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# RP-HPLC stability indicating assay and simultaneous estimation method for the combination of Glecapravir and Pibrentasvir in tablet dosage form

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## ABSTRACT

Glecaprevir & Pibrentasvir are directly acting antiviral agents and Hepatitis C virus (HCV) protease inhibitors which targets on viral RNA replication. The main objective of the present work is to determine a simple, precise, accurate RP-HPLC method. In RP-HPLC, the method was developed using Waters Alliance 2695 separation module with PDA detector, Altima 5µ C18 column 4.6×150mm & Empower Software. The mobile phase used was Methanol: TEA Buffer pH 4.5: Acetonitrile (50:35:15) at a flow rate of 1ml/min. The method was validated for System Suitability, Specificity, Linearity, Accuracy, Precision, Robustness & Forced Degradation Studies (Acidic, Basic, Oxidative). The %RSD was found to be <2.0% and all the other validation parameters were found to be within the limits. This method can be used for the routine determination of Glecaprevir and Pibrentasvir in bulk drug and in Pharmaceutical dosage forms.

**Keywords:** RP-HPLC, Glecaprevir, Pibrentasvir, Method Development, Validation.

## INTRODUCTION

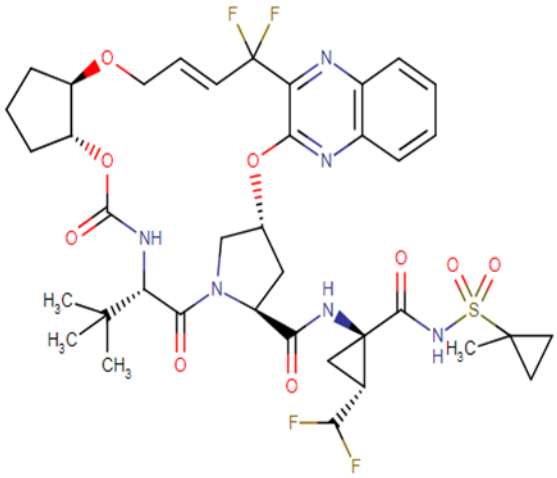
A drug incorporates all medications expecting for internal or external utilize for or within the conclusion, treatment, moderation or anticipation of infection or clutter in human creatures or other creatures, and fabricated only in agreement with the formulae specified in definitive books.[1]

## DRUG PROFILE

### Glecaprevir

Glecaprevir is a directly acting antiviral agent and HCV NS3/4A protease inhibitor it targets the viral RNA replication. In combination with Pibrentasvir, it is used to treat patients who experienced therapeutic failure from other NS3/4A protease inhibitors. It forms a high genetic barrier against resistance mutations of the virus.

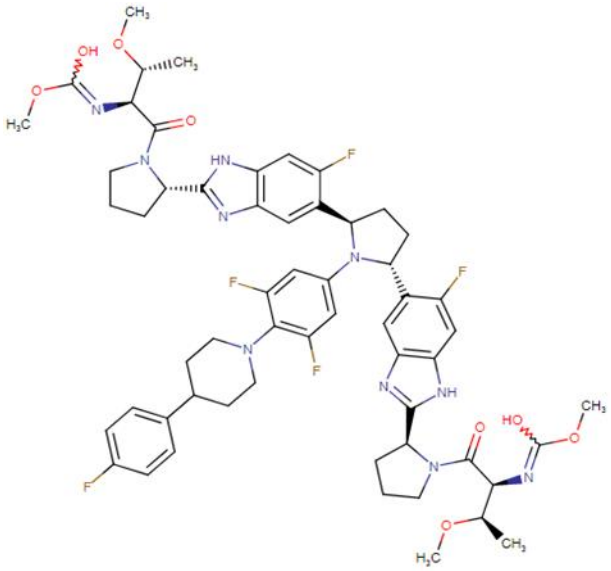
## Chemical Structure



## Pibrentasvir

Pibrentasvir is a directly acting antiviral agent and HCV NS5A inhibitor, it targets the viral RNA replication and viron assembly. In combination with Glecaprevir, it is used to treat patients who experienced therapeutic failure from other NS5A inhibitors Pibrentasvir along with Glecaprevir is available as an oral combination therapy under the brand name Mavyret. This FDA-approved combination is used to treat adults with chronic hepatitis C virus (HCV) genotypes 1-6 without cirrhosis (liver disease) or with mild cirrhosis, including patients with moderate to severe kidney disease and those who are on dialysis [3].

## Chemical Structure



### Aim

Development of new simultaneous RP-HPLC method for the estimation of Glecapravir and Pibrentasvir in tablet dosage form and then validation of the method.

### Plan of work

Glecapravir and Pibrentasvir are existing drugs. Literature reveals different methods for their analysis in their formulations. But our present plan is to develop a new, simple, precise& accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated.

Following are the objectives of present work**:**

- To develop analytical method
- To validate different parameters.
- Performing Stability Indicating Assay

## MATERIAL AND METHOD

The drug samples Glecapravir and Pibrentasvir were obtained from Sura Labs., Hyderabad. The solvents used were of HPLC grade methanol and water from Merck Co, Mumbai.

### Instrumentation

The HPLC system used was WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector, Altima C18 (4.6×150mm, 5µ) column.

## EXPERIMENTAL WORK

### HPLC method development

### Trails

### Preparation of standard solution

Accurately weigh and transfer 10 mg of Glecaprevir and Pibrentasvir standards into 10ml volumetric flasks, then add about 7ml of Methanol. Sonicate it and makeup the volume with Methanol.

Further pipette out 0.45ml of the above Glecaprevir and 1.12ml of the Pibrentasvir stock solutions into a 10ml volumetric flask and dilute it with Methanol.

### Procedure

Inject the samples and record the chromatograms, note the conditions of peak elution for performing validation parameters as per ICH guidelines.

### Mobile Phase Optimization

Initially the mobile phase used was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 50:35:15 v/v respectively.

### Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

## VALIDATION

### Preparation Of Buffer And Mobile Phase

### Preparation of Triethylamine (TEA) buffer (pH-4.5)

Dissolve 1.5ml of Triethyl amine in 250 ml water and adjust the $p^H$ to 4.5. Filter and sonicate the solution by vaccum filtration and ultrasonication.

### Preparation of mobile phase:

Accurately measured 400 ml (40%) of Methanol, 200 ml of Triethylamine buffer (20%) and 400 ml of Acetonitrile (40%) were mixed and degassed for 10 minutes and then filtered.

### Diluent Preparation:

The Mobile phase was used as the diluent.

### Optimised Chromatographic Conditions-(Standard)

Mobile phase : Methanol: TEA Buffer pH 4.5: Acetonitrile (50:35:15)
Column : Altima C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 225 nm

Column temp : 40ºC
Injection Vol : 10 µl
Run time : 7 minutes

## VALIDATION PARAMETERS

### System Suitability

Accurately weigh and transfer 10 mg of standards into 10ml volumetric flasks add about 7mL of Diluents, sonicate it and make up the volume with the same solvent. Further pipette out 0.45ml of the above stock solutions into a 10ml volumetric flask and dilute it up to the mark with Diluent.

### Linearity

From the standard stock solution aliquots are prepared and injected into chromatographic system and the peak area is determined. A graph is plotted and the correlation coefficient is calculated.

### Precision

**Repeatability** communicates the exactness beneath the same working conditions over a brief interim of time. Repeatability is additionally named intra –assay exactness.

## RESULTS & DISCUSSIONS

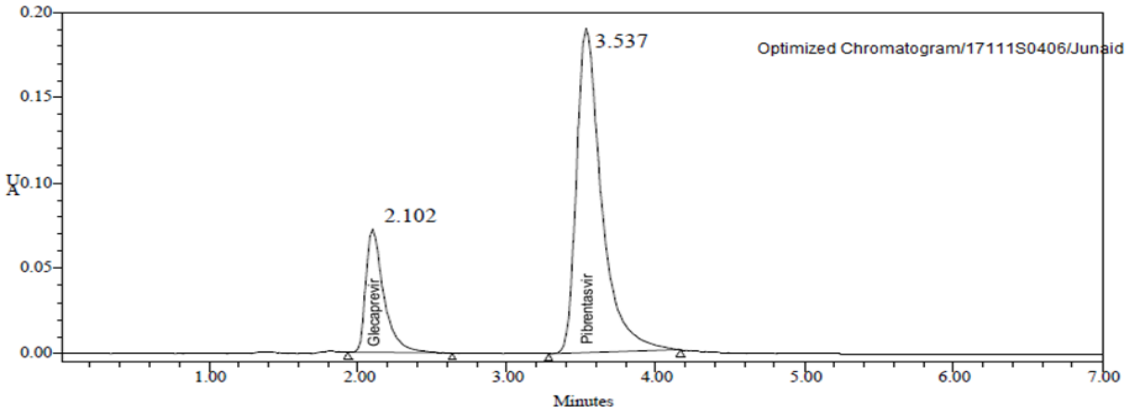
- **Intermediate Precision** communicates varieties inside research facilities, such as distinctive days, distinctive investigators, distinctive hardware, and so forward.
- **Reproducibility** communicates the exactness between research facilities

### Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

## RESULTS & DISCUSSIONS

### Optimized Chromatogram




### Peak results for Optimized Chromatogram

| Peak Name | $R_t$ | Area | USP Tailing | USP plate count |
|---|---|---|---|---|
| Glecaprevir | 2.102 | 4496421 | 0.96 | 5586.0 |
| Pibrentasvir | 3.537 | 5206219 | 1.22 | 5371.0 |

### Results of system suitability for Glecaprevir

| S.No | Name | Rt | Area | USP plate count | USP Tailing |
|---|---|---|---|---|---|
| 1 | Glecaprevir | 2.117 | 4496832 | 5641 | 1.9 |
| 2 | Glecaprevir | 2.118 | 4498657 | 5423 | 1.6 |
| 3 | Glecaprevir | 2.116 | 4500136 | 5143 | 1.6 |
| 4 | Glecaprevir | 2.109 | 4527238 | 5212 | 1.7 |
| 5 | Glecaprevir | 2.102 | 4543296 | 5419 | 1.6 |
| Mean | | | 4513231.8 | | |
| Std. Dev | | | 20933.87 | | |
| % RSD | | | 0.46% | | |

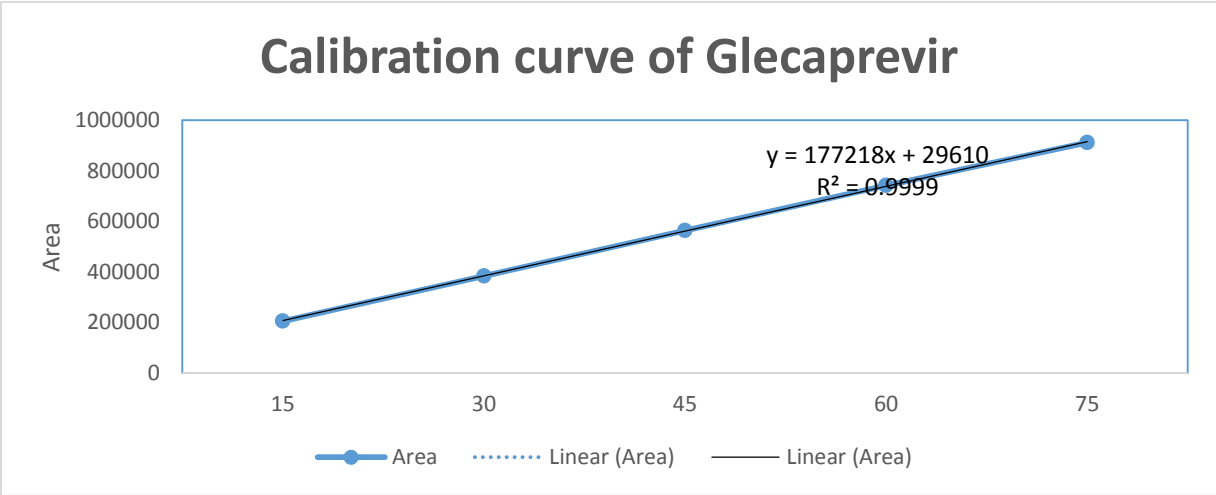
### Results of system suitability for Pibrentasvir

| S.No | Name | Rt | Area | USP plate count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|
| 1 | Pibrentasvir | 3.547 | 5204132 | 5031 | 1.2 | 2.07 |
| 2 | Pibrentasvir | 3.539 | 5222196 | 5435 | 1.4 | 2.05 |
| 3 | Pibrentasvir | 3.547 | 5242453 | 5983 | 1.5 | 2.0 |
| 4 | Pibrentasvir | 3.565 | 5263351 | 5849 | 1.6 | 2.01 |
| 5 | Pibrentasvir | 3.537 | 5282365 | 5378 | 1.6 | 2.01 |
| Mean | | | 5242899.4 | | | |
| Std. Dev | | | 31255.17 | | | |
| % RSD | | | 0.60% | | | |

## CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

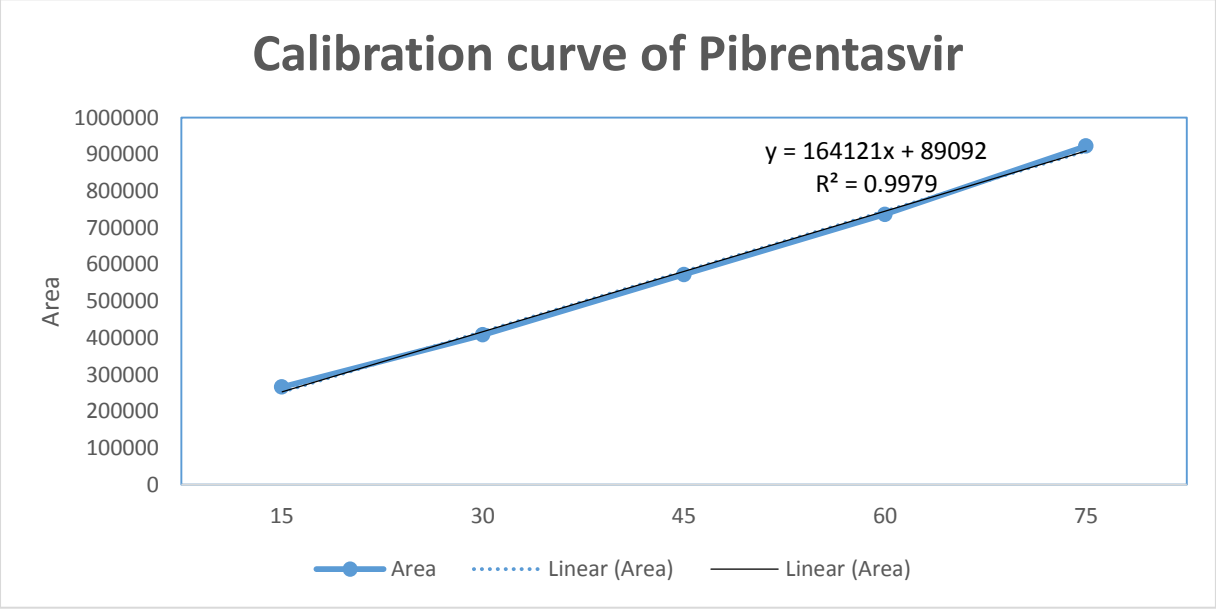
### Glecaprevir

| Concentration Level (%) | Concentration µg/ml | Peak Area |
|---|---|---|
| 1 | 15 | 205215 |
| 2 | 30 | 383527 |
| 3 | 45 | 563342 |
| 4 | 60 | 742347 |
| 5 | 75 | 911897 |



## Pibrentasvir

| Concentration Level (%) | Concentration µg/ml | Average Peak Area |
|---|---|---|
| 1 | 15 | 266324 |
| 2 | 30 | 408535 |
| 3 | 45 | 572957 |
| 4 | 60 | 736528 |
| 5 | 75 | 922934 |




## Results Of Repeatability For Glecaprevir

| S. No | Name | Rt | Area | USP plate count | USP Tailing |
|---|---|---|---|---|---|
| 1 | Glecaprevir | 2.108 | 4465236 | 2586 | 1.6 |
| 2 | Glecaprevir | 2.105 | 4465483 | 2947 | 1.4 |
| 3 | Glecaprevir | 2.113 | 4465691 | 2468 | 1.6 |
| 4 | Glecaprevir | 2.109 | 4465838 | 2146 | 1.9 |
| 5 | Glecaprevir | 2.109 | 4466002 | 2307 | 1.7 |
| Mean | | | 4465650.0 | | |
| Std. Dev | | | 300.08 | | |
| % RSD | | | 0.01% | | |

## Results Of Repeatability For Pibrentasvir

| S. No | Name | Rt | Area | USP plate count | USP Tailing |
|---|---|---|---|---|---|
| 1 | Pibrentasvir | 3.552 | 5206036 | 1.6 | 2371 |
| 2 | Pibrentasvir | 3.550 | 5206285 | 1.6 | 2841 |
| 3 | Pibrentasvir | 3.564 | 5206423 | 1.5 | 2816 |
| 4 | Pibrentasvir | 3.564 | 5206634 | 1.5 | 2872 |
| 5 | Pibrentasvir | 3.565 | 5206826 | 1.6 | 2845 |
| Mean | | | 5206440.8 | 1.6 | 2841 |
| Std. Dev | | | 305.84 | | |
| % RSD | | | 0.01% | | |

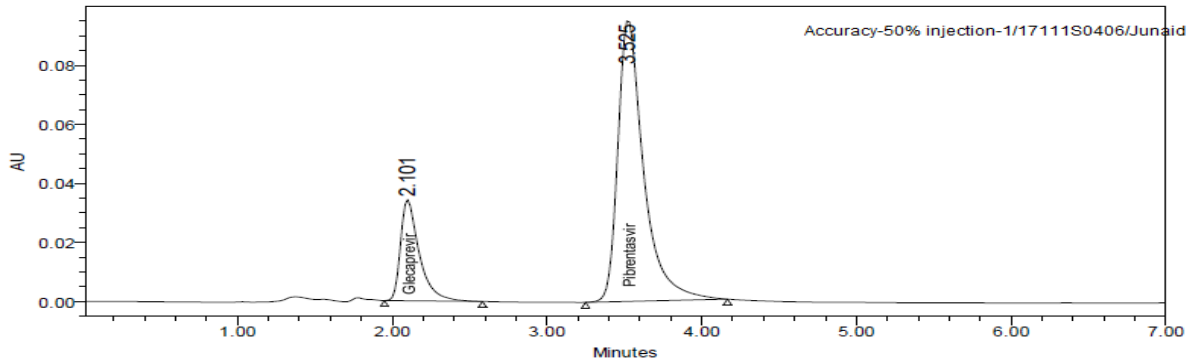
## The accuracy results for Glecaprevir

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 4496446 | 22.5 | 22.5 | 100 | 99.6% |
| 100% | 4632482 | 45 | 44.8 | 98.6 | |
| 150% | 4835724 | 67.5 | 67.42 | 99.5 | |

## The accuracy results for Pibrentasvir

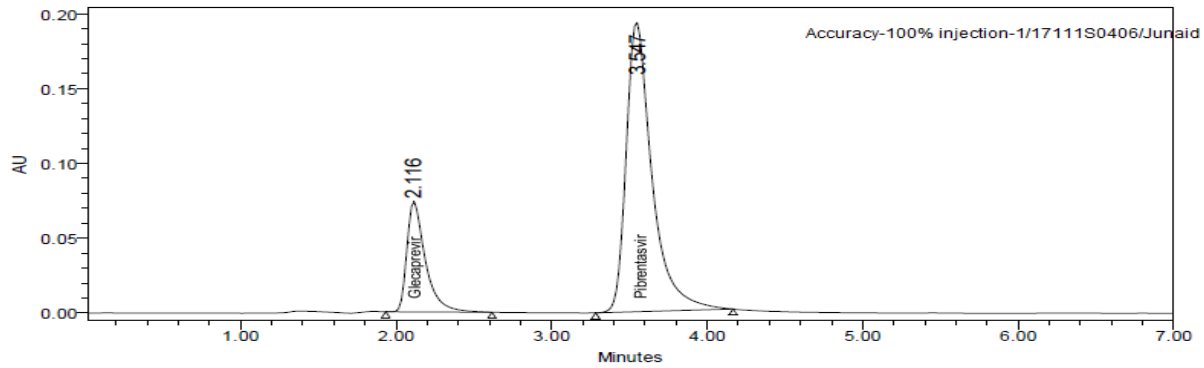
| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 5206229 | 56.25 | 56.249 | 100% | 100% |
| 100% | 5406587 | 112.5 | 112.48 | 99.9% | |
| 150% | 5606354 | 168.75 | 168.75 | 100% | |

## Accuracy 50%



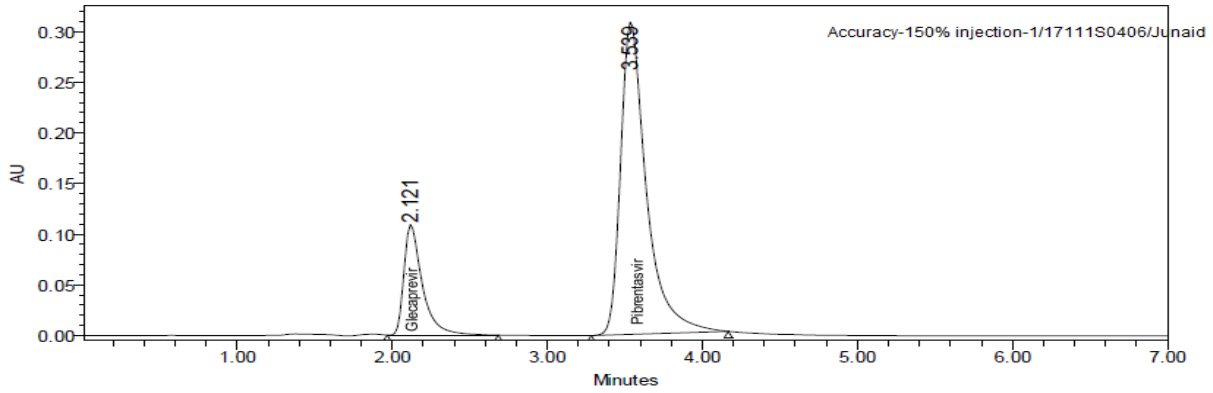

Chromatogram showing Accuracy-50% injection-1

## Accuracy 100%



## Chromatogram showing Accuracy-100% injection-1

## Accuracy 150%




## Chromatogram showing Accuracy-150% injection-1

## Results for Robustness

## Glecaprevir

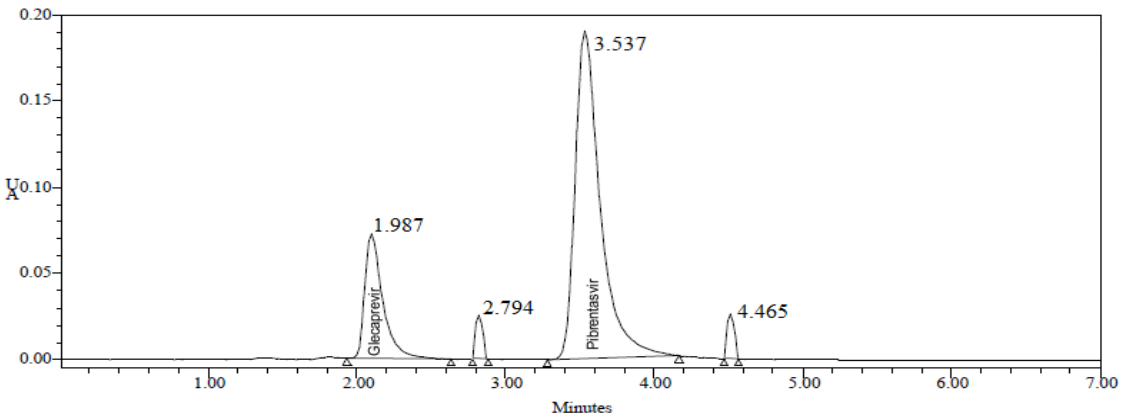
| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 4834327 | 2.102 | 5586 | 1.7 |
| Less Flow rate of 0.9 mL/min | 4834212 | 2.330 | 5231 | 1.7 |
| More Flow rate of 1.1 mL/min | 4835347 | 1.950 | 5234 | 1.7 |
| Less organic phase | 4835938 | 2.290 | 5643 | 1.4 |
| More organic phase | 4836352 | 1.998 | 5298 | 1.5 |

## Pibrentasvir

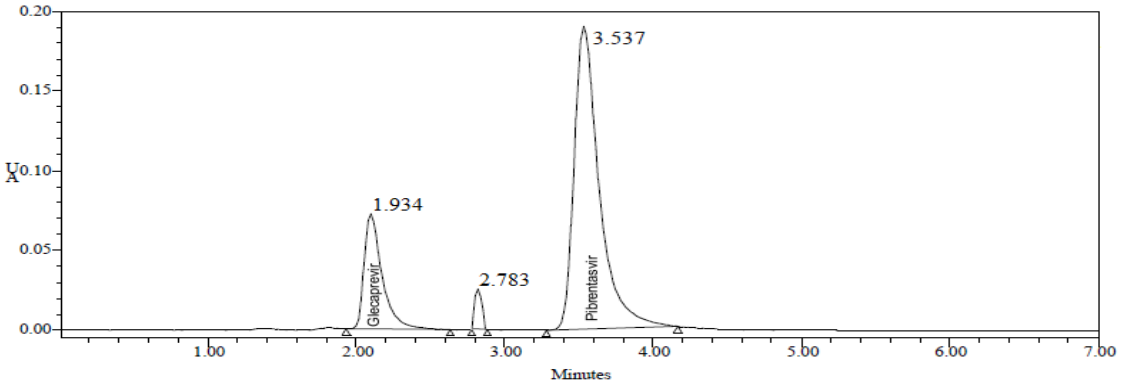
| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 5606275 | 3.537 | 5371 | 1.6 |
| Less Flow rate of 0.9 mL/min | 5605926 | 3.885 | 5324 | 1.7 |
| More Flow rate of 1.1 mL/min | 5606562 | 3.263 | 5098 | 1.7 |
| Less organic phase | 5606126 | 4.435 | 5239 | 1.2 |
| More organic phase | 5606643 | 3.009 | 5647 | 1.0 |

# EVALUATION OF METHOD

## Forced Degradation Studies



## Acidic Degradation




## Analysis

| Conditions | Sample Amount (µg/ml ) | Peak Area | % claim | % Degradation |
|---|---|---|---|---|
| **Sample Control** | 05.03 | 5606126 | 99.39% | - |
| **Acidic Degradation** | 04.79 | 5512345 | 97.73% | 1.66% |
| **Basic Degradation** | 04.92 | 5534338 | 98.12% | 1.27% |
| **Oxidative Degradation** | 05.38 | 5452326 | 96.66% | 2.73% |
| **Photolytic** | 05.41 | 5329451 | 94.48% | 4.91% |

## CONCLUSION

The estimation of Glecaprevir and Pibrentasvir was done by RP-HPLC. In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Glecaprevir and Pibrentasvir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Glecaprevir and Pibrentasvir was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA Buffer pH 4.5: Acetonitrile (50:35:15) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Glecaprevir and Pibrentasvir in bulk drug and in Pharmaceutical dosage forms.

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