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# Stability indicating RP-UPLC method for the determination of Aliskerin and its impurities in its bulk and pharmaceutical dosage forms.

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**ABSTRACT**

*A reversed-phase liquid chromatography (RP-UPLC) method is validated for the determination of aliskiren in tablet dosage form. The LC method is carried out on a Waters Acquity, BEH, C8 column (100 × 2.1 mm i.d.), maintained at 25°C. The mobile phase consisted of 2.72 grams of potassium dihydrogen ortho phosphate and 3.5 grams of 1-Octane sulfonic acid adjusted to pH 2.0 as aqueous phase and Acetonitrile as organic phase with gradient programme, run at a flow rate of 0.5 mL/min, with photodiode array detector set at 230 nm. The chromatographic separation is obtained with aliskiren retention time of 3.70 min, and it is linear in the range, with $R^2$ values greater than 0.9999. The specificity and stability-indicating capability of the method are proven through degradation studies, which also showed that there is no interference of the formulation excipients, showing that peak is free from any co eluting peak. The method showed adequate precision, with a relative standard deviation (RSD) values lower than 2.0%. Good values of accuracy were also obtained, with a mean value of 90-110%. Experimental design is used during validation to calculate method robustness. The proposed method is applied for the analysis of bulk drugs and the tablet dosage forms, contributing to improve the quality control and to assure the therapeutic efficacy.*

**Key words:** Aliskerin, RP-UPLC, Degradation products, Stability indicating.

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## INTRODUCTION

Hypertension, one among other associated disorders like diabetes, is the leading cause of morbidity and mortality, if left uncontrolled [1-2]. The main cause of hypertension is still yet unknown but from the understandings of the body physiological mechanism for maintaining blood pressure, it was known that the rennin angiotensin system (RAS) play a major and crucial role in maintaining a constant blood pressure. Apart from maintaining the blood pressure, the RAS system also helps in maintaining body electrolyte balance and had been thus recognized as an important target in treating hypertension and its associated disorders that result from untreated hypertension[3- 4]Many drugs like the so called Angiotensin converting enzyme inhibitors eg: captopril, enalapril,and Angiotensin receptor blockers like losartan, valsartan etc; are under current use and are the first choice drugs for treating hypertension5. However, one of these drugs suffers from adverse effects. So, long ago renin enzyme was a target for drug discovery companies. As a result of several efforts, aliskiren

Available as the hemifumarate salt resulted as a first in class drug for effective management of hypertension. It is 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 faces.

The literature survey reveals that there are available HPLC Methods UV Spectroscopic methods [6-17], Furthermore, to the best of our knowledge; no stability-indicating UPLC method is reported in the literature.

The objectives of the present manuscript describe the degradation behaviour of Aliskerin under hydrolysis (acid, base and neutral), oxidation, thermal and photolysis conditions. To optimize the liquid chromatography conditions to separate the drug from its degradation products on a reverse phase Acquity, BEH, C8 column and to establish a validated stability-indicating Assay and its impurities method by UV detection at 230 nm. These studies provide precious information about drug's inherent stability and assist in the validation of analytical methods to be used in stability studies [18]. The developed UPLC method was validated as per the International Conference on Harmonization (ICH) guidelines [19].

## MATERIALS AND METHODS

2.1 **Instrumentation:**
A Waters Acquity Ultra Performance liquid chromatography equipped with PDA Detector with Binary pump. The column utilised was Acquity UPLC, BEH, C-8, 2.1x100mm, 1.8µm.

2.2 **Materials:**
Aliskerin working standard and all four related substances (Table1) were produced from MSN Laboratories, Hyderabad. 1-Octane sulfonate salt, Acetonitrile and Potassium di hydrogen phosphate from Merck. , Tekturna® tablets were obtained from Novartis, Germany.

The compounds related to Aliskerin which could be expected as impurities or might appear as degradation products have been prepared and identified by MSN Laboratories and listed in below table.

**Table: 1 Impurities and their chemical names.**

| Name | Chemical Name |
|---|---|
| Hydroxy impurity (Impurity-01) | (2S,4S,5S,7S)-N-(2-Carbamoyl-2-methylpropyl)-5-amino-4-Hydroxy-2,7-diisopropyl-8-hydroxy-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamide hemi fumarate. |
| Acid impurity (Impurity-02) | (2S,4S,5S,7S)-N-(2-Carbamoyl-2-methylpropyl)-5-amino-4-Hydroxy-2,7-diisopropyl-8-hydroxy-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanoic acid. |
| Des methoxy impurity (Impurity-03) | (2S,4S,5S,7S)-N-(2-Carbamoyl-2-methylpropyl)-5-amino-4-Hydroxy-2,7-diisopropyl-8-hydroxy-8-[3-(3-methoxypropoxy)phenyl]- octanamide hemi fumarate. |
| N-BOC impurity (Impurity-04) | Tert-Butyl(3S,5S,6S,8S)-8-(3-amino-2,2-dimethyl-3-oxo propyl carbamoyl)-6-hydroxy-3-4-methoxy-3-(3-methoxy propoxy)benzyl)-2,9-dimethyldecan-5-yl,carbamate |

2.1 **Developing an UPLC Method:**
The UPLC method carried out in this study aimed at developing a chromatographic system capable of eluting and resolving Aliskerin and its impurities (related substances) from one another and that complies with the general requirements for system suitability.

All the Development related trails and observations are summarized and in below table.

**Table: 2 Method development trails and observations.**

| Trail No | Changes in trail | Results |
|---|---|---|
| 1 | Column: HSS C-18, 2.1x100mm, Acetonitrile and 0.01 M Potassium di hydrogen phosphate(pH-2.0) with gradient | The resolution Impurity -1 and Aliskerin was low. |
| 2 | Used methanol as organic modifier instead of Acetonitrile. | High tailing factor and longer retention times. |
| 3 | Changed the column to BEH,C-18,100mm | The resolution Impurity -1 and Aliskerin was low. |
| 4 | Added 1-Octane sulfonic acid salt to 0.02 M $KH_2PO_4$.BEH C18 column | Enhancement of peak symmetry and Increase in resolution with impurity-01 |
| 5 | When the pH was 3 to 7 | Decrease in retention times and resolution was poor. |

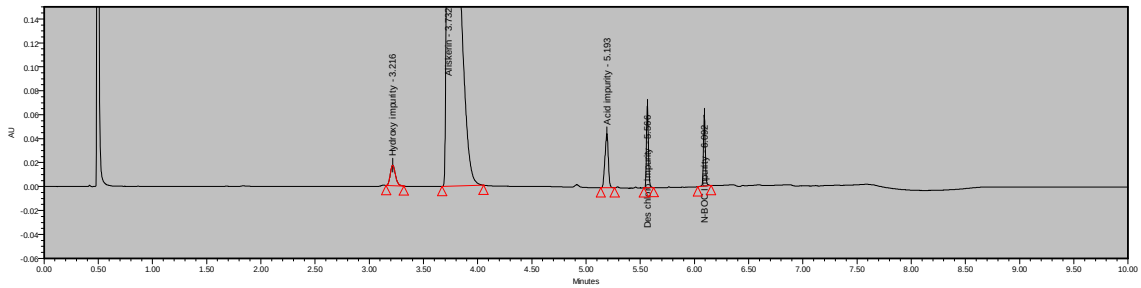


**Fig: 1: Impurity spiked sample:**

**2.2 Finalized conditions:**

Gradient elution technique was utilized with the column maintained at 45°C the Buffer phase used was Potassium dihydrogen ortho phosphate [0.02M] with 3.5 grams of 1-Octane sulfonic acid salt adjusted to pH 2.0 with dilute ortho phosphoric acid as aqueous phase and Acetonitrile was used as organic phase. The gradient elution was used the flow rate was 0.5 mL/min. Samples of 1µL was injected into the column and the detector was set at 230 nm with run time of 10 minutes. The relative standard deviation (R.S.D.) of six replicate injections of the standard preparation was not greater than 2.0% and the tailing factor was less than 2.0.

**Table 3: Gradient programme**

| Time(min) | Flow(mL/min) | %A | %B |
|---|---|---|---|
| 0.01 | 0.5 | 65 | 35 |
| 4.00 | 0.5 | 65 | 35 |
| 7.00 | 0.5 | 20 | 80 |
| 8.00 | 0.5 | 65 | 35 |
| 10.00 | 0.5 | 65 | 35 |

**2.3: Preparation of solutions:**

**2.3.1 Preparation of diluted standard solution**

An accurately weighed quantity of Aliskerin or related substances (Impurities 1, 2, 3 and 4) was dissolved in the diluent (water: Acetonitrile in 50:50 ratio) and diluted quantitatively. Serial dilutions were carried out, using the diluent, to obtain solutions of known concentrations to be used for the standard reparation (10 ppm for Impurities diluted standard and 100 ppm for assay).

**2.3.2: Preparation of Test solutions (Tekturna):**

Twenty tablets were weighed and powdered and accurately weighed portions equivalent to 200 mg Aliskerin were transferred to 100 ml volumetric flasks. Disintegrated with 30 mL of Diluent was added up to the volume. The solutions were sonicated and centrifuged as above and the supernatant was used as test solution for impurities with 2.0 mg per ml further transfer 5 mL of the above solution to 100 mL with the diluent for Assay (100 ppm).

**2.4 Quantification:**

Equal volumes, (1µL), of the standard preparations and the test preparations that contain Aliskerin in the diluent were injected into the chromatograph and the quantified for Aliskerin and its impurities by area normalization method.

**2.5 Linearity, Limit of detection and limit of quantification:**

Calibration graphs were constructed for Aliskerin and its impurities in either standard solution .The degree of linearity was assessed by the correlation coefficient, y-intercept, and slope. The limit of detection, LOD and the limit of quantitation LOQ have been estimated as 3 S.D. and 10 S.D. of the y intercept and slope.

**2.6 Precision:**

The precision was performed by preparing six individual preparations as per the method of analysis and evaluated for percentage of Aliskerin and its individual impurities and percentage of total impurities.

**2.7 Accuracy:**

The samples were prepared by spiking the Active substances and impurities stock solutions into the drug placebo mixture and the percent recovery was estimated.

**2.8: Solution stability:**
The solutions prepared in method precision were tested 24hrs and 48Hrs by maintaining at room temperature and estimated for impurity content.

**2.9: Robustness:**
Robustness was conducted by making the variations in flow rate, Column oven temperature and percentage of Acetonitrile.

**2.10: Ruggedness:**
The prepared solutions were filtered through 0.45 µ PVDF syringe filter and 0.45 µ PTFE syringe filter and evaluated against the centrifuged sample.

**2.11: Intermediate precision:**
The test was performed with another analyst on different day, different system and different column and the impurity contents were reported.

**2.12: Forced degradation studies:**
The forced degradation studies conditions and % degradations mentioned in the results (Table: 8) section.

## RESULTS AND DISCUSSION

**Table: 4: Validation characteristics of Assay method**

| Parameter | Results |
|---|---|
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. |
| Linearity | Established from 20 ppm to 300 ppm ($R^2$ value=0.9999) |
| Precision: | % RSD of impurity for six preparations= 0.24 |
| Accuracy | **%Level** **%Recovery**<br>20% 99.0<br>50% 99.7<br>80% 100.5<br>100% 99.1<br>300% 100.2 |
| Solution stability | 1)% Difference at 24 Hrs=0.18%<br>2)%Difference at 48 Hrs=0.20% |
| Robustness | Flow rate variation-System suitability passes<br>Temperature variation system suitability passes |
| Ruggedness | Filter validation:<br>Variation between PVDF &PTFE 0.45 micron filters=0.09% |
| Intermediate precision | %RSD=0.53% |

**Table: 5: Validation characteristics of Hydroxy impurity.**

| Parameter | Results |
|---|---|
| RRT | 0.83 |
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. |
| Linearity | Established from 1 ppm to 42 ppm ($R^2$ value=0.9995) |
| LOD and LOQ | LOD=0.23 ppm and LOQ=0.70 ppm |
| Precision: | % RSD of impurity for six preparations= 1.51 |
| Accuracy | **%Level** **%Recovery**<br>10% 105.4<br>20% 101.2<br>50% 97.0<br>100% 94.9<br>200% 95.7<br>400% 95.5 |
| Solution stability | 1)% Difference at 24 Hrs=0.01%<br>2)%Difference at 48 Hrs=0.01% |
| Robustness | Flow rate variation-System suitability passes<br>Temperature variation system suitability passes |
| Ruggedness | Filter validation:<br>Variation between PVDF &PTFE 0.45 micron filters=0.02% |
| Intermediate precision | Individual impurity variation=0.01%<br>Total impurity variation=0.03% |

**Table: 6: Validation characteristics of Acid impurity**

| RRT | 1.35 |
|---|---|
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. |
| Linearity | Established from 1 ppm to 42 ppm ($R^2$ value=0.9995) |
| LOD and LOQ | LOD=0.36 ppm and LOQ=1.10 ppm |
| Precision: | % RSD of impurity for six preparations= 0.81 |
| Accuracy | **%Level** **%Recovery**<br>10% 98.2<br>20% 96.9<br>50% 106.4<br>100% 96.0<br>200% 92.5<br>400% 90.5 |
| | |
| Solution stability | 1)% Difference at 24 Hrs=0.01%<br>2)%Difference at 48 Hrs=0.02% |
| Robustness | Flow rate variation-System suitability passes<br>Temperature variation system suitability passes |
| Ruggedness | Filter validation:<br>Variation between PVDF &PTFE 0.45 micron filters=0.01% |
| Intermediate precision | Individual impurity variation=0.02%<br>Total impurity variation=0.03% |

**Table: 7: Validation characteristics of Des methoxy impurity**

| RRT | 1.45 |
|---|---|
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. |
| Linearity | Established from 1 ppm to 42 ppm ($R^2$ value=1) |
| LOD and LOQ | LOD=1.42 ppm and LOQ=4.32 ppm |
| Precision: | % RSD of impurity for six preparations= 0.37 |
| Accuracy | **%Level** **%Recovery**<br>10% 103.4<br>20% 105.6<br>50% 110.6<br>100% 105.9<br>200% 104.3<br>400% 103.5 |
| Solution stability | 1)% Difference at 24 Hrs=0.01%<br>2)%Difference at 48 Hrs=0.03% |
| Robustness | Flow rate variation-System suitability passes<br>Temperature variation system suitability passes |
| Ruggedness | Filter validation:<br>Variation between PVDF &PTFE 0.45 micron filters=0.00% |
| Intermediate precision | Individual impurity variation=0.02%<br>Total impurity variation=0.03% |

**Table: 8: Validation characteristics of N-BOC impurity**

| RRT | 1.58 |
|---|---|
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. |
| Linearity | Established from 1 ppm to 42 ppm ($R^2$ value=1) |
| LOD and LOQ | LOD=0.77 ppm and LOQ=2.35 ppm |
| Precision: | % RSD of impurity for six preparations= 2.85 |
| Accuracy | **%Level** **%Recovery**<br>10% 104.6<br>20% 98.6<br>50% 106.0<br>100% 97.0<br>200% 97.2<br>400% 95.1 |
| Solution stability | 1)% Difference at 24 Hrs=0.00%<br>2)%Difference at 48 Hrs=0.00% |
| Robustness | Flow rate variation-System suitability passes<br>Temperature variation system suitability passes |
| Ruggedness | Filter validation:<br>Variation between PVDF &PTFE 0.45 micron filters=0.01% |
| Intermediate precision | Individual impurity variation=0.01%<br>Total impurity variation=0.03% |

**Table: 9: Forced degradation Results**

| Type | Condition &Duration | % Degradation | Peak purity | %Assay |
|---|---|---|---|---|
| Acid | 1N Hcl,24Hrs,50°C | 89% | Passes | 11.2 |
| Base | 1N NaoH,24Hrs,50°C | 32.58 | Passes | 67.2 |
| Peroxide | 10% $H_2O_2$,24Hrs,50°C | 32.28 | Passes | 67.7 |
| Water | Water,24Hrs,50°C | 0.31 | Passes | 99.6 |
| Thermal | 24Hrs,50°C | 0.29 | Passes | 99.7 |
| Photo | 1.2 million Lux hours | 0.21 | Passes | 99.8 |

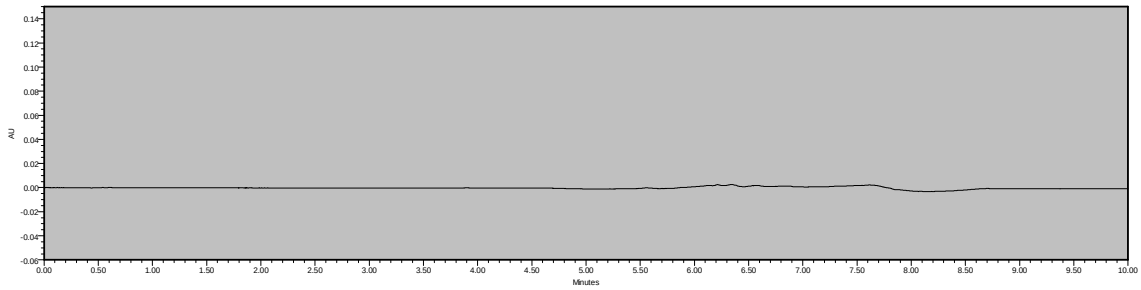


**Fig: 2: Blank chromatogram**

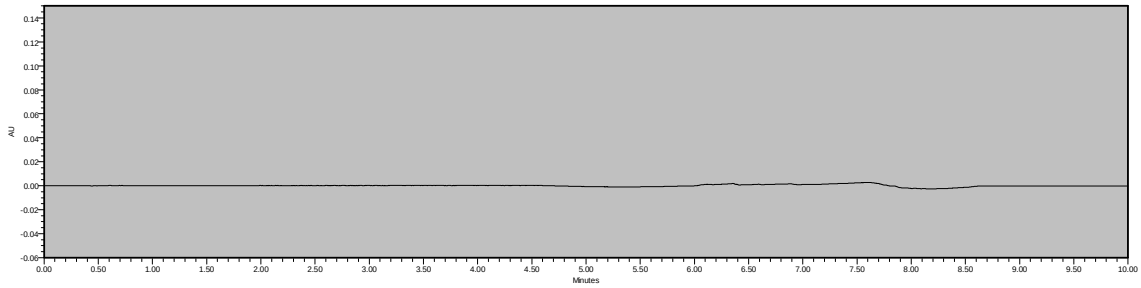


**Fig: 3: Placebo chromatogram**

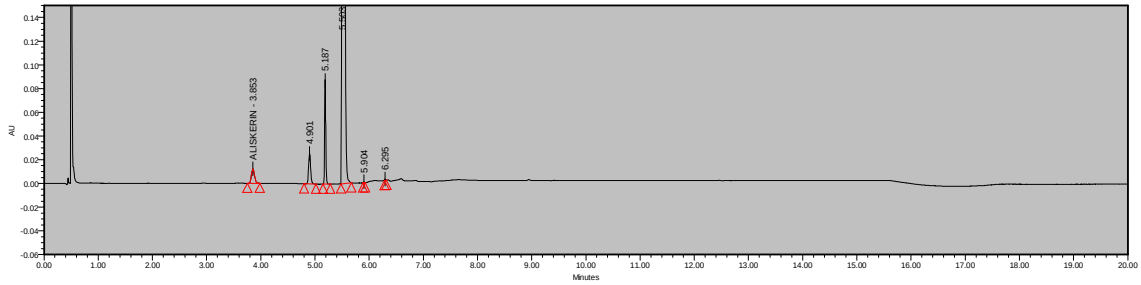


**Fig: 4: Acid stressed sample chromatogram**

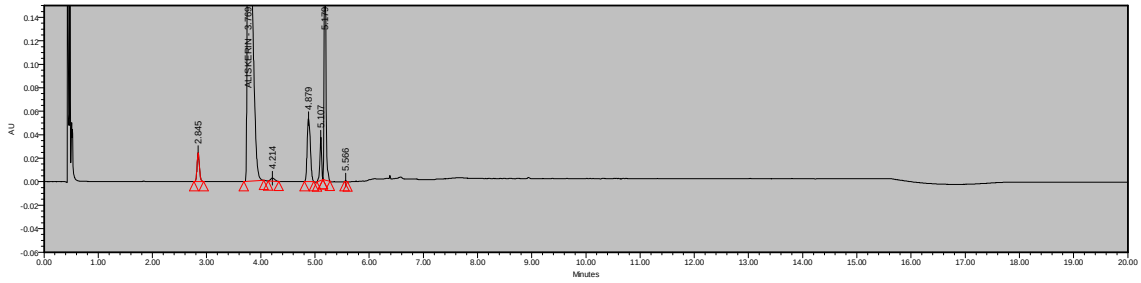


**Fig: 5: Base stressed sample chromatogram**

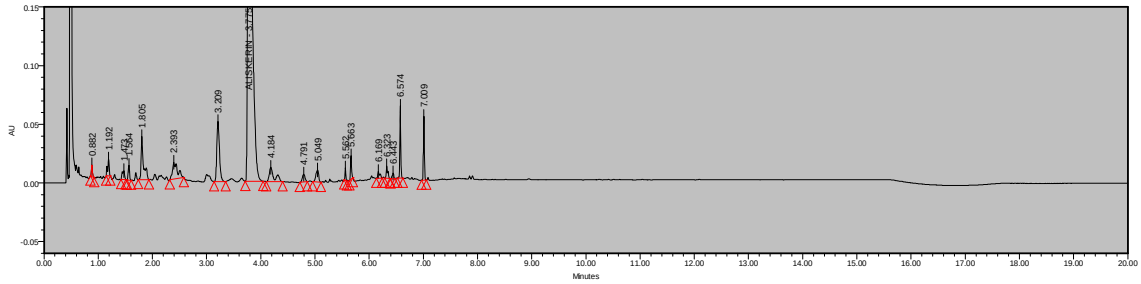


**Fig: 6: Peroxide stressed sample chromatogram**

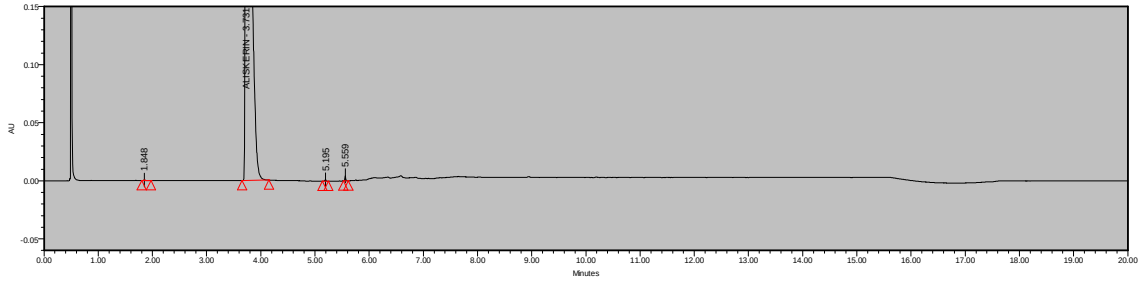


**Fig: 7: Water stressed sample chromatogram**

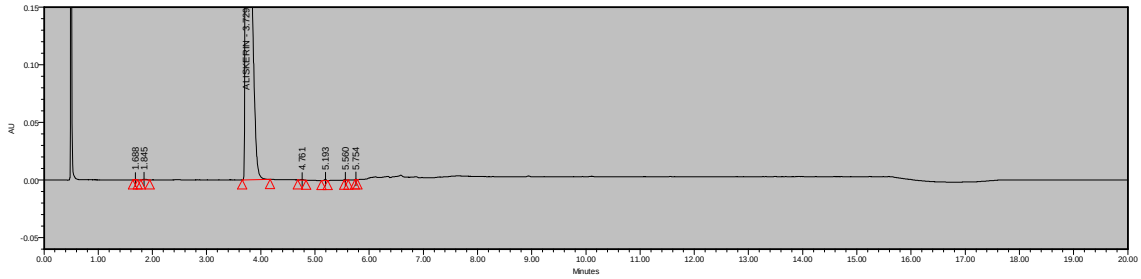


**Fig: 8: thermal stressed sample chromatogram**

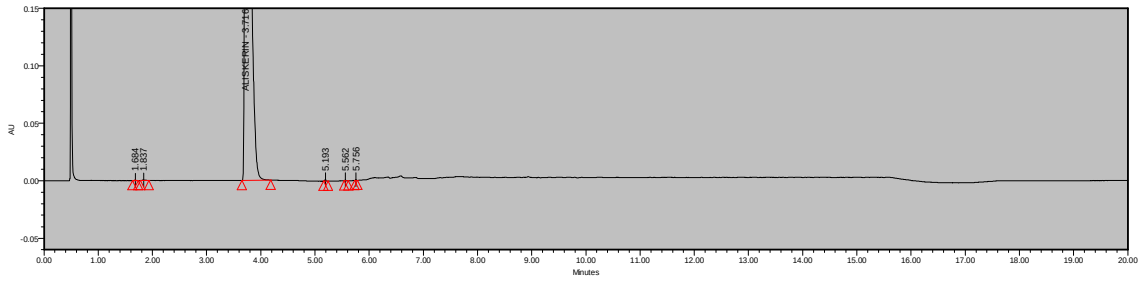


**Fig: 9: Light stressed sample chromatogram**

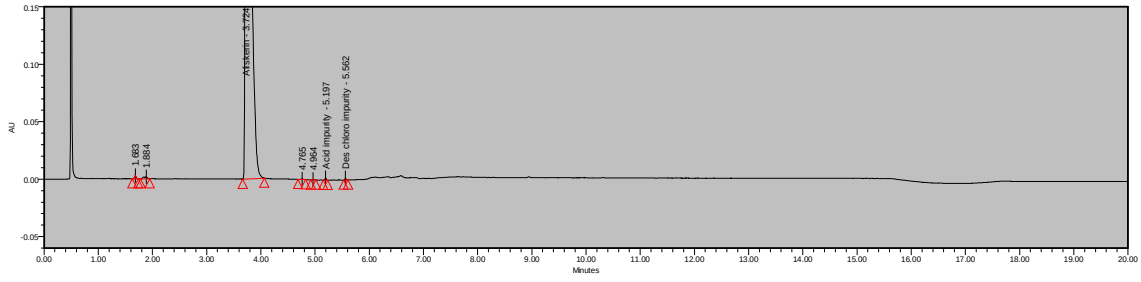


**Fig: 10: Tekturna sample chromatogram**

**Application to Tekturna samples:**

The validity of the method developed here for quantification of Aliskerin and the impurities that might interfere in the determination of Aliskerin was studied by Analyzing a commercial Aliskerin product Tekturna tablets

(Manufacturer Novartis, Germany) kept on stability at 40/75 Condition for 6 months. Two of the compounds related to Aliskerin appeared clearly on the chromatogram; this indicates that the proposed method can differentiate between the active moiety and its related impurities. Samples of Tekturna® (n=3) were analysed for Aliskerin and its impurities by this method and the results showed.

**Study for Uneluted peaks:**

Since the runtimes are lower, a study conducted on all the stressed samples for knowing the retained peaks by increasing the Acetonitrile to 90% for 20 minutes. The results showed there was no peak eluted at all.

**Equivalency study with HPLC method:**

The developed UPLC Method was compared with HPLC method (Obtained from API Vendor) results.

**Table 10: System suitability equivalence.**

| S.No | Method | Parameter | Criteria | HPLC method Result | Proposed Method |
|---|---|---|---|---|---|
| 1 | Assay | Tailing factor | Not more than 2.0 | 1.16 | 1.01 |
| 2 | Assay | Standard %RSD | Not more than 1.0 | 0.42 | 0.21 |
| 3 | Impurities | Tailing factor for Aliskerin- Peak | Not more than 2.0 | 1.84 | 1.37 |
| 4 | Impurities | Resolution between Aliskerin and Hydroxy impurity | Not Less than 1.5 | 1.8 | 3.4 |

**Table11: API Batch analysis Results (B.NO:AF0010412)**

| S.No | Method | Criteria | HPLC method Result | Proposed Method |
|---|---|---|---|---|
| 1 | Assay | 98.0-102.0% | 99.8 | 99.9 |
| 2 | Impurities | | | |
| 3 | Maximum individual impurity | Not more than 0.15% | 0.05 | 0.05 |
| 4 | Total impurity | Not more than 0.3% | 0.25 | 0.24 |

**Table12: Tekturna Tablet analysis Results (B.NO:F0294)**

| Details | HPLC method Results | UPLC Method results |
|---|---|---|
| Known impurity | 1)0.01% | 1)0.01% |
| Any unknown individual impurity | 2)0.04% | 2)0.04% |
| Total impurity | 3)0.08% | 3)0.08% |

## CONCLUSION

An UPLC method for related compounds in the commercial drug products and in the tablet formulation was validated in this study. Aliskerin and its impurities which may co exist with it as impurities or as degradants gave chromatograms of very well resolved peaks which indicate the specificity of the method and the possibility of using it as an indicator of stability. Slight changes in the experimental conditions did not affect significantly the resolution of the compounds of interest or their percent recoveries indicating the robustness of the method. All the statistical values (percent recovery, RSD, %D, the slope and the intercept, LOD and LOQ) calculated were within the acceptable limits. The method was shown to be equivalent with the HPLC method with the run time of 10 minutes. It can be used for estimation of Aliskerin and its related substances in bulk drugs, solid dosage form and quality control purposes.

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