elution of acetonitrile and 0.1% perchloric acid buffer (pH 2.6-2.8) in the ratio 60: 40 at a flow rate of 0.5 ml/min. The detection was carried out by using Acquity TUV ChA at 266nm. The total run time was 2.5 min and the retention time of amlodipine and Aliskiren were found to be 1.006 and 1.329 min respectively. According to ICH guidelines, forced degradation conditions were employed to establish the stability indicating method. The LOD and LOQ of Aliskiren and Amlodipine were found to be 0.17 μ g/ml, 0.51 μ g/ml and 0.03 μ g/ml, 0.10 μ g/ml respectively. The developed method was validated as per ICH guidelines for linearity, accuracy, precision, robustness and stability indicating studies and the method was successfully applied for the estimation of aliskiren and amlodipine in combined tablet dosage form.

Keywords: Aliskiren, Amlodipine, UHPLC, Stability indicating.

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INTRODUCTION

Hypertension is a chronic disease associated with other disorders like diabetics and many cardiovascular diseases including stroke, myocardial infarction and congestive heart failure. It leads to morbidity and mortality, if left uncontrolled, hence it is essential to control and manage blood pressure.^{1,2} There are several antihypertensive agents available with a different mechanism of action may play an important role in the optimal management of hypertension. It includes mono and combination therapy with β - blockers, diuretics, angiotensin- converting enzyme (ACE) inhibitors, angiotension II receptor blockers (ARB) and calcium channel antagonists.^{3,4}

Aliskiren chemically, (2S,4S,5S,7S)-5-amino-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-7-[[4-methoxy-3-(3-methoxypropoxy)phenyl]methyl]-8-methyl-2-propan-2-ylnonanamidehemifumarate.⁵ It is a non-peptide, first orally taken direct renin inhibitor simulating endogenous peptides approved for clinical use in the treatment of hypertension from the United States Food and Drug Administration (FDA).^{6,7} It reduces plasma renin activity through high-affinity binding and specificity via aromatic side chains.^{8,9}

The drug substance is a single diastereoisomerx having 4 chiral centers, all S-configured, presented as a white to slightly yellowish crystalline powder. The active is the hemifumarate salt of the corresponding amine, with a molecular weight of 609.8.

Its drug substance is highly soluble in water Acetate Buffer-pH 5.2, and Phosphate Buffer-pH 7.0.¹⁰ ¹¹Aliskiren reduces baseline systolic and diastolic blood pressure which is as effective as other first-line antihypertensive agents. Extra advantages can be reached when it is used in combination therapy.¹¹ Amlodipine besylate (AML; 3-ethyl 5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4- (2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate) (Fig.-1B) is a dihydropyridine long-acting calcium channel blocker that prevents the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle.¹² Amlodipine reduces blood pressure by acting directly on vascular smooth muscles leads to lessening in peripheral vascular resistance. Hence acts as a peripheral arterial vasodilator.^{13,14} The chemical structure of Aliskiren and Amlodipine are in the Fig.-1.

Fig.-1

A variety of methods are in faddy for estimation of Aliskiren as an isolated sample or together with other antihypertensive drugs in more complicated matrices such as pharmaceutical formulations and biological fluids. These methods have been based on spectrophotometry,¹⁵⁻¹⁸ spectrofluorometry,^{19,20} electrophoresis^{21,22} liquid chromatography,²³⁻²⁶ LC-MS/MS,²⁷ UPLC- MS/MS²⁸ and HPTLC.²⁹ Similarly some analytical methods have been presented for the estimation of Amlodipine as a single analyte³⁰ and in the presence of different antihypertensive agents.³¹⁻⁴⁰ Previously at the reference 35 and 36 there are two HPLC methods performed for the analysis of Aliskiren and Amlodipine but they are with longer run time of more than 10 minutes, stability test has not been performed in the reference 35 and in 36 reference stability studies have been performed but neutral studies not conducted and the retention time of both methods were above 3.5 and 5 minutes for Aliskiren and Amlodipine. But here in our method, we tried to develop with a total run time of 2.5 minutes. However, to the best of our knowledge, there was no reported RP – UHPLC stability method for simultaneous estimation of Aliskiren and Amlodipine in pharmaceutical formulations, previous to our work. Thus, efforts were made to develop fast, selective and sensitive analytical method for the estimation of Aliskiren and Amlodipine in their fixed dose binary formulation (Tekamlo) and drug substance using the reverse phase ultra-high performance liquid chromatographic method. Through the principle of UPLC and HPLC are the same but not the

performance. This is due to particle size of the column which is less than 2 μm in UPLC. Various advantages like higher resolution coupled with less runtime and enhanced sensitivity compared to HPLC. Solvent consumption is decreased, a decrease of the solvent disposal and its cost. Trough HPLC we can get good data but UPLC produces excellent data. The main advantage is increased resolution in half the runtime and one-tenth, the solvent cost.

In the current work, we developed a simple, environment-friendly, reliable and reproducible RP- UHPLC method which was duly validated by statistical parameters precision, accuracy, linearity and robustness. The method has been satisfactorily applied to the simultaneous estimation of Aliskiren and Amlodipine in bulk and pharmaceutical dosage forms.

According to the ICH guidelines, the developed was validated for the linearity, precision, robustness, specificity and stability studies.⁴¹

EXPERIMENTAL

Chemicals and Reagents

The pharmaceutical working standards of Aliskiren and Amlodipine were procured as a gift sample from spectrum pharma research solutions, Hyderabad. Fixed-dosage combination of a commercial tablet containing 300 mg Aliskiren and 10 mg Amlodipine (Tekamlo) was purchased from local market. The HPLC grade water and analytical grade perchloric acid which was procured from SDFCL chemicals, the HPLC grade Acetonitrile, methanol and analytical grade ortho-phosphoric acid were purchased from Rankem.

Chromatographic Conditions

The chromatographic separation was carried out by Waters Acquity UPLC with the binary solvent manager, equipped with Tunable UV detector and auto sampler. The Empower 2 software was used for signal monitoring, data collection and data processing. For photolytic degradation UV chamber was used and for thermal degradation hot air oven has been employed. Isocratic separation was achieved on an SD 3*100mm x 1.8 μm column.

Mobile Phase

Buffer and Acetonitrile were taken in the ratio of 40:60. The perchloric acid buffer was prepared by using 1 ml of perchloric acid, taken in a 1000 ml of volumetric flask and add about 900 ml of milli-Q water and sonicate it to degas and finally make up the volume with HPLC grade water. The flow rate of mobile phase was maintained at 0.5 ml/min. The column temperature was maintained at 30 $^{\circ}$ C and the detection was carried out at 266 nm with an injection volume of 0.5 μl .

Preparation of Standard Solution (300 $\mu\text{g/ml}$ Aliskiren & 10 $\mu\text{g/ml}$ Amlodipine)

Accurately Weighed and transferred 30 mg & 10 mg of Aliskiren and Amlodipine working Standards into a 10 ml and 100 ml clean dry volumetric flask respectively, add 5 ml and 50 ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1 ml was pipetted out into a 10 ml volumetric flask and then make up to the final volume with diluent. The diluent was prepared by using acetonitrile and water in 50: 50 ratio respectively.

Preparation of Sample Solution

20 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50 ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent.

RESULTS AND DISCUSSION

Method Development and Optimization of Chromatographic Conditions

In order to achieve good separation between the components present in the formulation, different buffer pH conditions and different proportions of solvents like methanol, acetonitrile, perchloric acid and water was tested. However, with perchloric acid buffer and acetonitrile mobile phase in the ratio 40: 60 achieved good satisfactory results at a flow rate of 0.5 ml/min measured at a detection of 266 nm.

Optimized conditions include:

Column: SD 3* 100mm×1.8 μ

Software: Empower 2

Temperature: 30°C

Flow rate: 0.5 ml/min

Injection volume: 0.5ml/min

Run time: 2.5 min

Detection: 266nm with Tunable UV detector

The chromatogram of the optimized standard mixture shown in Fig.-2. The system suitability parameters such as retention time, area, height, USP tailing and theoretical plates for optimized standard mixture chromatogram tabulated in Table-1.

Table-1: System Suitability Results

System Suitability Parameter	Amlodipine	Aliskiren
t _R (min)	1.006	1.329
Area	37383	172354
% Area	17.82	82.18
Height	24135	102857
USP Plate Count	11468.0	17390.4
USP Tailing	1.4	1.2
Resolution	-	8.2

Here t_R= Retention time

Method Validation

After the satisfactory development of the optimized method, it was subjected to method validation as per ICH guidelines.

System Suitability

System suitability is an integral part of the method validation to evaluate the parameters like tailing factor, theoretical plates, resolution and %RSD for replicate injections. The results were within the limits and were presented in Table-1. and Fig.-2. show the system suitability chromatogram.

Fig.-2: Optimized Standard Chromatogram of Aliskiren and Amlodipine

Accuracy

The accuracy of the proposed method was evaluated by calculating the recovery studies of the test drug at three different concentration levels (50%, 100%, 150%) by the standard addition method. A known amount of aliskiren and amlodipine was added to prequantified sample solution and three replicates of each concentration were injected in developed chromatographic conditions. The mean percentage recovery of aliskiren and amlodipine was varied between and indicating that the developed method was found to be accurate. The % recovery results were shown in Figs.-3,4,5 and Table-2.

Fig.-4: Chromatograms at 100 % Recovery Level (Triplicate Determination)

Fig.-5: Chromatograms at 150 % Recovery Level (Triplicate Determination)

Table-2: Accuracy Data

Recovery level	Amlodipine	Aliskiren
	Mean % recovery*	Mean % recovery*
50%	99.14 ± 0.93	99.59 ± 1.43
100%	98.46 ± 0.11	98.62 ± 0.58
150%	99.96 ± 0.24	99.86 ± 0.56

*% recovery from triplicate determinations

Precision

The precision of the method was investigated by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of Amlodipine and Aliskiren. System precision and method precision was found to be within permissible limits i.e., 1% and 2% respectively (Table-3). The intermediate precision of the method was also evaluated by performing analysis on different days.

Table-3: Results of Method Precision for Aliskiren and Amlodipine

Precision	Amlodipine		Aliskiren	
	Mean ± SD	%RSD	Mean ± SD	%RSD
Repeatability	36574 ± 156.5	0.4	171981 ± 1196.7	0.7
Intermediate precision	33435 ± 420	1.3	159622 ± 1460.4	0.9

Linearity and Range

Linearity was demonstrated from 25 to 150% of standard concentration using six calibration levels (25%, 50%, 75%, 100%, 125%, 150%) for both Aliskiren and Amlodipine. All the concentrations were prepared and injected into the system. A calibration curve was plotted for AUC against respective concentration. The plot was estimated using linear regression shown in Fig.-6, 7 and Table-4. From the results obtained the proposed method was found to be linear. The regression coefficient (r^2) was found to be 0.999 for both aliskiren and amlodipine.

Table-4: Results for Linearity

Level	Conc. of Amlodipine (ppm)	AUC for Amlodipine	Conc. of Aliskiren (ppm)	AUC for Aliskiren
25%	2.5	10294	75	45271
50%	5	19356	150	84898
75%	7.5	27507	225	130364
100%	10	36316	300	172647
125%	12.5	45777	375	212699
150%	15	54872	450	253462
	Slope	3607		563.3
	Y- intercept	673.6		1728
	R ²	0.999		0.999

Limit of Detection and Limit of Quantification

The LOD and LOQ of Aliskiren and Amlodipine were estimated by using the signal to noise approach and the signal-to-noise ratios are 3:1 and 10:1 respectively. The limit of detection and quantitation to be determined and for Aliskiren they are 0.17 µg/ml and 0.51 µg/ml, for Amlodipine they are 0.03 µg/ml and 0.10 µg/ml respectively.

Robustness

The robustness is a measure of minor deliberate changes in different chromatographic conditions like flow rate, mobile phase composition and column temperature did not significantly affect the recoveries, peak area and retention time of the drugs indicating that the proposed method was robust. % RSD was calculated, which found to be in the permissible limit (Table-5).

Fig.-6: Amlodipine – Linearity Curve

Table-5: Robustness Studies Results

Robustness Parameter	% RSD for Amlodipine	% RSD for Aliskiren
Flow rate (0.4 ml/min)	0.6	1.1
Flow rate (0.6 ml/min)	1.7	1.2
Mobile phase (35:65)	1.7	1.0
Mobile phase (45:55)	0.5	1.1
Temperature 25°C	0.8	0.6
Temperature 35°C	0.7	1.1

Fig.-7: Aliskiren – Linearity Curve

Analysis of Marketed Sample

The proposed method was applied for the analysis of Aliskiren and Amlodipine in tablet dosage forms. The marketed formulation (TEKAMLO tablets, 300 mg Aliskiren & 10 mg Amlodipine) was purchased from the local market. The % assay of the marketed formulation was found to be 99.8437% for Aliskiren and 99.3433% for Amlodipine and it was shown with the help of Fig.-8. and Table-6.

Fig.-8: Chromatogram of the Marketed Formulation

Table-6: Analysis of Tablet Formulation

Parameter	Aliskiren	Amlodipine
Mean Peak Area	172137	36406
Assay (%)	99.84	99.34
RSD (%)	0.8	0.8

Forced Degradation and Stability Indicating Test

Forced degradation / stress testing, defined as the stability testing of drug substance and drug product under conditions exceeding those used for accelerated testing. In order to establish whether the developed method was stability indicating both the drugs were stressed under various conditions (acid, base, oxidation, thermal) to perform forced degradation studies. An unstressed sample solution was also prepared as a blank in this study.

Acidic Degradation

To 1 ml of 0.1 N HCl add 6 ml of stock solution and was kept at 60°C for about 24 h in the water bath, cool made up the volume 10 ml with mobile phase. Filters the solution through 0.22 micron membrane filter.

Alkali Degradation

To 1 ml of 1.0 N NaOH add 6 ml of stock solution and was kept at 60°C for about 24 h in water bath, cool made up the volume 10 ml with mobile phase. Filters the solution through 0.22 micron membrane filter.

Neutral Degradation

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60°C. Cool it and made up the volume 10 ml with mobile phase. Filter the solution through a 0.22 micron membrane filter and inject into the system.

Oxidation Degradation

To 1 ml of 3%, H₂O₂ add 6ml of stock solution and was kept at 40°C for about 24 h in waater bath, cool made up the volume 10 ml with mobile phase. Filters the solution through 0.22 micron membrane filter.

Thermal Degradation

For thermal degradation hot air oven has been employed, the stock solution was kept at 65°C in a hot air oven for about 24 hours and injected into the chromatographic system.

UV Degradation

Photolytic degradation was carried out by using a UV chamber (UV light) and the sample was placed under it for a period of 24 hours and injected into a chromatographic system. The results of the stability studies were given in the Table-7.

Table-7: Forced Degradation Studies of Aliskiren and Amlodipine

Stress Condition	Amlodipine				Aliskiren			
	Area of Unstressed Sample	Area of Stressed Sample	% Degradation	Assay (%)	Area of Unstressed Sample	Area of Stressed Sample	% Degradation	Assay (%)
Acid degradation	36610	34919	4.61	95.29	172234	164045	4.75	95.15
Alkali degradation	36610	35724	2.42	97.48	172234	167748	2.6	97.30
Peroxide degradation	36610	36010	1.63	98.27	172234	169756	1.43	98.47
Photolytic degradation	36610	36282	0.89	99.01	172234	170893	0.77	99.13
Thermal degradation	36610	36403	0.56	99.34	172234	171387	0.49	99.41
Hydrolytic degradation	36610	36454	0.42	99.48	172234	170801	0.83	99.07

CONCLUSION

A simple, novel environmentally friendly UPLC method was developed and validated successfully for simultaneous determination of Aliskiren and Amlodipine. The method allows analysis of many samples in a short period of time and with less mobile phase. Both the drugs were found to be well resolved within a short run time of 2.5 mins. The developed method was validated according to ICH guidelines for accuracy, linearity, precision, stability and robustness. This method can be used successfully for the routine quality control analysis as well as for stability studies.

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