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# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF ALISKIREN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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**ABSTRACT**

*A stability-indicating LC assay method was developed for the quantitative determination of Aliskiren (ALISK) in pharmaceutical dosage forms. Chromatographic separation was achieved on Hyperchrom- ODS 5µ C18 column (250 X 4.6 mm). The mobile phase was prepared by mixing Acetonitrile and 0.05 M KH2PO4 Buffer (45:55v/v) and pH was adjusted to 2.5 with 10% Ortho –phosphoric acid. The flow rate was 1.0mL/min with detection wavelength 280nm. Aliskiren degraded in acidic and alkaline conditions, while it was more stable in neutral, oxidative, thermal and photolytic conditions. The described method was linear over a range of 10-60 µg/mL for determination of Aliskiren (r=0.9989) .The developed method was successfully validated according to ICH Guidelines. The mean recovery was found to be 99.96%. The method was found to be suitable for analysis in presence of its degradation products.*

***Keywords*** *– Aliskiren Hemifumarate, RP-HPLC Method, Stability indicating Assay Method*

## 1. INTRODUCTION

Aliskiren Hemifumarate (Alsk) chemically described as (2(2*S*,4*S*,5*S*,7*S*)-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[1] (Figure 1) is an orally active rennin inhibitor for the treatment of essential hypertension and heart failure.

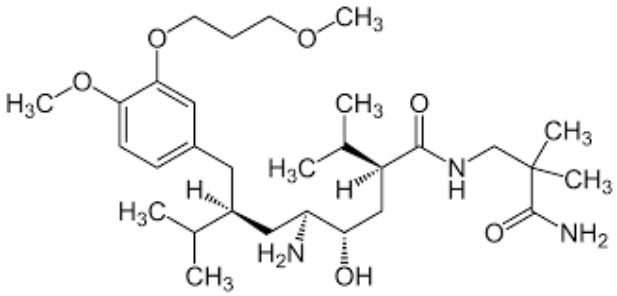

**Fig 1: Structure of Aliskiren**

Aliskiren metabolized slowly in the body resulting in stronger half lives which restrict it once a day dosing. The cytochrome P450 susceptibility is also less and a major proportion of the drug is eliminated unchanged via feces. Literature survey reveals that few

spectrophotometric methods[2] and HPLC methods[3-10] has been reported for the estimation of Aliskiren alone or in combination with other anti-hypertensive agents in pharmaceutical formulations. Literature survey does not reveal any simple Stability Indicating RP-HPLC Assay method for estimation of Aliskiren The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Aliskiren in pharmaceutical dosage form as per ICH guidelines[11].

## 2. MATERIALS AND METHODS

Aliskiren Hemifumarate was kindly obtained as a gift sample from Morepen Lab. Ltd. (Delhi). Commercial tablets containing Aliskiren 150mg was procured from the local chemist shop manufactured by Novartis. Water and acetonitrile, methanol used was of HPLC grade. Other chemicals were of either AR grade or GR grade.

### 2.1 Insrumentation

Chromatographic separation was performed on Analytical Technology Limited isocratic system consisted of hyperchrome ODS 5µ $C_{18}$ column (250 X 4.6 mm), Uv-3000 detector and P-3000 Pump, Rheodyne injector with 20µL capacity. The mobile phase comprised of 0.05M Potassium Dihydrogen phosphate Buffer: Acetonitrile, pH 2.5 (55:45) at flow rate 1.0 mL/min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15min. Analysis was performed at ambient temperature. The detection was monitored at 280nm.

### 2.2 HPLC Method Development

#### 2.2.1 Preparation of Mobile Phase

Preparation of 0.05M Phoshphate Buffer solution: Potassium Di hydrogen orthophosphate, 7g was dissolved in 1000 mL of water and Mixed, pH adjusted to 2.5 using ortho-phosphoric acid, sonicated to degas the buffer.

The mobile phase was prepared by mixing Acetonitrile and Buffer in the ratio of 45:55v/v. The mobile phase was filtered through nylon 0.45µm membrane filter. The same mobile phase was used as diluents.

#### 2.2.2 Aliskiren stock solution

A standard stock solution of Alisk was prepared in mobile having the concentration of 1mg/mL. A 1.0 mL portion of the above solution was further diluted up to 25.0 mL with mobile phase (40 µg/mL) (**Solution B1)**.

#### 2.2.3 Stress Degradation Studies

**a. Solution State Stability**

**i. Preparation of Standard Solution (Unexposed)**

An accurately weighed quantity of ALISK (~10.0 mg) was transferred to 25.0mL volumetric flask. To it 10.0 mL of mobile phase was added, shaken and volume made up to the mark with mobile phase. A 1.0 mL was further diluted to 25.0 mL with mobile phase so as to get the final concentration as 40µg/mL. A 20 µL volume of this solution was injected and chromatographed.

**ii. Preparation of Standard solution (- Stressed condition)**

Accurately weighed quantities of standard ALISK (~ 10mg ) were transferred to a series of 6 different 25.0 mL dry volumetric flasks and designated as flask a to f. To it 10.0mL of reagents (Acid, Alkali, 3% hydrogen peroxide, water) was added. The samples were kept at 50°C for three hours, samples were withdrawn at specified time as indicated, a (30min), b(60 min), c (90 min), d (120 min), e (150 min) and f (180 min).Samples were cooled to room temperature, mobile phase was added to each flask and volumes were adjusted up to the mark with mobile phase. The content in each flask was sonicated for 20 min. A 1.0 mL portion of each filtrate was separately diluted upto 10.0 mL with mobile phase and chromatographed.

**iii. Preparation of Sample Solution ( Stressed condition)**

Accurately weighed quantities of powder equivalent to (~10 mg Alsk) were transferred to 25.0mL dry round bottom flask. To it 10.0 mL of reagent (Acid, Alkali, 3% hydrogen peroxide, water) was added and kept for three hours at 50°C. The sample was withdrawn at the end of three hours, and volume was made up to the mark with mobile phase. The content in flask was sonicated for 20 min and the solution was filtered through whatmann filter paper (no.41). A 1.0 mL portion of filtrate was diluted upto 10.0 mL with mobile phase and chromatographed.

**iv. Preparation of Stressed sample solution for UV studies:**

The standard and sample marketed solutions were prepared following the above stress degradation study procedure. Each solution was scanned in the spectrum mode in the wavelength range 400-200 nm in 1.0 cm cell using mobile phase as blank.

**b. Solid State Stability**

**General procedure of preparation of Standard & Marketed Formulation**

**i. Preparation of Standard Solution (Exposed)**

An accurately weighed quantity exposed powder of ALISK (~25.0 mg) was withdrawn and transferred to 25.0 mL volumetric flask. To it 10.0 mL of mobile phase was added, shaken and volume was made up to the mark with mobile phase. A 1.0 mL was further diluted to 25.0 mL with mobile phase so as to get the final concentration as 40µg/mL. A 20 µL volume was injected and chromatographed.

**ii. Preparation of Sample Solution (Stressed condition)**

An accurately weighed quantity powder of marketed formulation equivalent 10.0 mg of ALISK was transferred to25.0 mL volumetric flask. To it 10.0 mL of mobile phase was added, shaken and volume was made up to the mark with mobile phase. The content was sonicated for 20 min & filtered through whatmann filter paper (no.41). A 1.0 mL portion of filtrate was further diluted to 10.0 mL with methanol so as to get the final concentration as 40µg/mL (on label claim basis). A 20 µL volume was injected and chromatographed.

**iii. Humidity studies (40°C/75% RH)**

An accurately weighed quantity of about 10 mg of Alisk standard and tablet powder equivalent to standard was transferred to a separate petri dish and uniformly spread and exposed to 40°C/75% RH for 14 days.

**iv. Photostability studies**

An accurately weighed quantity about 10 mg of Alisk standard and tablet powder equivalent to standard was transferred to a separate petri dish and uniformly spread and exposed to UV lamp at 254nm for 48 hours.

**v. Thermal studies (Dry Heat)**

It was performed by exposing the drug in oven at $50^0$C for three hours.

**2.3 Application of proposed method for assay of marketed formulation**

**2.3.1 Preparation of sample**

Twenty tablets were weighed and average weight was calculated. The tablets were triturated thoroughly and mixed. An accurately weighed quantity of tablet powder equivalent to 25.0 mg of ALISK was transferred to 25.0 mL volumetric flask, to it mobile phase was added, shaken for few minutes and volume was made up to the mark with mobile phase. The content was sonicated for 20 minutes and filtered through whatmann filter paper (no.41). A 1.0 mL potion of the filtrate was diluted to 25.0 mL with methanol. Five such samples were prepared. A 20 µL volume of the final diluted solutions were injected separately and the representative chromatograms were recorded.

**3. RESULTS AND DISCUSSION**

**3.1 Selection of Detection Wavelength**

The UV spectrum of working standard solution was recorded against methanol as a blank shown in Fig. 2.

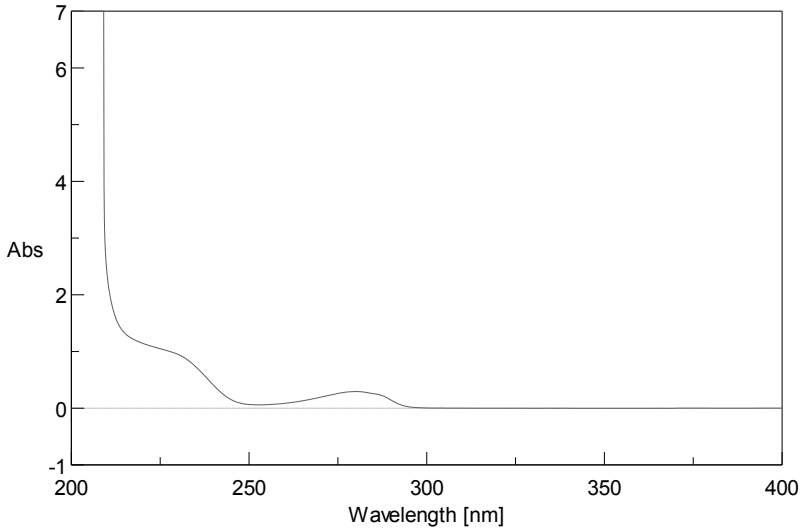


**Fig. 2: UV spectra of standard drug unexposed**

**3.2 HPLC method development and optimization**

The optimized chromatographic condition mentioned below was kept constant throughout the experimentation and mobile phase was allowed to equilibrate with stationary phase which was indicated by a steady line.

Column - Hyperchrome ODS 5 µ C18 column (250 X 4.6mm)

Mobile Phase - Acetonitrile and Buffer pH 2.5 (45:55 v/v)

Detection Wavelength - 280nm

Flow rate - 1.0 mL/min

Temperature (Ambient) - 28-30°C

Injection volume - 20 µL

A 20 µL solution of above mix standard was injected through manual injector and chromatogram was recorded. A standard chromatogram for Aliskiren and blank so recorded is shown in fig 3a-b.

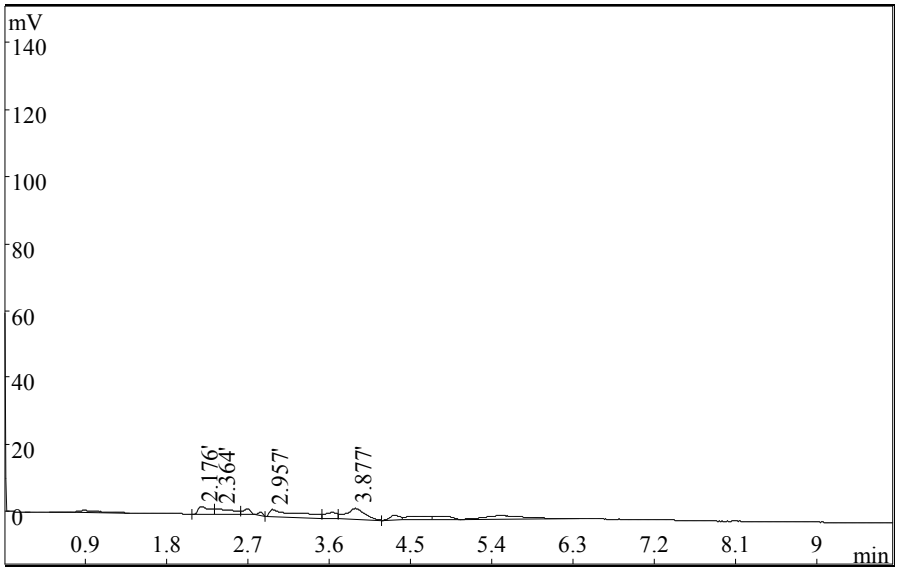


**Fig. 3**a : **Chromatogram of Blank**

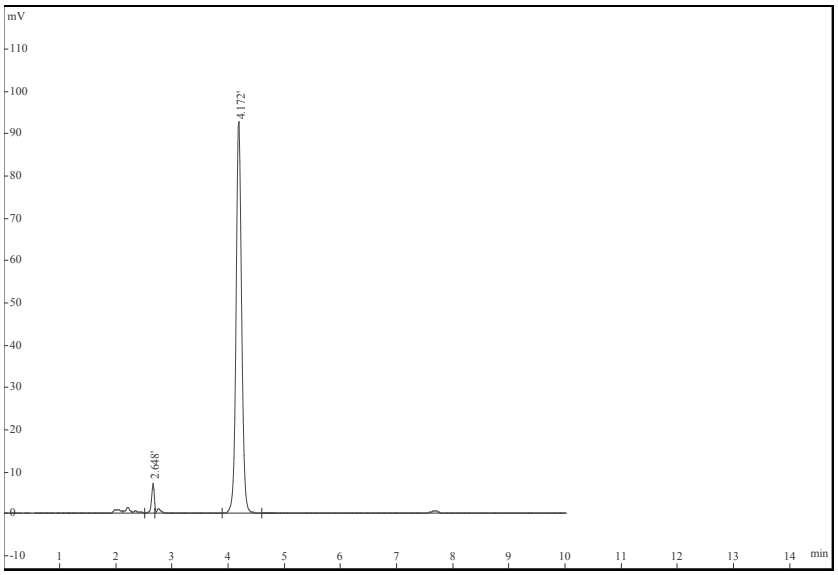


**Fig. 3b : Chromatogram of standard**

**3.3 Study of system suitability parameters**

After equilibration of column with mobile phase, five replicate injections of 20 µL solution of (B1) was injected through the manual injector and the chromatograms were recorded and the system suitability parameter were noted and values are shown in Table 1.

**3.4 Study of Linearity**

The graphs of concentration of drug vs. area under curve were plotted and the correlation coefficient was found to be (r=0.9986) for the drug.

**Table 1: Observation of system suitability parameters**

| Sr. No. | Standard Weight Taken | A.U.C of ALISK (mV) |
|---|---|---|
| 1 | ~25.0 mg | 919108 |
| 2 | | 917596 |
| 3 | | 908552 |
| 4 | | 917806 |
| 5 | | 907882 |
| Mean | | 914188.8 |
| ±S.D. | | 5487.279 |
| %RSD | | 0.6002 |
| Theoretical plate/column | | 8390 |
| Retention time | | 4.073 |
| Asymmetry | | 1.062 |
| Resolution | | 9.426 |

**3.5 Stability Indicating HPLC method Development**

**3.5.1 Solution State Stability**

**a. Acid hydrolysis (0.5N HCl)**

The study of chromatograms for acidic hydrolysis reveals that (fig. 4a-b) gradual degradation of the drug was observed after exposing it at 50°C for three hours. The study of chromatogram (Fig. 4a) reveals that the degradation of drugs under alkaline hydrolysis shows two additional peaks formed at [Deg 1 (tr – 5.3 min), and Deg 2 (tr – 7.7 min)] as they appear in chromatogram after refluxing for 30 min. The Deg 1& Deg 2 gradually increased up to 180 min of heating. The std drug exposed was found to be degraded to around 17.44% with appearance of two degraded products it is classified as Out of promotion to the condition. The degradation peaks was also formed in sample ALISK, and around16.56% of sample was degraded when subjected to similar conditions.

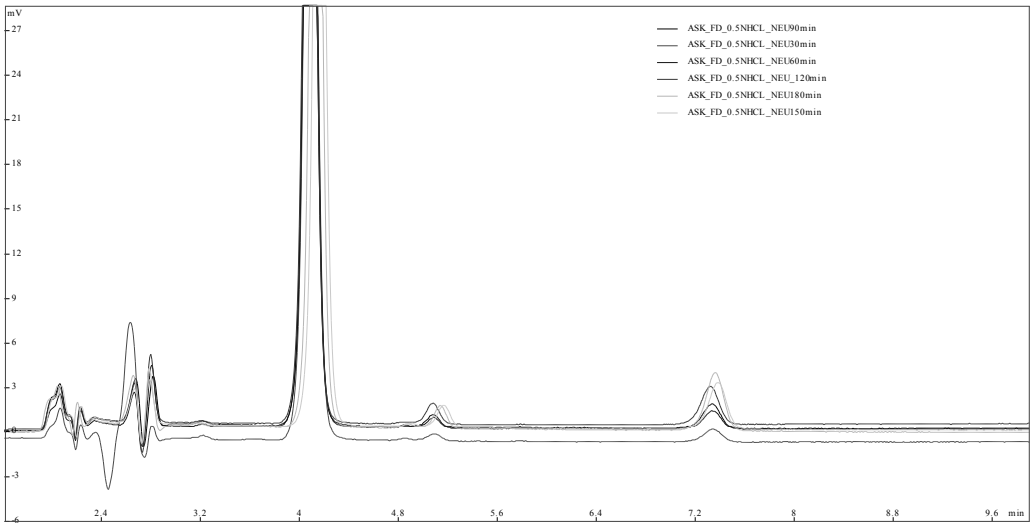


**Fig.4a : Overlain Chromatogram of standard drug under Acid hydrolysis**

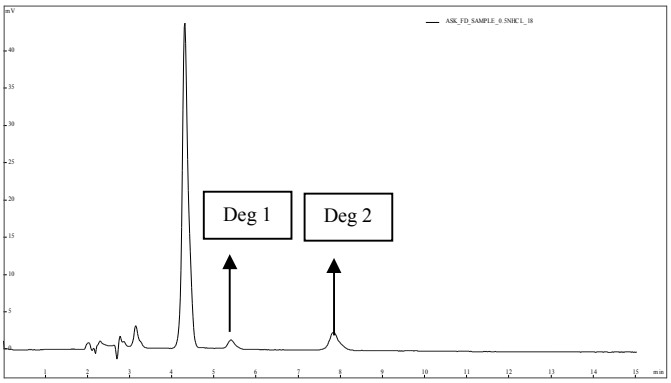


**Fig.4b : Chromatogram of Sample drug under Acid hydrolysis**

**b. Alkaline hydrolysis (0.5N NaoH)**

The study of chromatograms for alkaline hydrolysis reveals that (fig. 5a-b) gradual degradation of the drug was observed after exposing the drug at 50°C for three hours.

The study of chromatogram also reveals that the degradation of drugs under alkaline hydrolysis shows two additional peaks formed at [Deg1 (tr - 4.8 min), and Deg 2 (tr - 5.1min)] as they appear in chromatogram after refluxing for 30 min. The Deg 1 & Deg 2 was gradually increased up to 180 min of heating. The std exposed drug was found to be degraded to around 19.26% with appearance of two degraded products it is classified as Out of promotion to the condition. The degradation peaks was also formed in sample ALISK and around 17.44% was degraded when subjected to similar conditions.

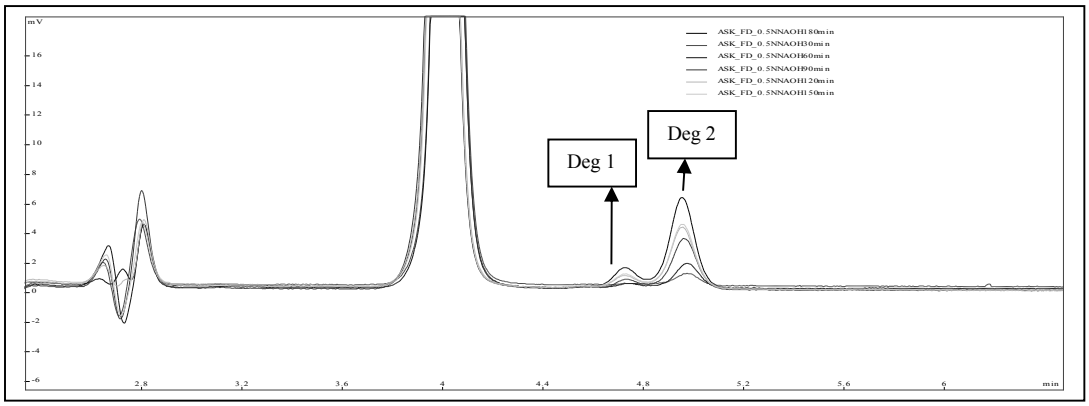


**Fig.5a : Overlain Chromatogram of Standard drug under Alkali hydrolysis**

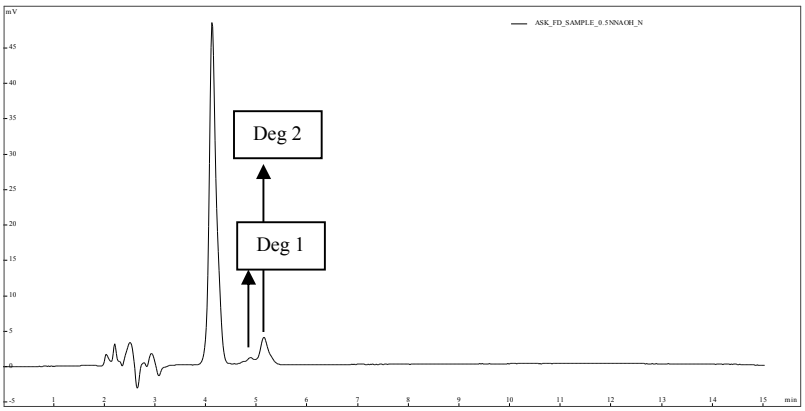


**Fig.5b : Chromatogram of Sample drug under Alkali hydrolysis**

**c. Neutral hydrolysis (distilled water)**

The study of chromatograms for neutral hydrolysis reveals (fig. 6a-b) that % of label claim of ALISK was found to be decreased up to heating for three hours. It was found to be false susceptible to neutral hydrolysis as no additional peaks were formed up to 180 min of heating. The drug was found to be degraded to around 10% with no appearance of extra peaks in standard as well as sample.

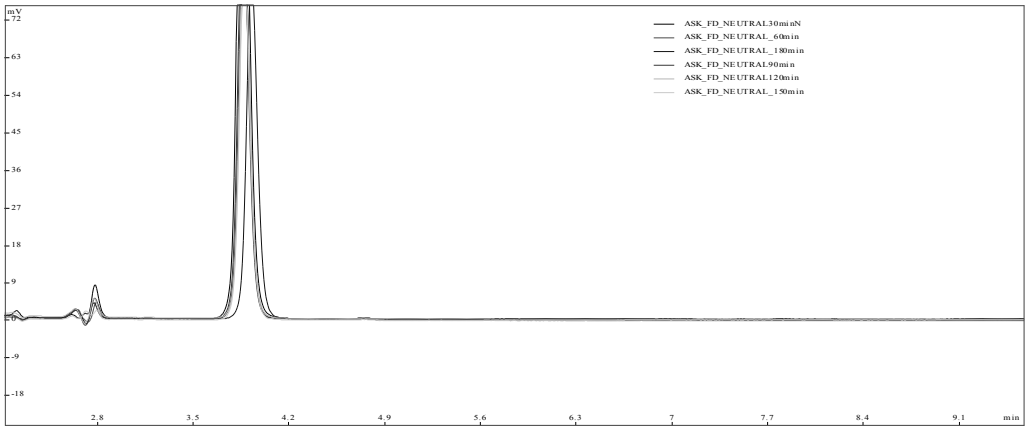


**Fig.6a : Overlain Chromatogram of Standard drug under Neutral hydrolysis**

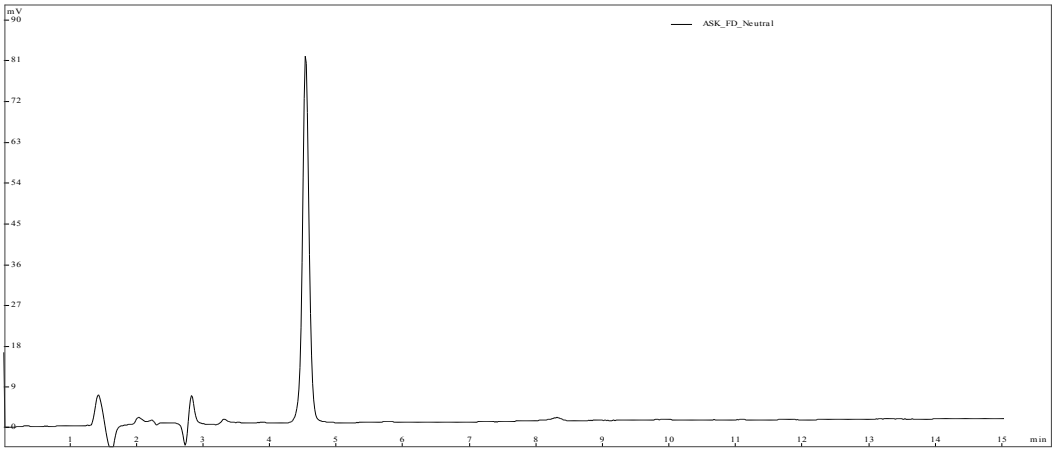


**Fig.6b : Chromatogram of Sample drug under Neutral hydrolysis**

**d. Oxidative hydrolysis (3 % $H_2O_2$)**

The study of chromatograms (Fig. 7a-b) reveals that the drug when subjected to oxidative hydrolysis, % of label claim of drug was found to be decreased on heating for three hours. It was found to be false susceptible to oxidative hydrolysis as no additional peak was observed in exposed Standard and samples. The degradation was found to be around 12%.

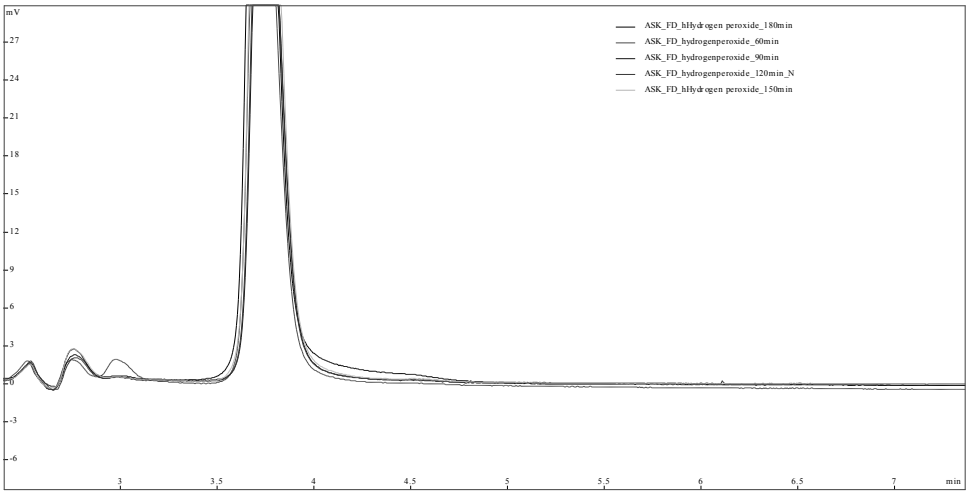


**Fig.7a : Overlain Chromatogram of Standard drug under Oxidative hydrolysis**

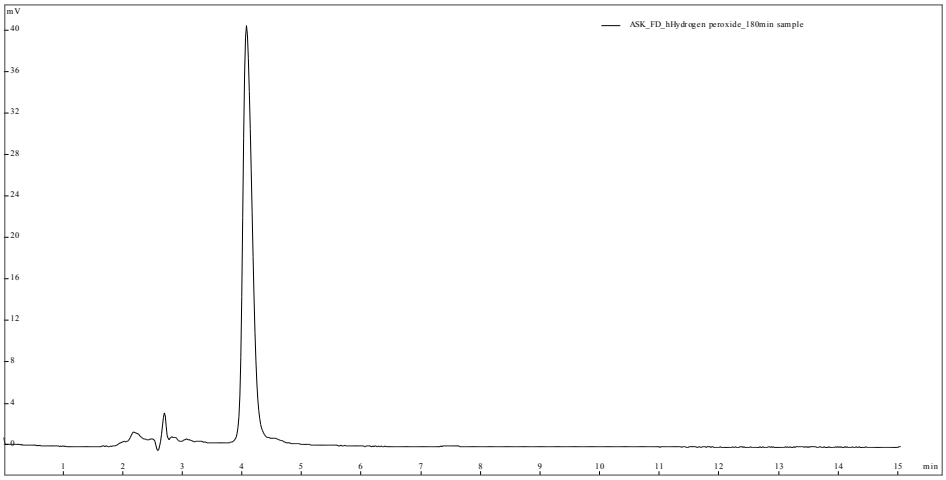


**Fig.7b : Chromatogram of Sample drug under Oxidative hydrolysis**

### 3.5.2 Solid State Stability Studies

#### a. Humidity Study (40ºC/75% RH) Marketed formulation (In powder form)

The humidity study was carried out on the standard drug and the marketed formulations by exposing it to 40ºC/75% RH for a period of 10 days.

The study of the chromatogram (Fig.8a-b) reveals that no additional peak was observed on exposure to 40ºC/75% RH of ALISK powdered sample for a period of 10 days, there was significant change in the area under curve for the drug up to 10th day. The area under the curve was decreased on 10th day but no additional peaks were seen in chromatogram. The results indicated that, on 10th day sample ALISK was degraded up to 30% i.e. degradation was found to be overdone.

Also when the standard drug was exposed to the similar condition, the chromatogram showed no additional peak generation but significant change in the area under curve. The drug was found to degrade around 43.77%.

Overlain UV spectra of sample solution recorded was compared with the UV spectrum of standard solution (fig 8c-d) which showed no change in the spectral pattern indicating that drugs have undergone no degradation but exhibit hypochromic effect at the λmax

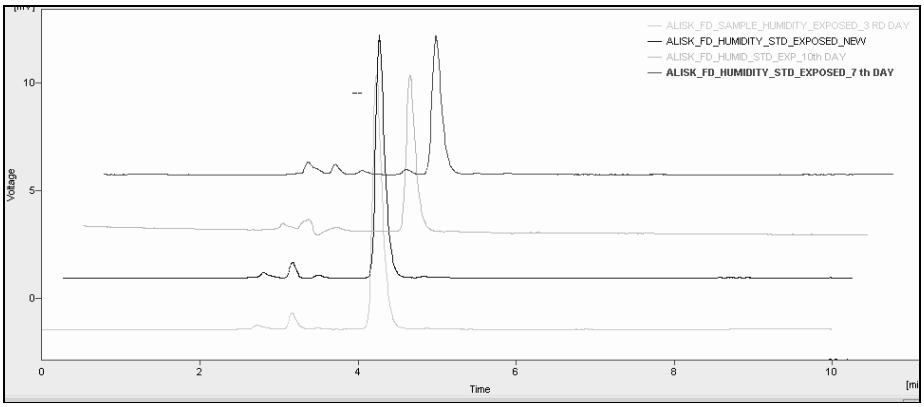


**Fig.8a : Overlain Chromatogram of Standard drug under Humidity studies**

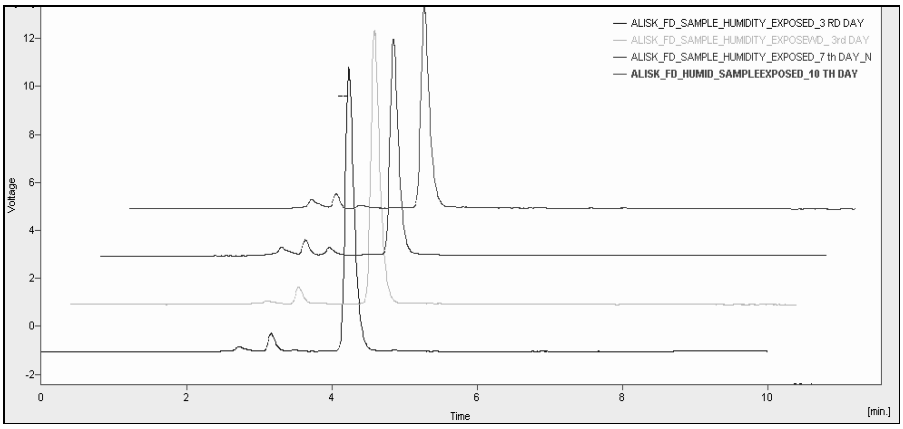


**Fig.8b : Overlain Chromatogram of Sample drug under Humidity studies**

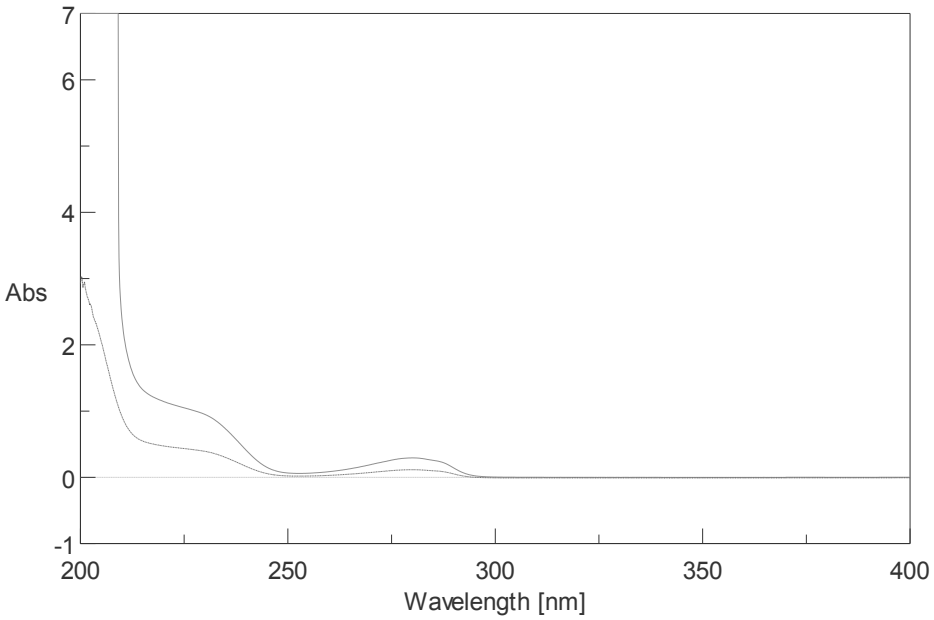


**Fig.8c : Overlay of Unexposed standard and 10th Day humidity standard (exposed)**

**b. Photo stability studies (UV Light)**

When standard drug & samples were exposed to UV light at 254 nm for a period of 2 days, the study of chromatogram (Fig.9a-b) reveals that there was no additional peak & no significant change in the area under curve for ALISK up to 2nd day i.e. ALISK was found to be false susceptible to degradation by UV light.

While the study of the chromatograms reveals that the area under the curve was decreased on 2nd day with no additional peak generation seen in the chromatogram in case of standard exposed & hence ALISK was found to be false (susceptible) to UV light. The results indicated that, on 2nd day standard exposed was degraded up to 11.66%.

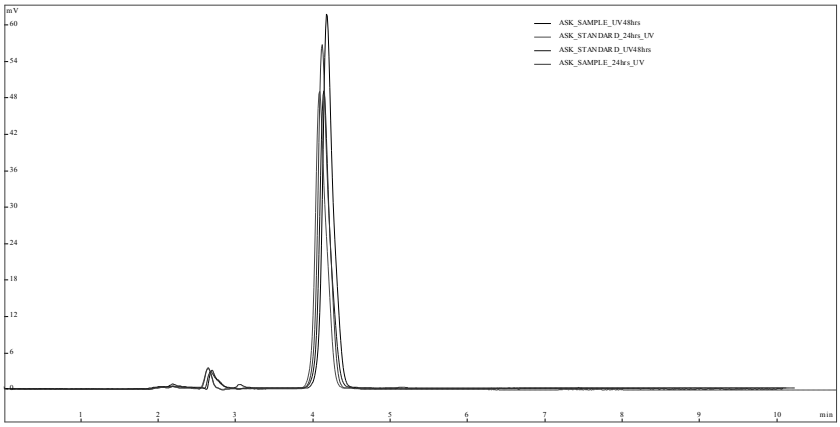


**Fig.9a :Overlain Chromatogram of Standard drug under UV LIGHT studies**

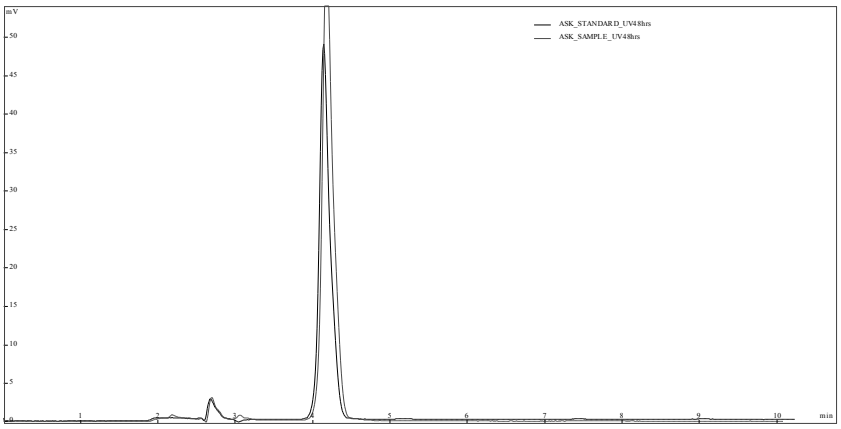


**Fig.9b: Overlain Chromatogram of Sample drug under UV LIGHT studies**

**c. Thermal studies (50ºC)**

When powdered sample was exposed to dry temperature at 50ºC for a period of 3 hours, there was no significant change in the area under curve for samples, no additional peaks were observed in the chromatogram (Fig. 10). The results indicates that after 3 hours ALISK was not degraded i.e. sample was found to be soft susceptible to thermal degradation. Summary of stability studies are shown in Table 2.

**Table 2: Summary of stability indicating Assay for ALISK**

| Sr. No. | Stressed condition | % Undegraded drug | |
|---|---|---|---|
| | | STD Exposed | ALISK |
| 1 | Alkaline reflux (180 min) | 80.72 | 82.54 |
| 2 | Acidic reflux (180 min) | 82.54 | 83.42 |
| 3 | Neutral reflux (180 min) | 90.49 | 88.79 |
| 4 | Oxidation reflux (180 min) | 90.61 | 88.98 |
| 5 | Humidity studies (10 days) | 56.21 | 70.65 |
| 6 | UV light (2 days) | 88.34 | 99.83 |
| 7 | Thermal studies (3hours) | 98.63 | 100.12 |

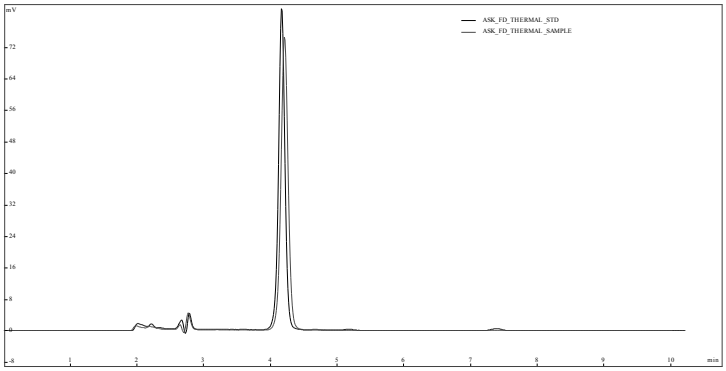


**Fig.10 : Overlain Chromatogram of Sample & Standard drug under Thermal Studies**

**3.6 Application of Proposed Method For Assay Of Marketed Formulation**

The 20 µL volume of the final diluted solution were injected separately, the representative chromatograms was recorded and shown in Fig. 11. The results of estimation are shown in Table 3.

**Table 3: Results of estimation in marketed formulation**

| Sr. No. | AUC of Standard (mV) | AUC of Sample (mV) | Amt. Estimated in Avg. wt. of Tab(mg) | % Label claim |
|---|---|---|---|---|
| 1 | 837474 | 896823 | 150.06 | 100.04 |
| 2 | | 830915 | 149.71 | 99.81 |
| 3 | | 839764 | 149.64 | 99.76 |
| 4 | | 908552 | 150.72 | 100.48 |
| 5 | | 899028 | 149.58 | 99.72 |
| | | | Mean | 99.982 |
| | | | ± SD | 0.29853 |
| | | | %RSD | 0.2985 |

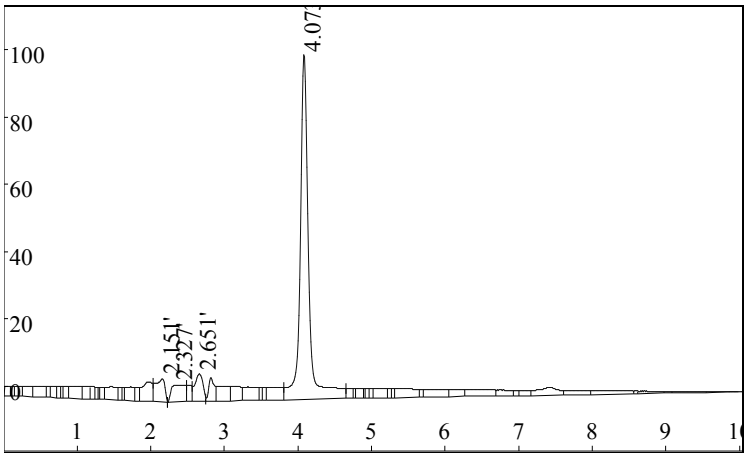


**Fig. 11 Chromatogram for ALISK in marketed formulation**

**3.7 Method Validation**

The method was validated as per the guidelines in terms of parameters like, precision, accuracy (recovery studies), system suitability parameters, linearity and range etc.

**i.Accuracy**

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. The mean % recovery was found to be 99.82. The % RSD was found to be 0.52055. Results of recovery study are summarized in Table 4.

**Table 4: Results of Accuracy Study**

| Sr. No. | Amt. Contributed by sample (mg) | Amt. of pure drug added (mg) | Total Amt. of Drug Estimated (mg) | Amount Recovered (mg) | % Recovery |
|---|---|---|---|---|---|
| 1 | 10.03 | 2.54 | 12.58 | 2.55 | 100.39 |
| 2 | 10.88 | 7.54 | 18.44 | 7.55 | 100.13 |
| 3 | 10.16 | 10.80 | 20.89 | 10.73 | 99.35 |
| 4 | 10.54 | 12.05 | 22.49 | 11.95 | 99.41 |
| | | | | Mean | 99.82 |
| | | | | ± SD | 0.51961 |
| | | | | %RSD | 0.52055 |

**ii.Precision**

Precision of proposed method was ascertained by replicate analysis of homogeneous samples. Precision of any analytical method is expressed as SD and RSD of series of measurements. The mean percent label claim was found to be 99.982. The % RSD was found to be 0.29855. Results are shown in Table 3.

**iii.Ruggedness**

Intermediate precision (Intraday and Inter-day) shows the % Label claim values within limits (% RSD not more than 2). The method was found to be précise. The ruggedness studies were carried out using different analyst variation. The results of intermediate precision parameter are shown in Table 5.

**Table 5: Results of Intermediate precision**

| Parameters | Mean % label claim ± S.D. of ALISK |
|---|---|
| Different Analyst (n=3) | 100.38 ± 0.15 |
| Intraday Variation (n=4) | 99.23 ± 0.88 |
| Interday Variation (n=3) | 92.98 ± 7.77 |

**iv. Linearity and range**

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110 and 120% of label claim (ALISK) were taken and dilutions were made as described under marketed formulation. Then, each solution was injected and chromatograms were recorded.

The correlation coefficient was found to be 0.9986 of ALISK.

**v. Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The system suitability parameter was evaluated for each varied condition. The amount of ALISK was calculated from sample solution in each varied condition. Results are shown in Table 6. The results of above study shown that the method were robust under varied conditions.

**Table No. 6: Observation and result of Robustness study**

| Sr.No | Deliberate Changes | R.T. | Asymmetry | % Label Claim |
|---|---|---|---|---|
| 1 | Standard Condition | 4.073 | 1.062 | 100.48 |
| 2 | Change in flow rate (1.1 ml) | 4.763 | 1.153 | 101.05 |
| 3 | Change in flow rate (0.9ml) | 3.920 | 1.109 | 101.45 |
| 4 | Change in Wavelength (285nm) | 4.047 | 1.029 | 98.77 |
| 5 | Change in Wavelength (275nm) | 4.030 | 1.021 | 99.12 |
| 6 | Change in pH (2.3) | 4.253 | 1.085 | 99.53 |
| 7 | Change in pH (2.7) | 4.237 | 1.029 | 98.86 |
| SD | | 0.279 | 0.049 | 1.092 |
| Overall SD | | 0.473 | | |

## 4. CONCLUSION

The proposed method was found to be simple, accurate, rapid and cost effective for determination of Aliskiren from pure drug and its dosage forms. The developed method is stability indicating method and can be conveniently use in routine and stability samples. The mobile phase is simple to prepare and economical. The sample recovery in a formulation was in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Aliskiren in pure form and its dosage forms.

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