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STABILITY PROFILE DEVELOPMENT USING SIMULTANEOUS ESTIMATION METHOD FOR FIXED DOSED COMBINATION OF ALISKIREN AND AMLODIPINE BY HPLC

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Aliskiren, Amlodipine, Forced Degradation, Linearity, Precision

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ABSTRACT: The Pharma market is getting flooded with fixed dose combination drugs due to better patient compliance, has but created a challenging situation for analytical chemists. The objective of study was developing an analytical method for simultaneously estimating aliskiren and amlodipine in combination with their forced degradation products obtained by treating the sample under tress conditions like acid and base hydrolysis (0.01N HCl, and 0.01 NaOH, oxidation(3% H_2O_2), photolytic and thermal degradation(80^oC). The linearity ranging was 2.5-25µg/ml and 10-100µg/ml of Amlodipine and Aliskiren and regression coefficient was found to be 0.998 and 0.996 respectively. The chromatographic separation of analyte was carried out using JASCO HPLC system with HiQ Sil C8 (4.6mmØ × 250mm, 5 μ) column. The mobile phase consists of acetronitrile and pH was adjusted to 4 with orthophosphoric acid solution. The analyte were detected at 210nm using UV detector with flow rate of 1ml/min.

INTRODUCTION: The Pharma market is getting flooded with fixed dose combination drugs [1, 2] as they have shown better patient compliance but created a challenging situation for analytical chemists [3]. The objective of study was developing an analytical method for simultaneously estimating pure drugs in combination with their degradation products [4, 5]. Aliskiren and Amlodipine used in fixed dose combination for treatment of hypertension [6]. Aliskiren act as rennin inhibitor [7, 8] and Amlodipine acts as calcium channel antagonist [9, 10].

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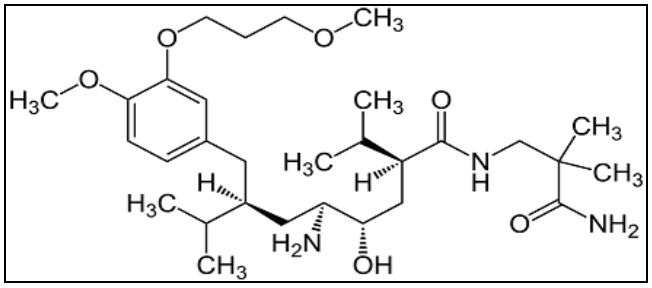
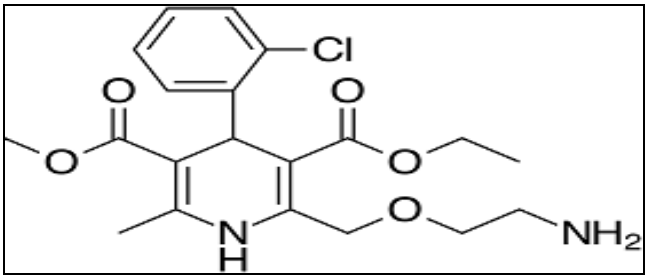
Chemically, Aliskiren is (2S, 4S, 5S, 7S)-N-(2-carbamoyl-2-methylpropyl)-5-amino - 4 - hydroxy-2,7-diisopropyl-8-[4-methoxy - 3 - (3 me -thoxy propoxy) phenyl] –octanamide (**Fig. 1a**) and Amlodipine is 3-ethyl-5-methyl 2-[(2-amino ethoxy)methyl]-4-(2-chlorophenyl)- 1, 4 - dihydro pyridine-6-methyl-3,5-dicarboxylate (**Fig. 1b**) [11, 12]. Analytical methods like HPLC, UV-Spectrophotometric were reported for the characterization of individual drug and combination with other drug.

However no reported stability indicating HPLC method for fixed dose combination of Aliskiren and Amlodipine. The present study was done to developed stability profile development by using simultaneous estimation method for fixed dose combination of Aliskiren and Amlodipine using HPLC.



(a)



(b)

FIG. 1: (a) CHEMICAL STRUCTURE OF ALISKIREN, (b) CHEMICAL STRUCTURE OF AMLODIPINE

MATERIALS AND METHODS:

Reagent and Chemicals: Amlodipine provided by Sun pharmaceuticals limited, HP and Aliskiren provided by Novartis healthcare Pvt. Limited. Methanol and Acetonitril (HPLC grade) provided by VETEC Bangalore. Distilled water prepared by glass distillation apparatus. Ortho phosphoric acid, Hydrogen peroxide, Sodium hydroxide, Hydrochloric acid was purchased.

Instrument: The chromatographic analysis was carried out on Jasco-HPLC with Pump 2080 Plus and Detector UV 2075 plus. With flow rate 1ml per minute. Brownis software was used for sample monitoring and processing. UV cabinet was used for photolytic degradation and Hot air oven was used for thermal degradation study.

Chromatographic Condition: The chromatographic separation of analyte was carried out using JASCO HPLC system with HiQ Sil C8 (4.6mmØ × 250mm, 5 μ) column. The mobile phase consists of Acetronitrile and pH was adjusted to 4 with orthophosphoric acid solution. The analyte were detected at 210nm using UV detector. The flow rate was set at 1ml/min.

Preparation of Stock Solution: Standard stock solution of Aliskiren and Amlodipine were prepared separately by dissolving 10 mg of Aliskiren and 10 mg of Amlodipine in 10 ml volumetric flask with mobile phase as diluents and sonicate for 5 min. from above solution prepare the deferent aliquots for mixture of Aliskiren and Amlodipine.

Forced Degradation Study: Forced degradation studies of the fixed dose combination of drug were carried out by treating the sample under tress conditions like acid and base hydrolysis, oxidation, photolytic and thermal degradation and resultant degradation products was investigated. These study help to know stability characteristics of the drug and the possibly degradation products [13].

Preliminary Study: In the preliminary examination, observations are made about sample stability, including exposure of solid state samples to heat and light and exposure of solutions to various pH and oxidative conditions. Preliminary study can also be used to aid in the development of an analytical method.

Acid Degradation Study (HCl): To the 5ml of stock solution of the Amlodipine and Aliskiren, 5 ml of 0.01N HCl was added and stand for 1 hour at room temperature. The resultant solution was neutralized by using 0.01N NaOH and diluted. The solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Base Degradation Study (NaOH): To the 5ml of stock solution of the Amlodipine and Aliskiren, 5 ml of 0.01N NaOH was added and stand for 1 hour at room temperature. The resultant solution was neutralized by using 0.01N HCl and diluted. The solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Oxidative Degradation (H_2O_2): To the 5ml of stock solution of the Amlodipine and Aliskiren, 5 ml of 3% H_2O_2 was added and stand for 30 min. at room temperature. The resultant solution was diluted. The solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Thermal Degradation: The standard stock solution was placed in the oven at 80 ºC for 2 days. The resultant solution was diluted. The solutions were injected into the system and the

chromatograms were recorded to assess the stability of the sample.

Photolytic Degradation: The photochemical stability of the drug was studied by exposing a standard stock solution to UV light by keeping the beaker in the UV chamber for 3 days. The resultant solution was diluted. The solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Method Validation: The method was validated for system suitability, linearity, accuracy, precision, Robustness, limit of quantitation and limit of detection as per ICH Q_2 (R_1) Guidelines [14].

Stability Study: The stability study of the FDC was carried out by using stability chamber for three months as per the ICH Q_1A (R_2) guidelines [15].

RESULTS AND DISCUSSION:

Method Development: A series of trials was conducted with different columns like C-8 and C-18 with different mobile phases to develop a suitable HPLC method for simultaneous estimation of Amlodipine and Aliskiren in Fixed dose combination. Finally a typical chromatogram was obtained with Acetonitrile and pH was adjusted to 4 with orthophosphoric acid at a flow rate of 1ml/min. Chromatographic separation was performed on HiQ Sil C-8 (4.6mmØ × 250mm, 5 μ) column and analyte were detected with UV detector at 210nm. The retention time of Aliskiren and Amlodipine was found to be 2.8 and 2min, respectively. Forced degradation study was also carried out using developed method and degradation compounds were effectively resolved from the Aliskiren and Amlodipine in fixed dose combination. The optimized separation conditions were given in **Table 1**.

TABLE 1: OPTIMIZED SEPARATION CONDITIONS

Chromatographic mode	Chromatographic condition
Instrument used	Jasco-HPLC with Pump 2080 Plus, Detector UV 2075 plus
Stationary phase	KYA TECH HIQ Sil C8 (4.6 mm × 250 mm) 5µm
Mobile phase	Acetonitrile pH 4
Standard solution	1000 µg/ml
Detection wavelength	210 nm
Flow rate	1 m/min
Sample size	20 µl
Run time	5 min

Method Validation: The validation was performed with above developed method HPLC method for simultaneous estimation of Amlodipine and Aliskiren according to ICH guidelines.

System Suitability: System suitability was performed to verify the acceptability of the resolution of the system. Parameters such as peak area, USP tailing, theoretical plates, retention time and peak symmetry were evaluated. The % RSD was found within limits. The results were shown in **Table 2**.

TABLE 2: RESULT FOR SYSTEM SUITABILITY

Parameters	Aliskiren	Amlodipine
Retention time	2.8 min.	2 min.
Plate count	7252.43	3527.44
tailing	1.29	1.81
Resolution	6.99	0.00

Linearity: The linearity of the method was determined by using different concentration ranging from 2.5-25µg/ml and 10-100µg/ml of Amlodipine and Aliskiren respectively. The linearity curve was constructed by plotting peak area versus concentration of analyte. From the results obtained proposed method found to be linear. The regression coefficient was found to be 0.998 and 0.996 for Aliskiren and Amlodipine respectively. The overlain chromatogram was shown in **Fig. 2**.

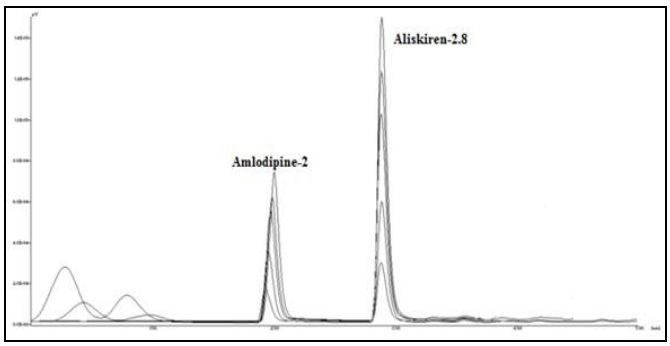


FIG. 2: THE OVERLAIN CHROMATOGRAM OF AMLODIPINE AND ALISKIREN

Accuracy: The accuracy of the proposed method was evaluated by calculating the recovery study of the drug at three different concentration levels (80%, 100% and 120%) by using spike method. The mean percentage recovery of Aliskiren and Amlodipine was varied between 99.1 and 101% indicate that the developed method was found to be accurate. The % recovery results were shown in **Table 3**.

TABLE 3: % RECOVERY RESULTS OF AMLODIPINE AND ALISKIREN

Spike level	Percentage recovery		Mean percentage recovery	
	Aliskiren	Amlodipine	Aliskiren	Amlodipine
	100.4	97.59		
80%	99.30	98.29	99.37	99.47
	98.43	102.53		
	95.36	100.3		
100%	100.49	99.76	98.07	100.04
	98.45	100.07		
	95.38	96.20		
120%	100.5	100.06	98.11	97.79
	98.46	97.12		

Precision: The precision of an analytical procedure define as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribe conditions. The %RSD was calculated from the chromatogram and result obtained were within the limit of 2% hence proposed method was found to be precise. The precision results were given in **Table 4**.

TABLE 4: RESULTS OF PRECISION STUDY OF AMLODIPINE AND ALISKIREN

Conc.(µg/ml)		Mean % conc. Estimated*		%RSD	
Aliskiren	Amlodipine	Aliskiren	Amlodipine	Aliskiren	Amlodipine
20	5	99.06	96.58	1.35	0.53
40	10	99.9	99.06	1.73	0.24
60	15	99.78	99.92	0.37	0.12

*****Average of three determination

LOD and LOQ: In the present study the LOD and LOQ of Aliskiren and Amlodipine were evaluated based on the Based on the Standard Deviation of the Response and the Slope. The limit of quantitation and Limit of detection is performed to know the lowest concentration level of the analyte that gives measurable response. The LOD was found to be 0.000478 and 0.0001037 and LOQ was found to be 0.001457 and 0.0003142 for Aliskiren and Amlodipine respectively.

Robustness: Robustness of the proposed method has been evaluated by small deliberate change in the system parameter such as flow rate. It was found that there is no change in the peak area and retention time by small change like ±0.1 ml change in flow rate.

The % RSD was found to be within the range and method was found to be robust. The robustness results were shown in **Table 5**.

TABLE 5: RESULTS OF ROBUSTNESS STUDY OF ALISKIREN AND AMLODIPINE

Parameters	Aliskiren		Amlodipine	
	Peak area	Retention time	Peak area	Retention time
Flow rate 0.8 ml/min.	317930.96	2.89	175254.75	1.95
Flow rate 1.2 ml/min.	317922.5	2.88	175231.7	1.96

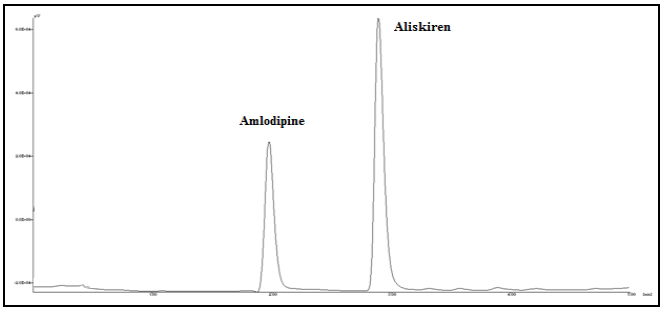
Forced Degradation Studies: In the present study Forced degradation studies were carried out to develop stability profile for the fixed dose combination of Amlodipine and Aliskiren and ensure the effective separation of both drugs from degradation products. Degradation was observed by generation of different peaks with different retention time with respective original peaks of drug. The percentage assay of degradation was calculated from the peak area obtained in degradation conditions and it was compared with assay of non degraded conditions. From the chromatograms (**Fig. 3**), it was found that both the drugs are susceptible to Acid, Base and photolytic degradation and percentage assay degradation was found to be within the range. The Amlodipine was more susceptible to thermal degradation than the Aliskiren and the Aliskiren is more susceptible to oxidative degradation than Amlodipine.

In all the conditions the purity angle was found to be less than that of purity threshold which indicates

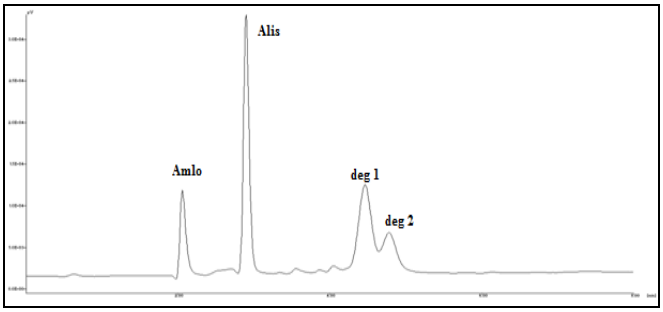
that the developed method was stability indicating. The forced degradation studies were performed without intending to identify the degradation products but merely to show that they are not interfering with active molecule if any present. The results of forced degradation study were shown in **Table 6**.

TABLE 6: RESULTS OF FORCED DEGRADATION STUDY FOR AMLODIPINE AND ALISKIREN

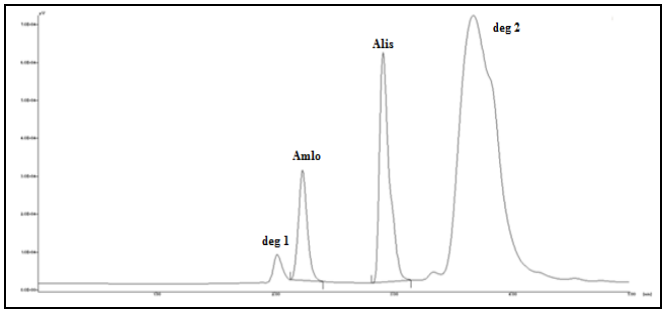
Stress condition	Concentration	Time	% Degradation	
			Amlodipine	Aliskiren
Acid degradation	0.01 N HCl	60 min	5.94	16.82
Base degradation	0.01 N NaOH	60 min	9.49	13.97
Oxidative degradation	3% H_2O_2	30 min	2.97	10.92
Photolytic degradation	-----	3 day	13.78	21.96
Thermal degradation	80 ^{0}C	2 day	32.79	2.39



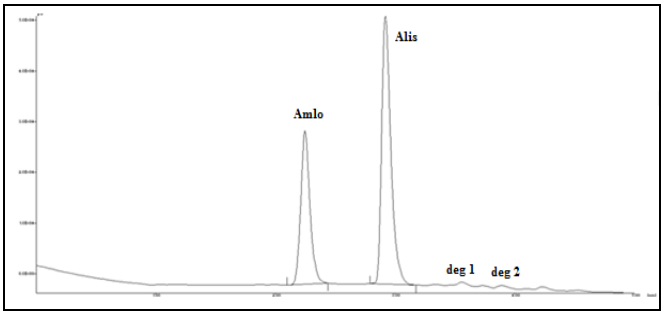
CHROMATOGRAM OF ALISKIREN AND AMLODIPINE WITHOUT DEGRADATION



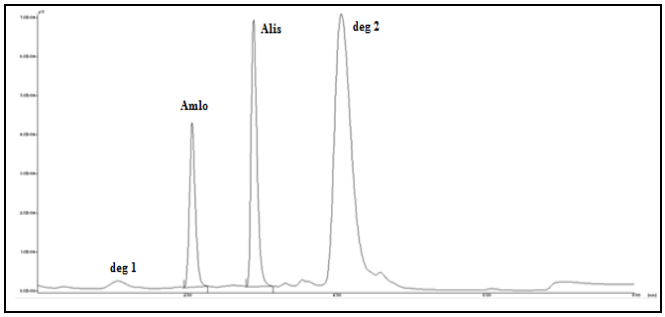
ACID DEGRADATION



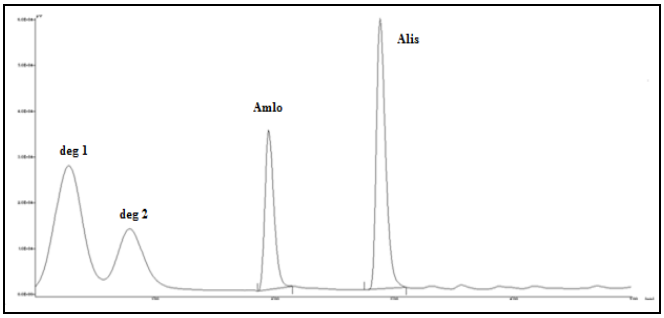
BASIC DEGRADATION



PHOTOLYTIC DEGRADATION



THERMAL DEGRADATION



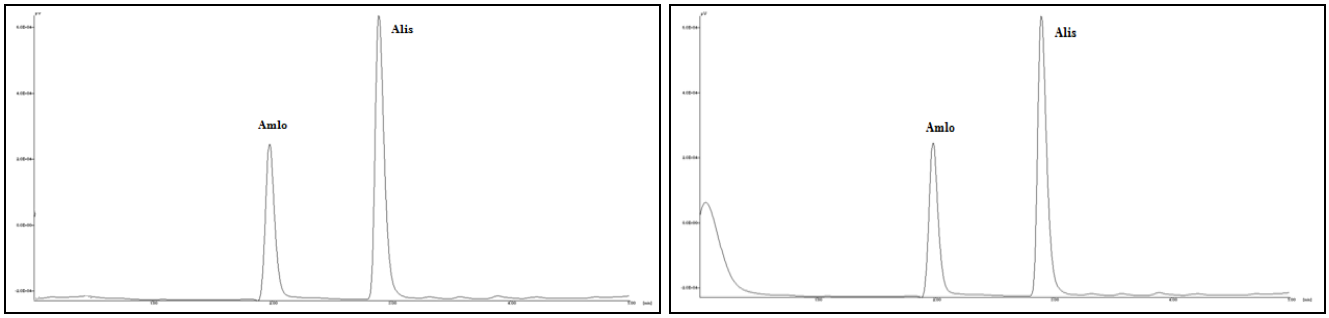
OXIDATIVE DEGRADATION

FIG. 3: CHROMATOGRAMS OF FORCED DEGRADATION STUDIES

Stability Study: The fixed dose combination of Amlodipine and Aliskiren does not undergo any substantial changes during 3 months stability testing as per ICH guidelines. Hence we can say that the combination of Amlodipine and Aliskiren has shown good short term stability.

TABLE 7: RESULT OF STABILITY STUDY FOR COMBINATION OF AMLODIPINE AND ALISKIREN

Parameters	Aliskiren		Amlodipine	
	Before	After	Before	After
Retention time	2.89	2.88	2	1.96
Area under curve	317930.96	317922.5	175254.75	175231.7
Plate count	7252.43	7255	3527.44	3520.22



BEFORE STABILITY TESTING **AFTER 3 MONTHS STABILITY TESTING**

FIG. 4: CHROMATOGRAMS OF STABILITY STUDY

CONCLUSION: On the basis of results and statistical evaluation it can be concluded that, the prime objective of developing simple, accurate, precise, specific and selective analytical methods for simultaneous estimation of Amlodipine and Aliskiren from FDC, as well as in the presence of their degradation product was a successfully achieved. The developed methods can be applied for routine testing of drug stability.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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