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# Stability-Indicating Method Development and Validation for the Simultaneous Estimation of Glecaprevir and Pibrentasvir in Pharmaceutical Dosage Form by UPLC

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**Abstract**

***Aim:*** The proposed study aimed to develop a stability-indicating ultra-performance liquid chromatography (UPLC) method for the estimation of Glecaprevir and Pibrentasvir in Pharmaceutical dosage form and validate the method in accordance with the International Conference on Harmonization guidelines.

***Methods:*** The optimized conditions for the developed UPLC method are Acquity UPLC CHS C18 (100mm × 1.8mm, 2.0µm) column maintained at $30^0$C with a mobile phase consisting of methanol and water in the ratio of 45:55%v/v on isocratic mode at a flow rate of 1ml/min.

***Results and conclusion:*** The sample was detected at 260nm. The retention time for Glecaprevir and Pibrentasvir was deemed at 0.40min and 0.69min. The developed method was validated for accuracy, precision, specificity, ruggedness, robustness, and solution stability. The method obeyed Beer's law in the concentration range of 25µg/ml to 150µg/ml for Glecaprevir and 10µg/ml to 60µg/ml for Pibrentasvir with a correlation coefficient of 0.999for Glecaprevir and Pibrentasvir respectively. Forced degradation studies were conducted by exposing the drug solution to numerous stress conditions such as acidic, basic, peroxide, neutral, photolytic, and thermal conditions. The net degradation was considered within the limits, indicating that the drug is stable in stressed conditions. The developed method for the estimation of Glecaprevir and Pibrentasvir can be utilized for the routine analysis of pharmaceutical dosage form.

**Keywords:** Glecaprevir, Pibrentasvir, stability-indicating, Method development, validation, Ultra-performance liquid chromatography.

## INTRODUCTION

Hepatitis C [1] is a liver disease caused by the hepatitis C virus. Hepatitis C is a major cause of liver cancer. Globally, an estimated 70 million people suffering from hepatitis C virus infection.

Glecaprevir [2] is a novel direct acting antiviral agent and Hepatitis C virus (HCV) NS3/4A protease inhibitor. It is chemically (1*R*,14*E*,18*R*,22*R*,26*S*,29*S*)-26-*tert*-butyl-*N*-[(1*R*,2*R*)-2-(difluoromethyl)-1-[(1-methylcyclopropyl)sulfonylcarbamoyl]cyclopropyl]-13,13-difluoro-24,27-dioxo-2,17,23-trioxa-4,11,25,28-tetrazapentacyclohentriaconta-3,5,7,9,11,14-hexaene-29-carboxamide. It targets the viral RNA replication. Its molecular formula is $C_{38}H_{46}F_4N_6O_9S$, and its molecular weight is 838.87g.mol$^{-1}$

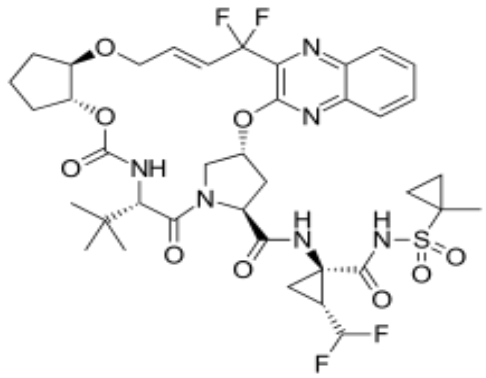

**Fig.1. Chemical structure of Glecaprevir**

Pibrentasvir [3] is an antiviral agent and Hepatitis C virus NS5A inhibitor. It is chemically Methyl((2S,3R)-1-((2S)-2-(5-((2R,5R)-1-(3,5-difluoro-4-(4-(4-fluorophenyl)piperidin-1-yl)phenyl)-5-(6-fluoro-2-((2S)-1-(N-(methoxycarbonyl)-O-methyl-L-threonyl)pyrrolidin-2-yl)-1H-benzimidazol-5-yl)pyrrolidin-2-yl)-6-fluoro-1H-benzimidazol-2-yl)pyrrolidin-1-yl)-3-methoxy-1-oxobutan-2-yl)carbamate. It targets the viral RNA replication. Its molecular formula is $C_{57}H_{65}F_5N_{10}O_8$, and its molecular weight is 1,113.20g.mol$^{-1}$.

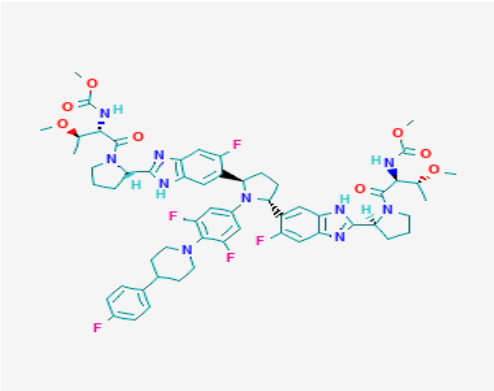

**Fig.2. Chemical structure of Pibrentasvir**

Ultra-performance liquid chromatography (UPLC) is specially designed to withstand higher system pressure during chromatographic analysis so that it enables a significant decrease in separation time and solvent consumption. UPLC columns packed with 2µm sized particles provide not only increased linear velocity without loss of efficiency, providing both speed and resolution. Using advantages of UPLC, a number of applications in different fields including industrial pharmacy, clinical

analysis, food analysis, and pesticide analysis have been recorded.

The Literature survey reveals that there are only a few methods developed for the estimation of Glecaprevir and Pibrentasvir using HPLC [4-8]

As there was no method developed using UPLC, the present study aimed to develop and validate a UPLC stability-indicating method for the estimation of Glecaprevir and Pibrentasvir in a pharmaceutical dosage form.

## METHODS

### Reagents and chemicals

Glecaprevir and Pibrentasvir working standards were procured from Spectrum pharma research solutions, Hyderabad as a gift sample.

The Mavyret tablets were procured from a local pharmacy. All the chemicals used were of AR grade purchased from Merck, Mumbai.

### Chromatographic conditions and instruments

The Acquity UPLC system equipped with a binary solvent manager, sample manager, Ultraviolet (UV) detector, and CHS C18 (100mm×1.8mm, 2.0µm)column was used for the determination of Glecaprevir and Pibrentasvir. The optimized conditions included Methanol: Water (45:55%v/v) as mobile phase run on an isocratic mode at a flow rate of 1ml/min. The column was held at $30^0$C and detection was done at 260nm. Further, a piece of equipment is used are ultrasonic bath sonicator and weighing balance (Denver).

### Preparation of the mobile phase

A Mixture of Acetonitrile and Water in the ratio of 50:50%v/v was used as mobile phase.

### Preparation of standard and sample solutions

About 25mg of Glecaprevir and 10mg of Pibrentasvir working standards was dissolved in 25ml of diluent. Then, 1 ml of the above stock solution was diluted to 10ml using diluent to get a concentration of 100µg/ml of Glecaprevir and 40µg/ml respectively.

5 Tablets (Mavyret) were weighed accurately and the average weight was calculated. An amount equivalent to 25mg of Glecaprevir and 10mg of Pibrenstasvir was dissolved in 25ml of diluent, filtered the solution, and diluted 1 ml of the above solution to 10ml with diluent.

### Method Validation

The developed method was validated in accordance with the International Conference on Harmonization (ICH) guidelines [9, 10].

### Specificity

The specificity of the method was determined by comparing the drug solution to the placebo solution and observed for the interference of placebo peak with drug peak.

### Accuracy

Accuracy of the method was determined in accordance with percentage of recovery. The drug solution along with sample was prepared in three concentration levels, i.e., 50%, 100%, and 150%. Then, the percentage recovery was calculated.

### Precision

The Precision of the method was established by injecting the standard solution 6 times into the UPLC system and percentage relative standard deviation (%RSD) was calculated.

### Linearity

The Linearity of the method was determined by preparing a series of dilutions ranging from 10µg/ml to 150µg/ml and injecting them into a UPLC system.

### Ruggedness

Ruggedness was determined by injecting the standard solution into UPLC 6 times for different days. The percentage of RSD was calculated.

### Robustness

Robustness of the method was determined by varying the optimized analytical conditions, such as mobile composition by ±5%, flow rate by ±0.1ml/min and column oven temperature by $\pm5^0$C.

### Solution stability

Solution stability determined on the basis of analyzing the standard drug solution after storage for 24h under laboratory conditions.

### Forced degradation studies

Forced degradation studies [11] were carried out for drug by exposing the drug solution to the various stress conditions such as acidic (2N hydrochloric acid for 30 min for $60^0$C) , basic(2N sodium hydroxide for 30 min for $60^0$C), peroxide(20% hydrogen peroxide {$H_2O_2$}for 30 min for $60^0$C) , neutral (refluxing the drug in water for 6h at $60^0$C), photolytic (exposing the drug solution to UV light by keeping the beaker in UV chamber for 7 days or 200 t h/$m^2$ in photostability chamber, and thermal (exposing the drug solution in hot air oven at $105^0$C for 6 h) conditions.

## RESULTS AND DISCUSSION

From the UV spectrum, detection wavelength for Glecaprevir and Pibrentasvir was found to be 260nm.

For the development of a method for the estimation of Glecaprevir and Pibrentasvir in pharmaceutical dosage form, initially many mobile phases and many columns were tried to elute the drug peak with less tailing factor, more plate count and better resolution. Acquity UPLC CHS C18 100mm×1.8mm, 2.0µm column, and %v/v mobile phase were selected based on peak parameters. The detection wavelength was found to be 260nm.

Prepared standard solution, sample solution, and the blank solution were injected into the UPLC system, and system suitability parameters were noted as summarized in Table 2 along with chromatograms as showed in Fig 3, 4, and 5 respectively.

The developed method was identified to obey Beer's law in the concentration range of 25µg/ml to 150µg/ml for Glecaprevir and 10µg/ml to 60µg/ml Pibrentasvir with a correlation coefficient of 0.999 each.

A linearity graph was plotted between concentration and peak area as showed in the Fig7 and Fig8 results as presented in Table2.

The method was found to be accurate as the percentage recovery was 99.00-101.00% for Glecaprevir and 98-102% for Pibrentasvir and was within the limits. The

percentage of RSD was determined to be 0.54%RSD and 1.13%RSD, which indicates that the method was precise. The method was shown to be specific, as there is no interference of retention time of placebo peak with that of drug peak. The placebo chromatogram was displayed in Fig 6.

Forced degradation studies results indicate that the drug was reported to be stable in various stress conditions as net degradation was to be within the limits. The chromatograms were illustrated in Fig 9-15 and results were outlined in Table3.

**Table 1: Optimized chromatographic conditions for determination of Glecaprevir and Pibrentasvir**

| S.No. | Parameter | Description |
|---|---|---|
| 1 | Stationary phase | CHS C18 (100×1.8, 2.0µ) |
| 2 | Mobile phase | Methanol: Water (45:55) |
| 3 | Flow rate | 1ml/min |
| 4 | Detection wavelength | 260nm |
| 5 | Detector | UV |
| 6 | Injection volume | 10µl |
| 7 | Column temperature | $30^0$C |
| 8 | Run time | 3min |
| 9 | Diluent | Water: Acetonitrile |

**Table 2: System suitability and validation parameter results**

| Parameter | Result | |
|---|---|---|
| | **Glecaprevir** | **Pibrentasvir** |
| USP Plate count | 1907.2 | 3946.1 |
| USP Tailing factor | 1.5 | 1.5 |
| USP Resolution | 3.6 | - |
| Precision (%RSD) | 0.9 | 0.9 |
| Accuracy | 99.10-100.50 | 98-1.02 |
| Specificity | Specific, No interference | |
| Linearity range (µg/ml) | 25-150 | 10-60 |
| Correlation coefficient, $r^2$ | 0.999 | 0.999 |
| LOD (µg/ml) | 1.52 | 0.82 |
| LOQ (µg/ml) | 4.61 | 2.48 |
| Robustness (%RSD) | | |
| Flow rate – | 0.6 | 0.6 |
| Flow rate + | 0.5 | 0.5 |
| Column temperature – | 0.7 | 0.9 |
| Column temperature + | 1.3 | 1.2 |
| Mobile phase – | 1.3 | 1.3 |
| Mobile phase + | 1.3 | 1.2 |
| Solution stability (%RSD) | | |
| (0 hrs) | 1.0 | 0.6 |
| (24 hrs) | 0.4 | 0.6 |

**Table 3: Forced degradation studies result**

| Stress condition | Glecaprevir | | Pibrentasvir | |
|---|---|---|---|---|
| | **%Assay** | **%D** | **%Assay** | **%D** |
| Acid | 94.14 | 5.86 | 94.85 | 5.15 |
| Base | 94.11 | 5.89 | 96.03 | 3.97 |
| Neutral | 99.61 | 0.39 | 99.53 | 0.47 |
| Peroxide | 95.90 | 4.10 | 96.77 | 3.23 |
| Photolytic | 98.47 | 1.53 | 97.92 | 2.08 |
| Thermal | 96.76 | 3.24 | 98.52 | 1.48 |

%D- Percentage Degradation

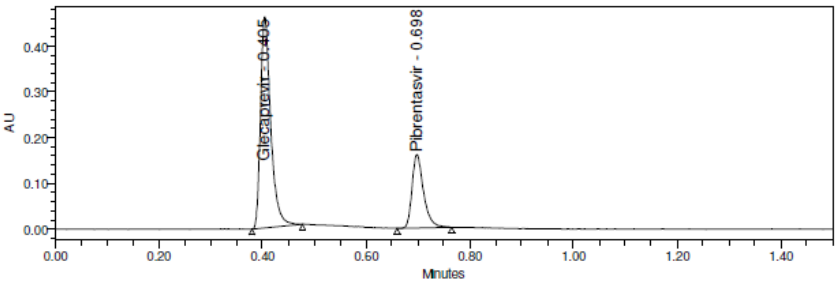


**Fig 3 Standard Chromatogram of Glecaprevir and Pibrentasvir**

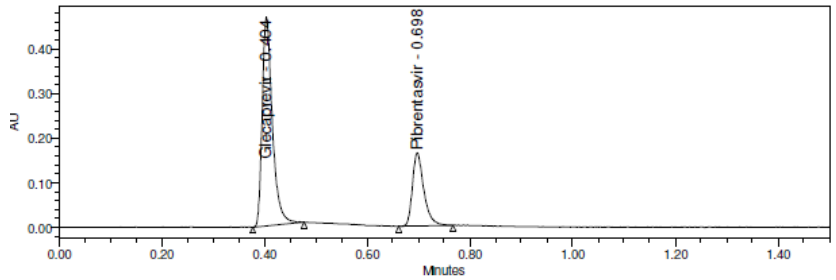


**Fig 4 Sample Chromatogram of Glecaprevir and Pibrentasvir**

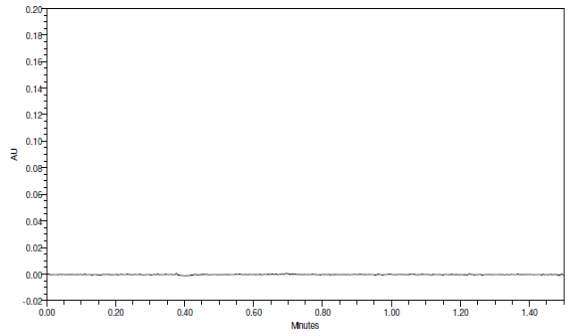


**Fig 5 Blank chromatogram'**

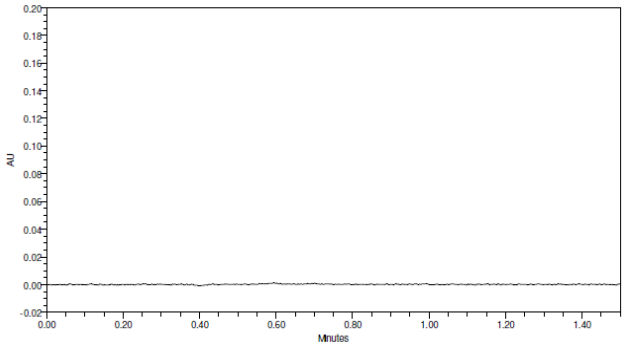


**Fig 6 Placebo chromatogram**

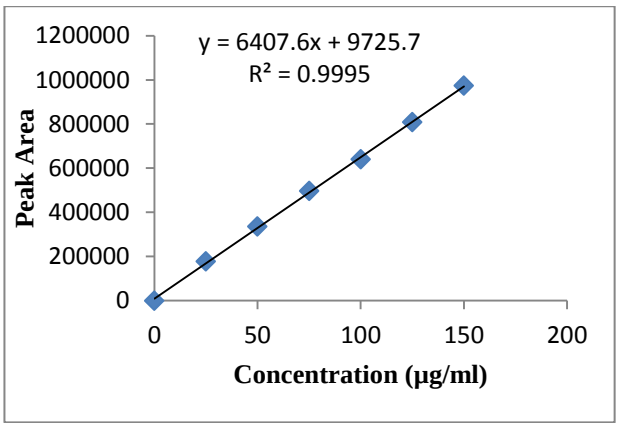


**Fig 7 Linearity of Glecaprevir**

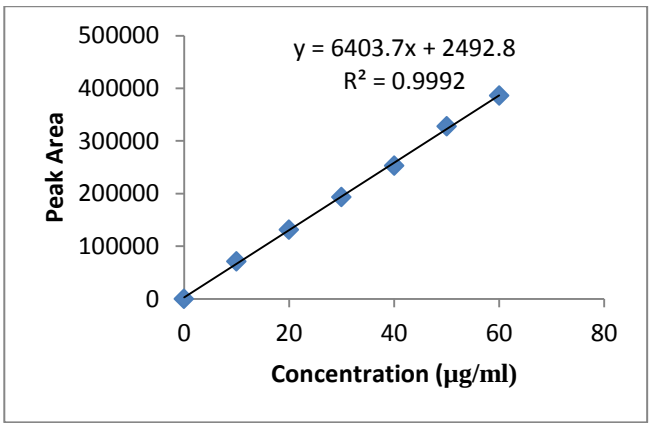


**Fig 8 Linearity of Pibrentasvir**

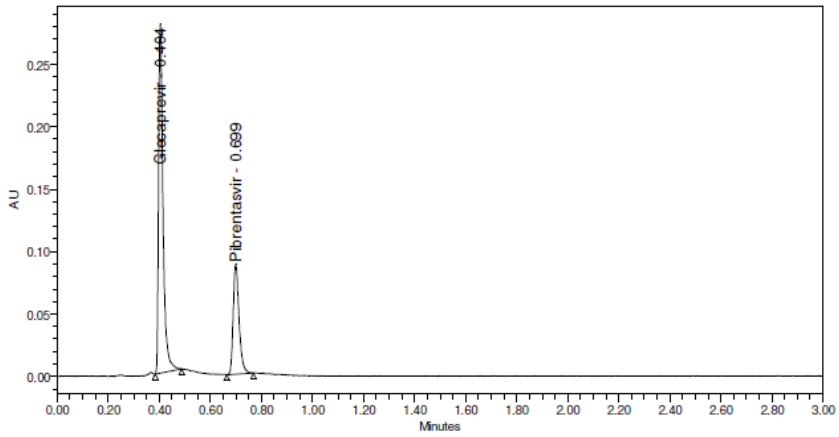


**Fig 9 Acid Degradation chromatogram**

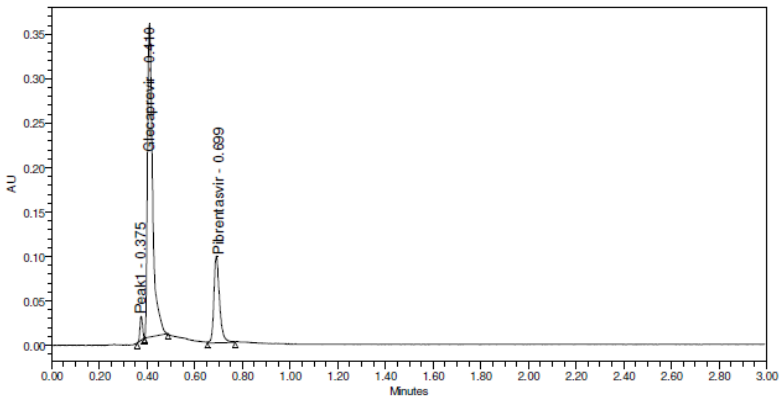


**Fig 10 Base Degradation chromatogram**

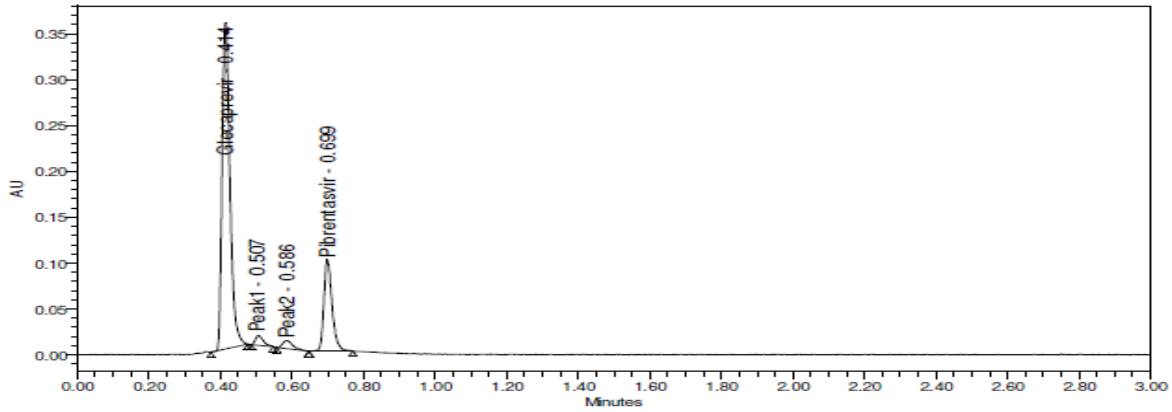


**Fig 11 Peroxide Degradation chromatogram**

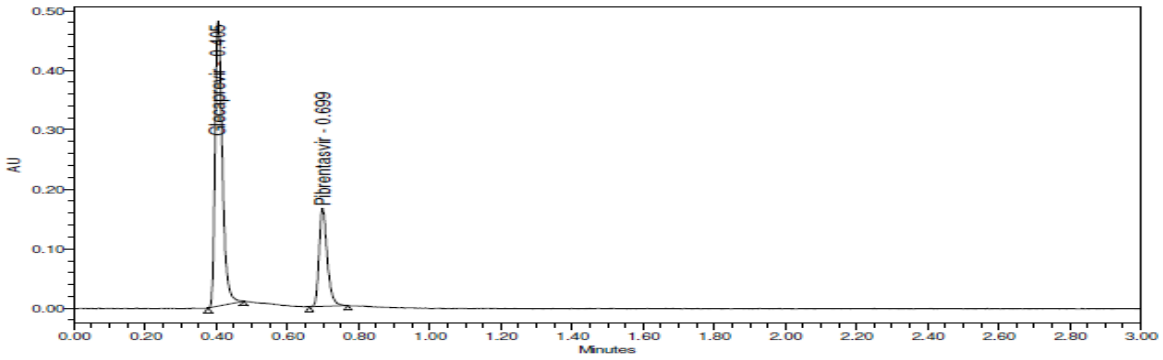


**Fig 12 Water stress study chromatogram**

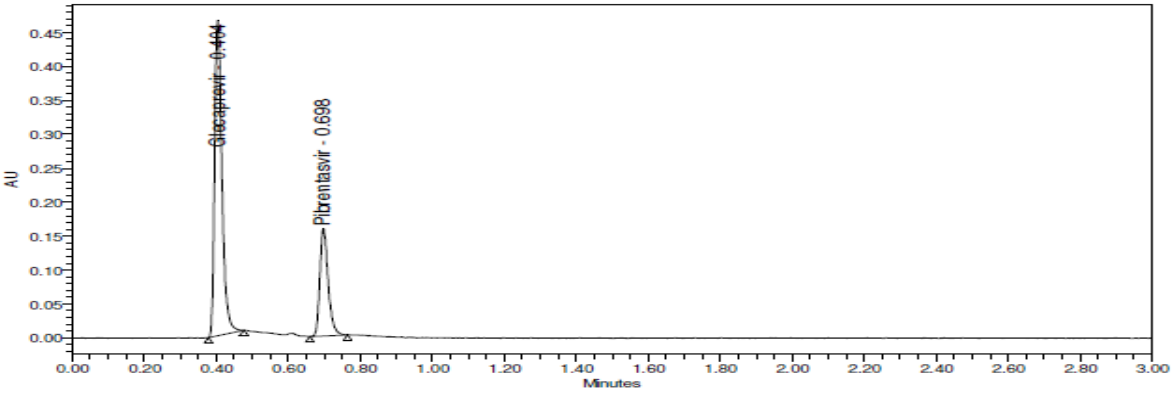


**Fig 13 Photo stability Degradation chromatogram**

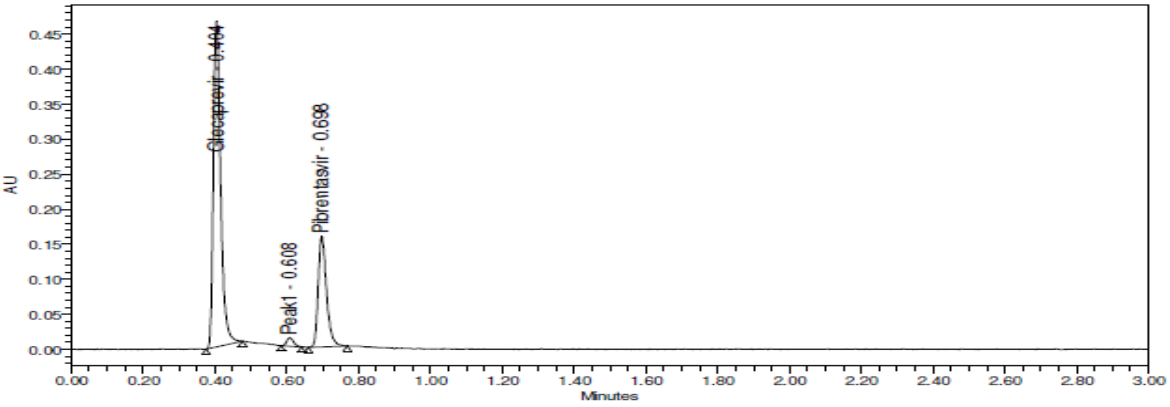


**Fig 14 Dry heat study chromatogram**

## .CONCLUSION

A specific, accurate, and precise stability-indicating method was developed for the estimation of Glecaprevir and Pibrentasvir in pharmaceutical dosage form using UPLC. The method was validated using numerous validation parameters, and the method was found to be linear, precise, accurate, specific and robust. From the degradation studies conducted, it infers that Glecaprevir and Pibrentasvir were stable at high concentrations of acid, base, peroxide, thermal, UV and water stress conditions. The run time was 2min, which enables rapid quantification of many samples in routine and quality control analysis of tablet formulation.

**Acknowledgement**

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**Author's Contributions**

All the authors contributed equally to this manuscript.

**Conflicts of Interest**

The authors claim that they have no conflict of interest. It has not meant to publish elsewhere. Moreover, it has not meant simultaneously presented for publication elsewhere. All authors have decided to the submission to the journal.

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