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SIMULTANEOUS ESTIMATION OF ALISKIREN AND AMLODIPINE BY RP-HPLC AND ITS VALIDATION

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ABSTRACT

A simple, accurate, economical and precise reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous determination of Aliskiren and Amlodipine. The separation was achieved on Intersil C18 column (250 x 4.6 mm, 5 µm) as stationary phase with a mobile phase comprising of Methanol: KH_2PO_4 (80:20) in an isocratic mode, at a flow rate of 1 ml/min. The detection was monitored at 239 nm. The retention time of Aliskiren and Amlodipine were 2.869 min and 3.942 min respectively. The linearity was found to be in the range of 20-80 µg/ml for Aliskiren and Amlodipine respectively with correlation coefficient of 0.999. The proposed method was validated according to ICH guidelines for parameters like linearity, accuracy, precision and specificity. All validation parameters were within the acceptable range. The developed method was successfully applied for the estimation of Aliskiren and Amlodipine in pure and pharmaceutical dosage form.

KEYWORDS: Aliskiren, Amlodipine, RP-HPLC, Validation, Simultaneous estimation, ICH guidelines.

INTRODUCTION

Aliskiren (Figure 1) (2(*S*),4(*S*),5(*S*),7(*S*)-*N*-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2, 7-diisopropyl-8- [4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamide hemifumarate[1] is used alone or together with other medicines to treat high blood pressure (hypertension). Aliskiren is a direct renin inhibitor, decreasing plasma renin activity (PRA)

and inhibiting the conversion of angiotensinogen to Ang I. Whether aliskiren affects other RAAS components, e.g., ACE or nonACE pathways, is not known. All agents that inhibit the RAAS, including renin inhibitors, suppress the negative feedback loop, leading to a compensatory rise in plasma renin concentration. When this rise occurs during treatment with ACEIs and ARBs, the result is increased levels of PRA. During treatment with aliskiren, however, the effect of increased renin levels is blocked so that PRA, Ang I and Ang II are all reduced, whether aliskiren is used as monotherapy or in combination with other antihypertensive agents. Aliskiren is more expensive than most other antihypertensive agents.[2]

Amlodipine (Figure 2) (*RS*)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate[3] (as besylate, mesylate or maleate) is a long-acting calcium channel blocker (dihydropyridine class) used as an anti-hypertensive and in the treatment of angina. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle.[4]

Literature survey of Amlodipine and Aliskiren revealed few methods based on UV Spectrophotometry[5-6] and Chromatography[7-8] have been reported for determination of both drugs in single and combined dosage forms. The present work describes the development and validation as per ICH guidelines[9] of reverse phase high performance liquid chromatographic (RP-HPLC) method, which can quantify these components simultaneously.

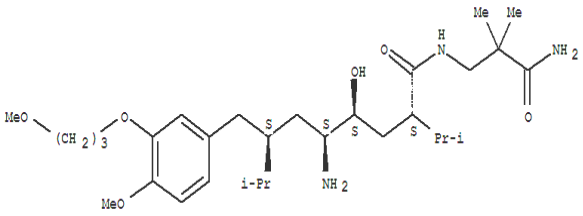

Fig.1: Chemical structure of Aliskiren.

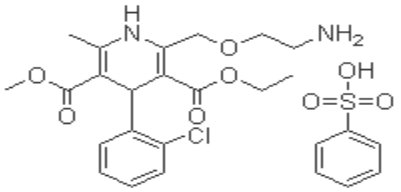

Fig.2: Chemical structure of Amlodipine.

2. EXPERIMENTAL

Materials and Methods

Reagents required

Methanol: HPLC grade, Merck

Water: HPLC grade, Merck

K_2HPO_4: AR grade.

KH_2PO_4: AR grade.

Drugs used

The gift samples of Aliskiren and Amlodipine were kindly provided by Novartis Pharmaceutical Ltd and the marketed formulations containing Aliskiren (150 mg) and Amlodipine (5 mg) were procured from local pharmacy (trade name: TEKAMLO).

Instrumentation and Chromatographic Conditions

The developed method HPLC system with UV detector data were acquired and processed by Empower software. The separation was carried out at ambient temperature by using a Intercil C_{18} (4.6 x 250mm, 5µm) column. The mobile phase consisting of Methanol: Buffer (80:20v/v). The flow rate was 1 ml/min. The injection volume was 20 µL and detection at a wavelength of 239 nm.

Preparation of buffer

3.8954g of di-sodium hydrogen phosphate and 3.4023 of potassium dihydrogen phosphate were weighed accurately and transferred into a beaker containing 1000ml of distilled water and dissolved completely. Then p^H was adjusted to 3.0 with orthophosphoric acid and then filtered through 0.45µm membrane filter.

Preparation of Mobile phase

Mix the 800 ml of Methanol (80%) and 200 ml of buffer (20%). Filter through 0.45 µ filter under vacuum filtration.

The mobile phase liquid is also used for making working dilution of drugs.

Preparation of stock solutions

Stock Solution A of Aliskiren and Amlodipine (1mg/ml and 1mg/ml respectively).

The stock solution of Aliskiren and Amlodipine were prepared by weighing accurately 100 mg of Aliskiren and 100 mg of Amlodipine pure drug and transferred to a 100ml volumetric flask and dissolved in the mobile phase and made up to the mark with mobile phase. Stock solution A was prepared.

Working Solution of Aliskiren and Amlodipine (100µg/ml and 100µg/ml respectively).

From the above Stock Solution A 10 ml was taken and transferred to a 100ml volumetric flask and made up to the mark with mobile phase. Working stock solution was prepared.

Linearity of pure standard solution

The linearity of the samples of Aliskiren and Amlodipine was prepared by suitably diluting working solution and found to be linear response of drug over a range of 20-60 µg/ml concentration for Aliskiren and Amlodipine respectively. The three such linearity's of Aliskiren and Amlodipine were taken for correlation co-efficient and standard deviation calculation.

Table 1: Area Of Different Concentration Of Aliskiren And Amlodipine Obeying Beer's Law.

S.No	Aliskiren(µg/mL)	Area(mV.s)	Amlodipine(µg/mL)	Area(mV.s)
1	20	159849	20	252726
2	30	245940	30	379850
3	40	334617	40	504286
4	50	430308	50	629167
5	60	511282	60	738585

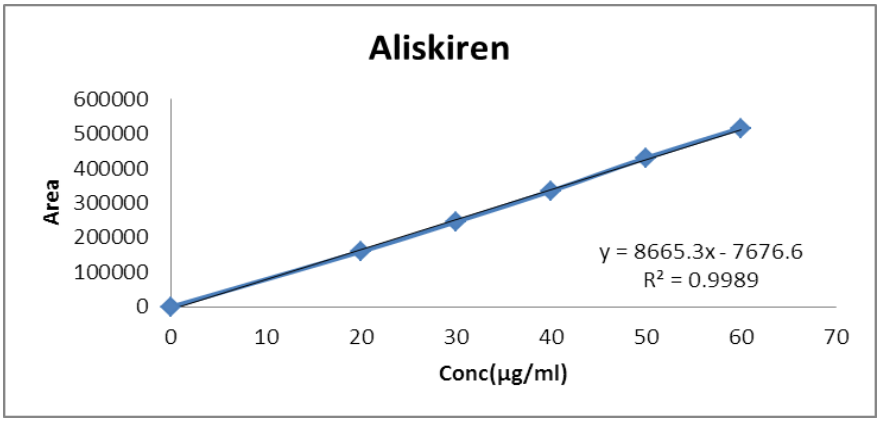

Fig 3: Graph showing Linearity of Aliskiren.

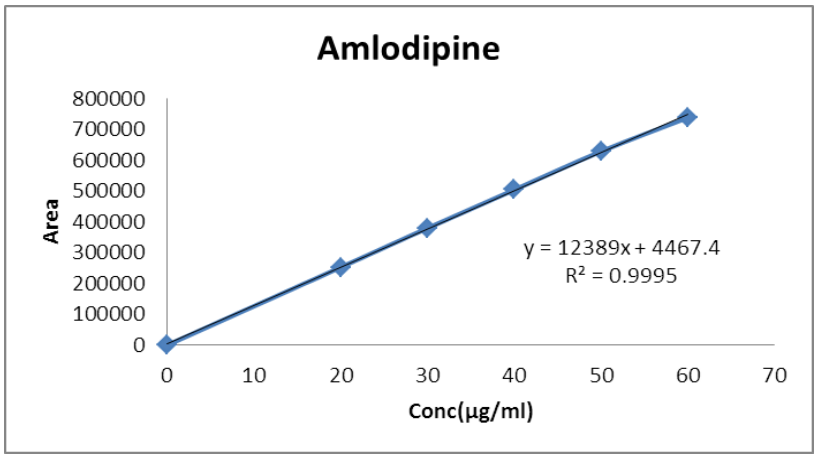

Fig 4: Graph showing Linearity of Amlodipine.

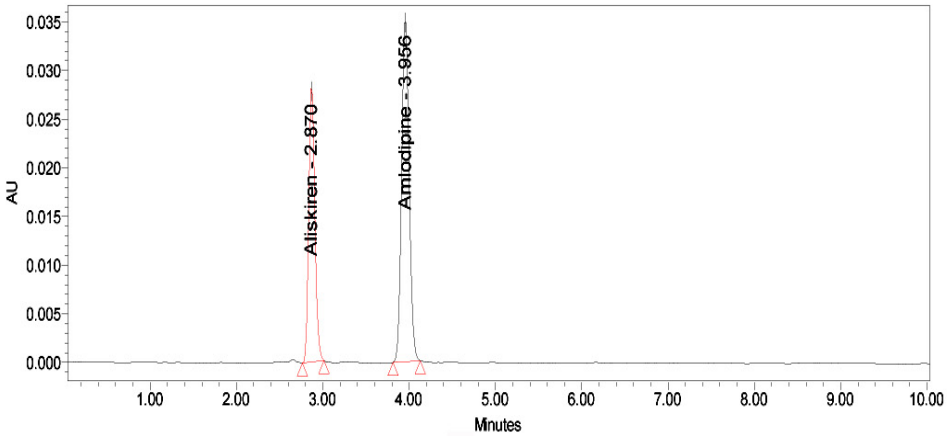

Fig. 5: Chromatogram showing peaks of Aliskiren and Amlodipine.

Preparation of Sample Solution

TEKAMLO of strength 150 mg of Aliskiren and 5 mg of Amlodipine. respectively. Average weight of twenty tab were taken and crushed to make powder, weighed powder containing 150 mg Aliskiren was transferred to 100ml of volumetric flask and volume was made up to the mark with diluent (Methanol: Buffer) (80:20) and filtered through whatmann filter paper in to another 100ml volumetric flask and make up to mark with same diluent which gives the solution of 1.5 mg/ml concentration of Aliskiren and 5 µg/ml of Amlodipine, further dilutions of the solution made to obtain the concentration of 150 µg/ml concentration of Aliskiren and 50 µg/ml of Amlodipine with the same diluent and again filter it with milli pore filter through syringe filter and used for further analysis. The same procedure as mentioned for the pure drug was followed for the formulation. The concentrations of both Aliskiren and Amlodipine were determined by measuring peak area at 239 nm.

Assay for Marketed formulation

The Assay performed by the marketed formulation of Aliskiren and Amlodipine (TEKAMLO). The prepared standard and sample solutions were injected into HPLC and peak areas were recorded. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of samples.

Table 2: Assay of marketed formulation.

Drug	Labeled amount (mg)	Amount found mg/tab	% Recovery	%RSD (n=5)
ALISKIREN	**150 mg**	**149.53**	**99.69**	**0.0012**
AMLODIPINE	**5 mg**	**4.92**	**98.40**	**0.0022**

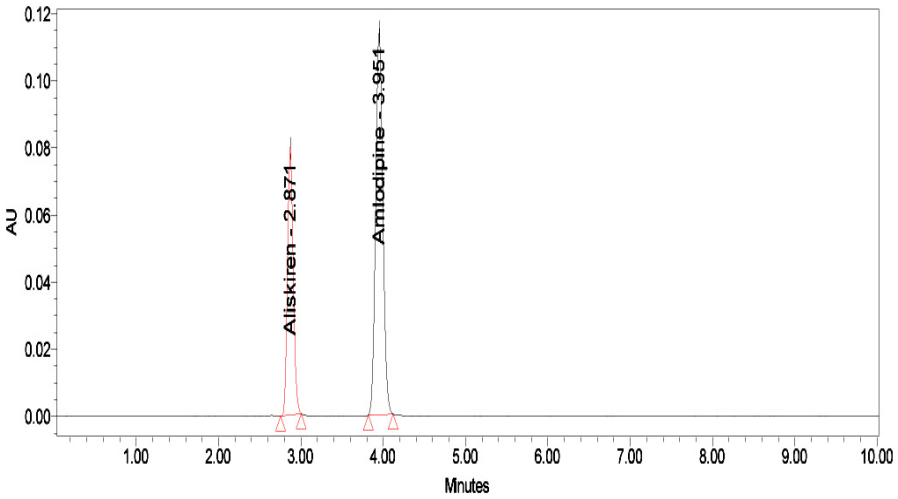

Fig.6: Chromatogram showing peaks of Aliskiren and Amlodipine of formulation.

VALIDATION OF HPLC METHOD

The HPLC method was validated in accordance with ICH guidelines.

Precision

System Precision for Aliskiren and Amlodipine: The system precision was evaluated by measuring the peak responses of Aliskiren and Amlodipine for five replicate injections of standard solution, prepared as the proposed method. The results shown in the table-3 indicate that the precision of the system is with in the limit. (Acceptance criteria: % rsd nmt 2.0%).

Method Precision for Aliskiren and Amlodipine was determined by preparing a sample solution of single batch Aliskiren and Amlodipine Tablet five times and analyzing as per

the proposed method. The results shown in Table-3 indicate that the proposed method is precise. (Acceptance criteria: % RSD NMT 2.0%.

Accuracy

To check the accuracy of the developed method and to study the interference of formulation excipients, recovery study was carried out by using standard addition method by adding 100% concentration to a fixed amount of the pre analysed sample and the amount of drug were analyzed by the proposed method. Results from the recovery studies are given in table 4 & 5.

Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of detection and limit of quantification were estimated from signal to noise ratio. LOD is the lowest concentration resulting in a peak area of three times the baseline noise and the equation is LOD = 3.3 x ASD/S. LOQ is the lowest concentration that provide signal to noise ratio more than 10 and the equation is LOQ = 10 x ASD/S, where 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The important parameter to be studied was the resolution factor between two peaks. Robustness of the method was carried out by deliberately made small variation in the flow rate, pH of mobile phase, organic phase ratio and column oven temperature by using 40 µg mL^{-1} of Aliskiren and Amlodipine, respectively.

Linearity

The linearity of the method was determined by comparing the known concentration Vs response, a series of calibration standards 20,30,40,50,60 **µg/ml** of Aliskiren and Amlodipine were prepared. The solutions were injected into the chromatographic system and peak area of each peak at each concentration was noted. The calibration curve was plotted using peak area versus concentration of the standard solution.

Degradation Studies

Acid degradation

About 100 mg of Aliskiren and Amlodipine were transferred to a 100 ml volumetric flask and dissolved in minimum quantity of diluent, 5 ml of 0.1 N HCL was added and the volume was

made up to the mark with diluent, the solution was heated at 60-70^{o}c for 1 hour. Cool the solution at room temperature further dilutions of the solution made to obtain the concentration of 40 µg/ml concentration of Aliskiren and Amlodipine respectively with the same diluent and used for further analysis. The same procedure as mentioned for the pure drug was followed for the formulation.

Base Degradation

About 100 mg of Aliskiren and Amlodipine were transferred to a 100 ml volumetric flask and dissolved in minimum quantity of diluent, 5 ml of 0.1 N NaoH was added and the volume made up to the mark with diluent, the solution was heated at 60-70^{o}c for 1 hour. Cool the solution at room temperature further dilutions of the solution made to obtain the concentration of 40 µg/ml concentration of Aliskiren and Amlodipine respectively with the same diluent and used for further analysis. The same procedure as mentioned for the pure drug was followed for the formulation.

Oxidative Degradation

About 100 mg of Aliskiren and Amlodipine were transferred to a 100 ml volumetric flask and dissolved in minimum quantity of diluent, 5 ml of 1% H_2O_2 was added and the volume made up to the mark with diluent, the solution was heated at 60-70^{o}c for 1 hour. Cool the solution at room temperature further dilutions of the solution made to obtain the concentration of 40 µg/ml concentration of Aliskiren and Amlodipine respectively with the same diluent and used for further analysis. The same procedure as mentioned for the pure drug was followed for the formulation.

Thermal Degradation

About 100 mg of Aliskiren and Amlodipine were placed in a china dish. The dish was covered by aluminium foil and kept in hot air oven at 60-70ºC for 1 hour and transferred to 100 ml volumetric flask and dissolved in minimum quantity of diluent and the volume made up to the mark with diluent, the solution was heated at 60-70^{o}c for 1 hour. Cool the solution at room temperature further dilutions of the solution made to obtain the concentration of 40 µg/ml concentration of Aliskiren and Amlodipine respectively with the same diluent and used for further analysis. The same procedure as mentioned for the pure drug was followed for the formulation. Results of degradation data was given in table no-7.

Table: 3 Precision Data.

Drug	Concentration (µg/ml)	System Precision % Recovery (%RSD, n=5)	Method Precision % Recovery (%RSD, n=5)
Aliskiren	40	100.00 (1.10)	99.28 (0.83)
Amlodipine	40	99.91 (0.35)	98.48 (0.35)

Table: 4 Results of recovery study of ALISKIREN.

Amount of Aliskiren in sample(µg)	Amount of standard Aliskiren added(µg)	Total amount of Aliskiren (µg)	Total amount Aliskiren found Mean±SD	Total amount recovered (µg)	% Recovery (%RSD)
20	-	20	20.04±1.80	-	100.03(0.18)
20	20	40	40.05±1.03	20.01	100.05(0.10)

Table 5: Results of recovery study of AMLODIPINE.

Amount of Amlodipine in sample(µg)	Amount of standard Amlodipine added(µg)	Total amount of Amlodipine (µg)	Total amount Amlodipine found Mean±SD	Total amt recovered (µg)	% Recovery (%RSD)
20	-	20	20.15±1.92	-	100.75(0.19)
20	20	40	40.01±1.72	19.86	97.86(0.17)

Table 6: Optical Characteristics Of The Proposed Method For Aliskiren And Amlodipine.

Parameter	ALISKIREN	AMLODIPINE
λmax (nm) selected	239	239
Beer's law limits (µg/ml)	20-60	20-60
Correlation coefficient (r)	0.998	0.999
Relative standard deviation (%)	0.0012	0.0022
% Error at 99%(0.01 level)	0.242	0.237
% Error at 95%(0.05 level)	0.368	0.354
Limit of detection (µg/ml)	0.57	0.56
Limit of Quantification (µg/ml)	1.74	1.69

Table 7: Degradation Data.

Drug	Degradation	Area (mV.s)	% Recovered	% Degraded
Aliskiren	Acid	312236	93.31	6.69
	Base	333278	99.60	0.40
	Oxidative	325749	97.35	2.65
	Thermal	319090	95.36	4.64
Amlodipine	Acid	475643	94.32	5.68
	Base	497024	98.56	1.44
	Oxidative	485879	96.35	3.65
	Thermal	458950	91.01	8.99

CONCLUSION

The proposed RP-HPLC method was found to be simple, accurate, precise, linear and specific for quantitative estimation of Aliskiren and Amlodipine in bulk and its formulation. The proposed RP-HPLC method is cost effective and less time consuming. Hence the proposed HPLC method is suitable for routine analysis of Aliskiren and Amlodipine in raw materials and in pharmaceutical formulations in the quality control laboratories.

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