ISSN- 2231–5667 (Print) **www.asianpharmaonline.org**
ISSN- 2231–5675 (Online) **www.ajpaonline.com**

RESEARCH ARTICLE

Development and validation of zero absorbance method for simultaneous estimation of aliskiren and amlodipine in combined dosage form

Patel Samixa R.[1]*, Patel Chhaganbhai N.[2]
[1]School of Pharmacy, RK University, Rajkot, India
[1]Atmiya Institute of Pharmacy, Department of Pharmaceutical Chemistry, Yogidham Gurukul, Kalawad Road, Rajkot-360005. Gujarat, India.
[2]Shri Sarvajanik Pharmacy College, Mehsana, 384001, Gujarat, India.
*Corresponding Author E-mail: **samixa.patel@gmail.com, samixa.patel@yahoo.co.in**

ABSTRACT:
A simple, accurate, precise, economical and reproducible method was developed for simultaneous estimation of aliskiren and amlodipine in Combined Pharmaceutical Dosage Forms. The excipients in the commercial tablet preparation did not interfere with the assay. At 360 nm, Amlodipine showed some absorbance while aliskiren showed zero absorbance so that amlodipine was estimated at 360 nm. While at 287 nm aliskiren showed some absorbance while amlodipine showed zero absorbance so that aliskiren was estimated at 287nm. Linearity in concentration range of 10-120 µg/mL for aliskiren and 4-28 µg/mL for amlodipine with the mean recoveries were 100.08 ± 1.32 and 99.76 ± 1.05 % for aliskiren and amlodipine, respectively. Validation of the proposed method was carried out according to ICH guidelines. Thus the present study gives an excellent method for the determination of the two drugs in combined dosage formulation without their prior separation

KEYWORDS: Amlodipine besylate, Aliskiren, Spectrophotometric method.

INTRODUCTION:
Aliskiren, (2(S), 4(S), 5(S), 7(S)-N- (2-carbamoyl- 2-methylpropyl) -5- amino-4-hydroxy2,7 diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy) phenyl] octanamide hemifumarate) 1-3 (Fig.1).The first oral direct rennin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension. Aliskiren blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation of angiotensin I and angiotens in II [1].

Literature survey revealed HPLC[2], RP-HPLC[3-5], simultaneous UV spectrophotometric[6] and spectroflurimetric[7] methods are reported for the estimation of aliskiren hemifumarate alone or in combination with other anti-hypertensive agents. Amlodipine ((RS)-3-ethyl 5-methyl 2-[(2- aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate)[8] is long acting calcium channel blockers used as an antihypertensive and in the treatment of angina. It acts by relaxing the smooth muscle in the atrial wall, decreasing total peripheral resistance and hence reducing blood pressure, in angina it increases blood flow to the heart muscle. Various analytical methods have been reported for the assay of Amlodipine in pure form as well as in pharmaceutical formulation. They include HPLC [9, 10], HPTLC [11], RP-HPLC [12, 13], Gas chromatography [14], mass-spectrometry [15], and flourimetry[16]. Aliskiren and Amlodipine combination is not official in any

Received on 18.05.2016 Accepted on 28.07.2016

Asian J. Pharm. Ana. 2016; 6(3): 138-142.
DOI: 10.5958/2231-5675.2016.00021.1

pharmacopeias so any official analytical method is not available for estimation of these two drugs. One spectroscopy method is available for estimation of Aliskiren and Amlodipine in tablet dosage form [17]. But no any absorbance correction method is available for determination of Aliskiren and Amlodipine in combine dosage forms. So aim of the present study was to develop accurate, precise and selective absorbance correction method assay procedure for the analysis of Aliskiren and Amlodipine in combined dosage forms.

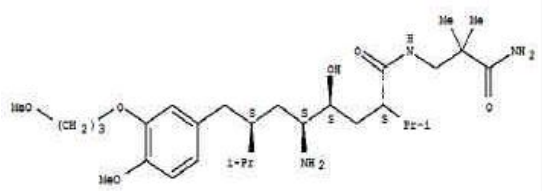

Figure: 1 Structure of Aliskiren

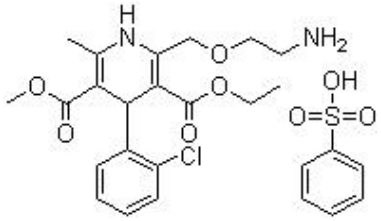

Figure: 2 Structure of Amlodipine

EXPERIMENTAL:

Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, Sartorius CP224S analytical balance (Shimadzu, Japan),Ultra sonic cleaner (Life care eq. PVT. LTD, Mumbai, India), Corning volumetric flasks, pipettes of borosilicate glass were used in the study.

Reagents and Materials

Pharmaceutical grade of Aliskiren (ALK) was kindly supplied as a gift samples from Novartis India limited, India and Amlodipine besylate were kindly supplied as a gift samples from Torrent research centre, Gujarat (India),The pharmaceutical formulation (Tekamlo™) containing 150 mg ALK and 5 mg AML was procured from the local pharmacy, AR grade Methanol (S.D. Fine Chemical Ltd., Mumbai, India), Whatman filter paper no. 41 (Whatman International Ltd., England)

Preparation of ALK and AML Standard Solutions

A mixed stock solution of ALK (2000 µg/mL) and AML (1000 µg/mL) was prepared by accurately weighing ALK (200 mg) and Amlodipine besylate (138 mg) which is equivalent to AML (100 mg), dissolving in methanol and diluted to 100 mL with methanol in the same volumametric flask

Preparation of Sample Solutions

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 150 mg of ALK and 5 mg of AML was transferred to a 100 mL volumetric flask. The content was mixed with methanol (60 mL), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtered through a Whatman filter paper no. 41. The volume was adjusted up to the mark with methanol. An aliquot of this solution (0.6 mL) was transferred in to a 10 mL volumetric flask and Addition of 1 ml AML standard solution (100µg/ml) to the same volumetric flask. Methanol was transferred to this volumetric flask and Volume was made up to the mark to give a solution containing 90 µg/ml ALK and 13µg/ml AML. This solution was used for the estimation of ALK and AML.

Determination of the analytical wavelengths

The standard solutions of ALK (40 µg/mL) and AML (16 µg/mL) were scanned separately in the UV range of 200-400 nm. It appeared that ALK showed zero absorbance at 360 nm while AML showed some absorbance and at 287 nm AML showed zero absorbance while ALK showed some absorbance. The absorbance value at 360 nm is due to AML only in the combined mixture of both drugs and the absorbance value at 287 nm is due to ALK only in the combined mixture of both drugs.

METHOD VALIDATION [18]:

Calibration curve (Linearity)

Calibration curves were plotted over a concentration range of 10-120 µg/mL for ALK and 4-28 µg/mL for AML. Accurately measured standard working solutions of ALK (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL) and AML (0.4, 1.0, 1.6, 2.0, 2.4, and 2.8 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with methanol, and absorbance were measured at 360 nm for ALK and 287 nm for AML. The calibration curves were constructed by plotting absorbance vs concentrations.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of ALK and AML by the standard addition method. Standard solutions of ALK (20, 40, 60 µg/mL) and AML (5, 10, 15 µg/mL) were added to prequantified sample solutions of ALK (40 µg/mL) and AML (10 µg/mL) tablet dosage form. The amounts of ALK and AML were estimated by applying obtained values to the regression equation of the calibration curve.

Method Precision (% Repeatability)
The precision of the instruments was checked by repeated scanning and measurement of absorbance of solution of (n = 6) of ALK and AML (20 µg/mL) without changing the parameter.

Intermediate Precision (Reproducibility)
The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of ALK (40, 80, and 120 µg/mL) and AML (16, 20, and 24 µg/mL). The results were reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification
LOD and LOQ of the drug were calculated using the following equations designated by International Conference on Harmonization (ICH) guideline:

$$LOD = 3.3 \times \delta/S$$
$$LOQ = 10 \times \delta/S$$

Where δ = the standard deviation of the response
S = Slope of calibration curve.

ANALYSIS OF ALK AND AML IN COMBINED DOSAGE FORM:-
The response of formulations was measured at 360 nm and 287 nm for AML and ALK, respectively by proposed method as described above. The amounts of ALK and AML present in sample solution were determined by fitting the responses into the regression equation of calibration curve of the proposed method.

RESULTS AND DISCUSSION:

Method development
The utility of dual wavelength data processing program is its ability to calculate unknown concentration of component of interest in a mixture containing an interfering component. For elimination of the effects of an interfering component, two specific wavelengths are chosen.

1. First wavelength λ_1 at which absorbance of AML was observed and there was no interference of ALK at this wavelength (360nm).
2. Second wavelength λ_2 at which absorbance of ALK was observed and there was no interference of AML at this wavelength (287.0 nm).

These two selected wavelengths were employed to determine the concentration of ALK and AML (Figure 3).

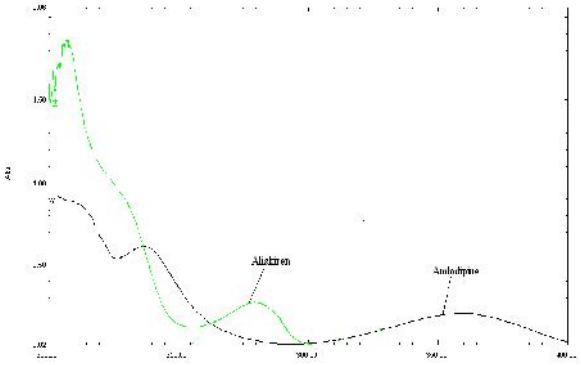

FIGURE: 3 Overlain absorption spectra of standard solution of Aliskiren and Amlodipine in methanol

Validation of proposed method
The proposed method has been validated for the simultaneous determination of ALK and AML in tablet dosage form using following parameters.

Linearity
Linear correlation was obtained between absorbance Vs concentrations of ALK in concentration range 10-120 µg/mL and AML in concentration range of 4-28 µg/mL for proposed method. Regression parameters are mentioned in table 1 and the calibration curves of these two drugs are shown in Fig.2, Fig.3.

Accuracy
The recovery experiment was performed by the standard addition method. The mean recoveries were 100.08 ± 1.32 and 99.76 ± 1.05 % for ALK and AML, respectively (Table 1). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 2.

TABLE 2 Recovery Data for the Proposed Method

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (%)	Mean % Recovery ± SD*
ALK	I	40	50 %	98.91 ± 0.95
	II	40	100 %	101.2 ± 1.49
	III	40	150 %	100.13 ± 1.53
AML	I	10	50 %	98.52 ± 0.31
	II	10	100 %	100.5 ± 1.29
	III	10	150 %	99.87 ± 1.15

*Mean % Recovery ± SD of six observations.

Method precision (% Repeatability)
The % RSD values for ALK and AML were found to be 1.88 and 1.69 for Absorbance correction method (Table 1 and Table 3). The low values of RSD indicate the proposed methods are repeatable.

TABLE 3 Precision Data for Aliskiren and Amlodipine

ALK and AML (20 µg/ml)	Absorbance at 287 nm	Absorbance at 360 nm
1	0.079	0.188
2	0.077	0.185
3	0.079	0.181
4	0.08	0.185
5	0.076	0.19
6	0.078	0.184
Mean	0.078	0.185
S.D.	0.0014	0.0031
%CV	1.88	1.69

Intermediate precision

The low RSD values of interday (1.45-1.96 % and0.66-1.73%) and intraday (0.32-1.02 % and 0.47-1.23 %) variations for ALK and AML, respectively, reveal that the proposed method is precise (Table 1).

LOD and LOQ

LOD for ALK and AML were found to be 1.36 µg/mL and 0.58 µg/mL, respectively. LOQ for ALK and AML were found to be 4.14 µg/mL and 1.77 µg/mL, respectively (Table 4.1). These data show that method is sensitive for the determination of ALK and AML.

Assay of the pharmaceutical formulation

The proposed validated method was successfully applied to determine ALK and AML in their combined dosage form. The spectrum of sample is shown in Fig. 4. The results obtained for ALK and AML were comparable with the corresponding labeled amounts (Table 4).

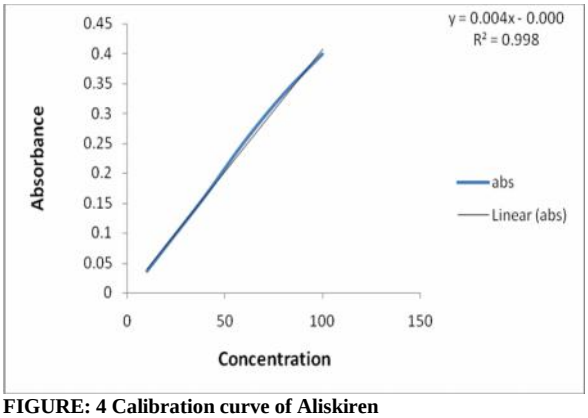

FIGURE: 4 Calibration curve of Aliskiren

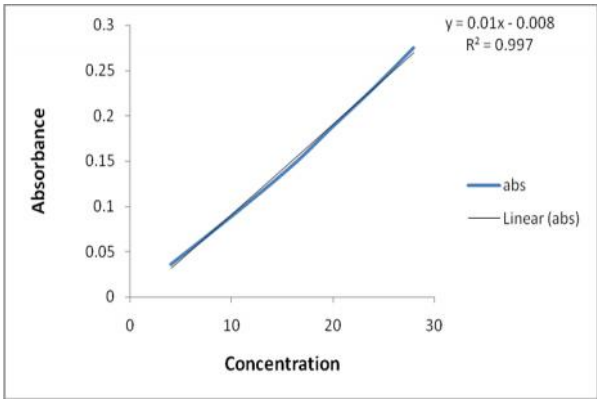

FIGURE: 5 Calibration curve of amlodipine

TABLE 1 Regression analysis data and summary of validation parameters for the proposed method.

PARAMETERS	ZERO ABSORBANCE METHOD	
	ALK	**AML**
Concentration range (µg/mL)	20-120	10-60
Slope	0.004	0.010
Intercept	0.00	0.008
Correlation coefficient	0.998	0.997
LOD (µg/mL)	1.36	0.58
LOQ (µg/mL)	4.14	1.77
% recovery (Accuracy, n = 6)	100.08 ± 1.32	99.76 ± 1.05
Repeatability (% RSD, n = 6)	1.88	1.69
Precision (%RSD)		
Interday (n = 6)	0.45-1.96	0.66-1.73
Intraday (n = 6)	0.32-1.02	0.47-1.23

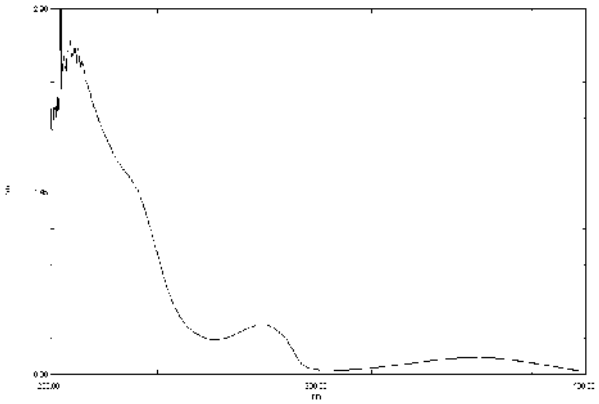

FIGURE 6 Absorption Spectra of sample solution of Aliskiren and Amlodipine in methanol

TABLE 4 Analysis of Marketed Formulation of Aliskiren and Amlodipine by Proposed Method (n = 6)

Sample No.	Label Claim		Amount Found		% Label Claim	
	ALK (mg/tab)	AML (mg/tab)	ALK (mg/tab)	AML (mg/tab)	ALK (mg/tab)	AML (mg/tab)
1	150	5	153.19	5.06	102.13	101.27
2	150	5	150.13	4.91	100.09	98.23
3	150	5	148.20	5.08	98.80	101.60
4	150	5	149.10	5.09	99.40	101.89
5	150	5	151.87	4.92	101.25	98.40
6	150	5	148.90	5.07	99.27	101.40
Mean			150.23	5.02	100.15	100.46
S.D.			1.93	0.08	1.28	1.23

CONCLUSION:

In this proposed method the linearity is observed in the concentration range of 10 – 120 µg/mL with co-efficient of correlation, (r^2) = 0.998 and the concentration range of 4 - 28 µg/mL with co-efficient of correlation (r^2) = 0.997 for ALK and AML, respectively.The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the ALK and AML in combined dosage form without any interference of the excipients.

REFERENCES:

1. Dieterich H, Kemp C, Vaidyanathan S, Yeh C. Aliskiren, the first in a new class of orally Efective rennin inhibitors, has no clinically significant drug interactions with digoxin in healthy volunteers. Clinical Pharmacology and Therapeutics.2005; 79: 64–64.
2. Shalini P, Sarvesh P, Kona.SS, Yogendra S, Varun J. Development and validation of HPLC method for analysis of some antihypertensive agents in their pharmaceutical dosage forms. Journal of Pharmaceutical Sciences and Research. 2010 ;2 (8): 459-464.
3. Sangoi MW, Sangoi MS, Oliveira PR, Secretti LT, Rolim CM. Determination of aliskiren in tablet dosage forms by a validated stability indicating RP-LC method. Journal of Chromatographic Science 2011; 49
4. Kumara SG , Sheshagiri RJ, Rajendra JM, Ashok KU, Vinay DK. Analytical ethod development and validation of aliskiren in bulk and tablet dosage form by RP-HPLC method. Journal of Pharmacy Research 2011, 4(3), 865-867.
5. Satish BK, Rao NS, and Vijaya BK. A simple and sensitive method for the determination of aliskiren hemifumarate using HPLC-UV detection. Raisin J Chemistry. 2011;4(2) :285-288
6. Micheli WS, Leonardo TS. Development and validation of an UV spectrophotometric method for the determination of aliskiren in tablets. Isabel Fração Diefenbach e Clarice Madalena Bueno Rolim. Quim. Nova,2010;33(6):1330-1334.
7. Wrasse SM, Sangoi MS, Oliveira PR, Secretti LT, Rolim MB. Spectrofluorimetric determination of aliskiren in tablets and spiked human plasma through derivatization with dansyl chloride. Journal of Chromatographic Science, 2011; 49
8. The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals, 13th ed., Merck and Co.Inc., White House Station, New Jersey, 2001,pp. 488, 865.
9. Halker UP, Bhandari NP, Rane SH. High performance liquid chromatographic simultaneous determination of amlodipine and enalapril maleate from pharmaceutical preparation. Indian Drugs. 1988; 35: 168.
10. Shimooka K, Sawada Y, Tatematsu H. Analysis of amlodipine by a sensitive high performance liquid chromatography method with amperometric detection. J. Pharm. Biomed. Anal. 1989; 7: 1267.
11. Agrekar AP, Powar SG. Simultaneous determination of atenolol and amlodipine in tablets by high performance thin layer chromatography. J. Pharm. Biomed. Anal. 2000; 21: 1137
12. European Pharmacopoeia, 3rd ed. Council of Europe, Strasbourg, 2001. pp. 431, (Supplement).
13. Avadhanulu AB, Srinivas JS, Anjaneyulu Y. Reversed phase HPLC determination of Amlodipine in drugs and its pharmaceutical dosage forms. Indian Drugs. 1996; 33: 36.
14. Bresford AP, Marcrac PV, Stopher DA. Analysis of amlodipine in human plasma by gas chromatography. J Chromatogr. 1987; 420: 178.
15. Feng Y, Meng Q, Guo X. Human plasma Amlodipine GC– MS determination, Guandong Yaoxueyuan Xuebao. 1998;
16. Mohamed YE, Naglaa ME, Bahia AM, Nasshwa GM. Fluorimetric determination of amiodrone, amlodipine and propafenone. Bull. Fac. Pharm. 1998; 36: 1.
17. Das P, Patel S, Radhika PP, Subramanyam EV,Sharbaraya DA. Simultaneous Estimation of Aliskiren and Amlodipine in Tablet Dosage form by UV Spectroscopy. International Journal of Drug Development and Research. 2012; Vol. 4(2): 265-270
18. International Conference on Harmonization (ICH), Q2R1, Validation of Analytical Methods: Methodology, Yokohama, Japan, (2005)