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*Original Article*

# A New Force Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Pibrentasvir and Glecaprevir in Bulk and its Tablet Dosage Form

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## ABSTRACT

**Background**: A simple reversed phase high performance liquid chromatographic method was developed for the simultaneous estimation of the Pibrentasvir and Glecaprevir in bulk and its solid dosage form. **Materials and Methods**: The method was developed on water's $C_{18}$ column capacitate 250X4.6 mm, 5 µm particle size, wavelength was fixed at 225 nm with photo diode array detection. The mobile phase was consisted mixture of 0.5 mM Ortho phosphoric acid buffer: Acetonitrile in the ratio of 75:25 v/v, pH 4.3 was adjusted with hydrochloric acid and flow of mobile phase through the column was maintained 1mL/min. **Results:** The retention times of Pibrentasvir and Glecaprevir were found to be 3.04 and 4.45 min respectively. The method was statistically validated with concern of precision, linearity, range and robustness of method was found for Pibrentasvir and Glecaprevir. The above method was afforded excellent percentage recovery was found to be 99.87-100.22% and 99.68-100.17% for Pibrentasvir and Glecaprevir respectively. The Limit of detection and Limit of quantification were found 0.12 and 0.43µg/mL and 0.008 and 0.027µg/mL for Pibrentasvir and Glecaprevir. The forced degradation studies were performed. **Conclusion**: The method was developed and validated for the estimation of Pibrentasvir and Glecaprevir. The method was sensitive, precise, accurate, and robust, showed good linearity with different concentrations. The method is used for the routine analysis of Pibrentasvir and Glecaprevir in its bulk and pharmaceutical dosage form.

**Key words:** Pibrentasvir, Glecaprevir, RP-HPLC, Ortho phosphoric acid buffer.

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## INTRODUCTION

The combination of Glecaprevir and Pibrentasvir was highly efficacious and well tolerated in patients with HCV genotype 1 infection and prior failure of directly acting antiviral containing therapy; RBVco administration did not improve efficacy.[1] Although direct acting antiviral therapies for chronic HCV infection have demonstrated high rates of sustained virologic response, virologic failure may still occur, potentially leading to the emergence of viral resistance, which can decrease the effectiveness of subsequent treatment.[2,3] Glecaprevir is a next-generation HCV NS3/4A with potent pangenotypic activity. In enzymatic assays, Glecaprevir exhibited a high level of selectivity for HCV NS3/4A protease over human proteases. Glecaprevir is an HCV NS3/4A protease inhibitor, and Pibrentasvir is an NS5A inhibitor.They have been co formulated Glecaprevir and Pibrentasvir in clinical trials for the treatment of all six major HCV genotypes.[4-6] The treatment of HCV with glecaprevir/pibrentasvir leading to first global approval in the European Union and subsequent approval in the United States of America for chronic Hepatitis C Virus infection.[7] The chemical structures of Glecaprevir and Pibrentasvir were showed in Figures 1 and 2.

## MATERIALS AND METHODS

All the chemicals and reagents were of analytical grade. Water was double distilled and filtered with a membrane filter. Acetonitrile – Hihg Performance Liquid Chromatography grade (Merck, India), hydrochloric acid and ortho phosphoric acid (SD fine chem, India) were used to prepare mobile phase. Pharmaceutical grade standard drugs viz., Pibrentasvir and Glecaprevir were kindly gifted by Ajanta Pharma Limited, Mumbai, India. The combined tablet formulation contains 40 mg of Pibrentasvir and Glecaprevir 100 mg of (Mavyret) purchased from local market of Kurnool.

### Preparation of standard solution

10 mg of Pibrentasvir and Glecaprevir were weighed and transferred to individual 10ml volumetric flasks with small quantity of mobile phase. The solutions were sonicated for 10 min and volume was made with mobile phase to give concentration 1000 µg/mL. This solution was further diluted with the mobile phase to get final concentrations of 80 µg/mL and 200 µg/mL working standard solutions for Pibrentasvir and Glecaprevir respectively.

### Preparation of sample solution

Twenty tablets were weighed and finely powdered. The average weight of tablets was determined. The powder equivalent to 10 mg of Pibrentasvir was weighed and transferred to a 10 mL volumetric flask. 10 mL of diluent was added to dissolve contents of tablets completely by using ultra sonicator for 10 min. The aliquot portion of the filtrate was further diluted to get final concentrations of 80 µg/mL of Pibrentasvir and 200 µg/mL of Glecaprevir. This solution was filtered through membrane filter. The 20 µL of this solution was injected in to High Performance Liquid Chromatographic system.

### Chromatographic Conditions

The mobile phase was consisted of 0.5 mM Ortho Phosphoric acid buffer: Acetonitrile in the ratio of 75:25 v/v and pH 4.3 was adjusted with hydrochloric acid. The mobile was filtered through 0.45µ membrane.

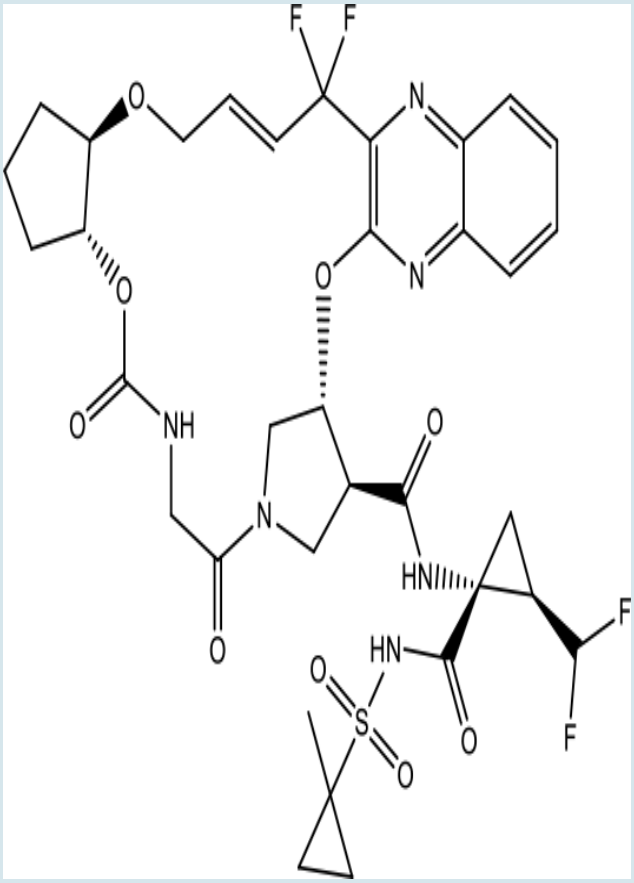

**Figure 1:** Chemical structure of Glecaprevir.

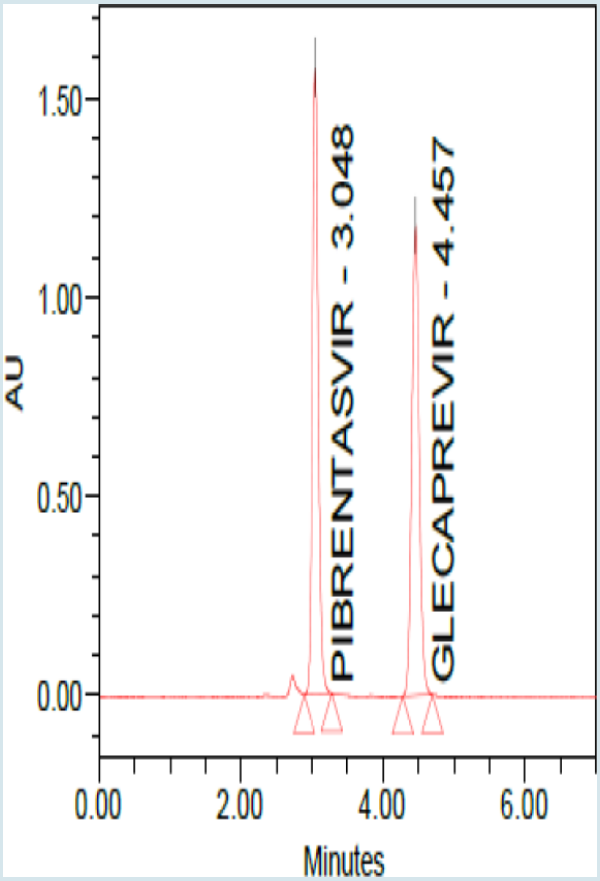


**Figure 3:** Standard chromatogram of Pibrentasvir and Glecaprevir.

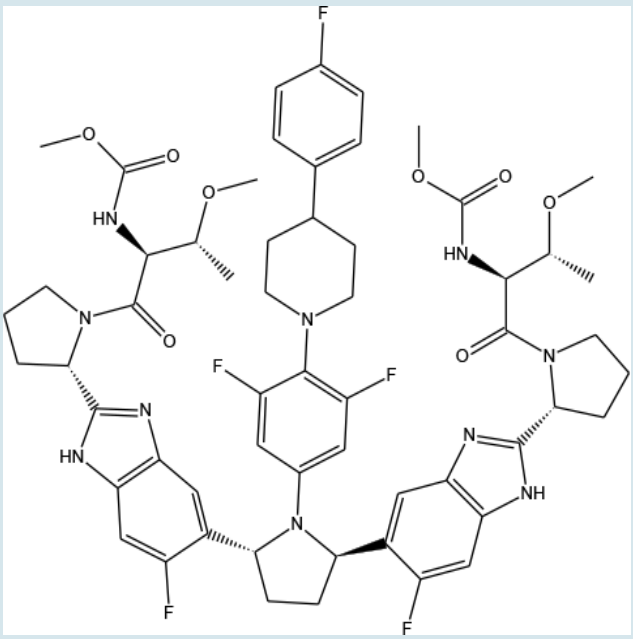

**Figure 2:** Chemical structure of Pibrentasvir.

The mobile phase was pumped at a flow rate of 1mL/min. The effluents were monitored at 225 nm with Photo diode Array detection. The 20 µL solution was injected in to High performance liquid chromatographic system.

## Method validation

The method validation was performed according to International Council for Harmonization guidelines. The following method validation parameters resembling specificity, precision, accuracy, linearity, robustness, limit of detection and limit of quantification.[8-10]

## Specificity

The specificity was studied by injecting the mobile phase (blank), standard and sample solution prepared as per the developed method and injected into the HPLC system and study the any interference with retention times of Pibrentasvir and Glecaprevir.

## System suitability parameters

To make certain critical parameters were m *et al* l system suitable requirements conducted on all the days. The chromatogram was eluted and showed symmetrical peaks. The standard, sample and blank chromatograms showed in Figures 3, 4, 5 and results were tabulated as in [Table 1].

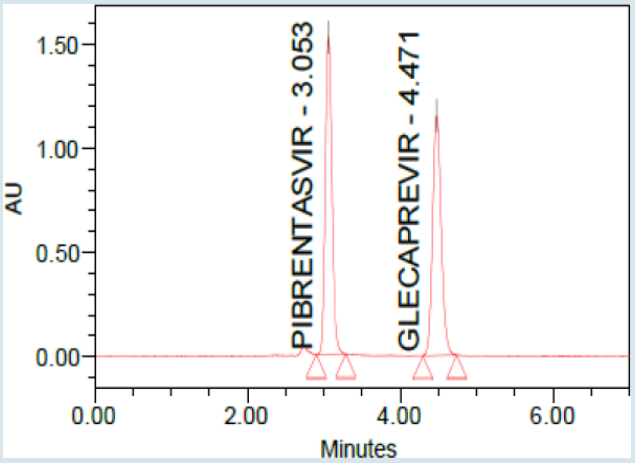


**Figure 4:** Sample chromatogram of Pibrentasvir & Glecaprevir.

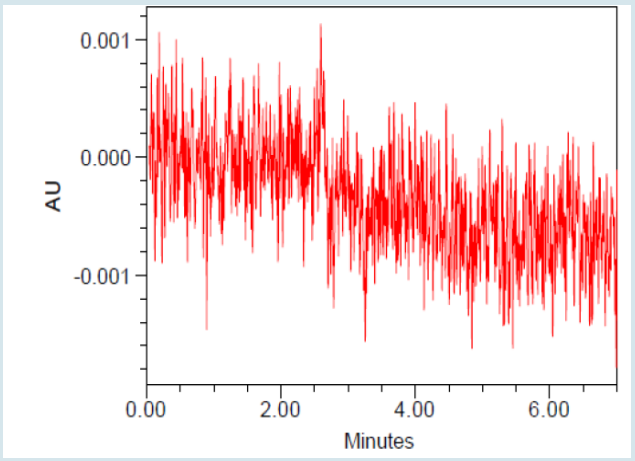


**Figure 5:** Blank chromatogram of Pibrentasvir and Glecaprevir.

## Precision

The precision was assessed through assay with respect to intermediate precision and intraday precision. The repeatability of the system was studied by injecting analyte with 6 replicate injections. The %RSD values varied from 0.47-0.03%. The results were showed good intra-day precision. The results were tabulated in [ Table 2 ]

## Linearity

The linearity of the method was obtained through calibration curve (peak area vs concentration). The pure solution was checked in the concentration range of 20-120 µg/ml for Pibrentasvir and 50-300 µg/ml for Glecaprevir. The calibration curve was showed linear over concentration range and $R^2$ values were found to be 0.999 for Pibrentasvir and 1 for Glecaprevir, that results indicated good linearity between peak area and concentration. The data of graphs were showed in Figures 6 and 7. Acceptance criteria: Correlation coefficient should be not less than 0.999.

## Accuracy

The accuracy of the method was studied by spiking standard solution with analyzed sample solution at three concentrations levels 50%, 100%,

**Table 1: System suitability results of Pibrentasvir & Glecaprevir.**

| S. No | Parameter | PNR | GPR |
|---|---|---|---|
| 1 | Theoretical Plate Count | 3985 | 6425 |
| 2 | Peak Area | 9057740 | 8707518 |
| 3 | Peak Height | 2151554 | 240210 |
| 4 | RT | 3.048 | 4.457 |
| 5 | Tailing | 1.147 | 1.096 |
| 6 | Resolution | - | 7.97 |
| 7 | S/N | 2415 | 128 |

**Table 2: Precision results of Pibrentasvir & Glecaprevir.**

| S.NO | PEAK AREA | | %ASSAY | |
|---|---|---|---|---|
| | PNR | GPR | PNR | GPR |
| 1 | 9110742 | 8707008 | 100.40 | 99.90 |
| 2 | 9061995 | 8706154 | 99.95 | 99.89 |
| 3 | 9054193 | 8705139 | 99.86 | 99.88 |
| 4 | 9038029 | 8701982 | 99.19 | 99.84 |
| 5 | 8994188 | 8700572 | 99.20 | 99.82 |
| 6 | 9058562 | 8703774 | 99.90 | 99.86 |
| AVRAGE | 9052951.50 | 8704104.83 | 99.75 | 99.87 |
| STDEV | 37763.41 | 2481.68 | 0.47 | 0.03 |
| % RSD | 0.42 | 0.03 | 0.47 | 0.03 |

**Table 3: Accuracy results of PNR & GPR.**

| Parameters | Peak Area | Amount added(µg) | Amount recovered (µg) | % of recovery | % mean recovery |
|---|---|---|---|---|---|
| PIBRENTASVIR | | | | | |
| 50% | 4524143 | 39.90 | 39.94 | 100.12 | 100.12 |
| 100% | 9055022 | 79.80 | 79.98 | 100.22 | 100.22 |
| 150% | 13534934 | 119.70 | 119.54 | 99.87 | 99.87 |
| GLECAPREVIR | | | | | |
| 50% | 4500718 | 39.90 | 39.95 | 100.14 | 100.14 |
| 100% | 9018173 | 79.80 | 79.54 | 99.68 | 99.68 |
| 150% | 13593541 | 119.70 | 119.90 | 100.17 | 100.17 |

**Table 4: Results of Limit of Detection & Limit of Quantification results.**

| S.NO | Parameter | PNR | GPR |
|---|---|---|---|
| 1 | Slope | 10886 | 10886 |
| 2 | STDEV | 0.47 | 0.03 |
| 3 | LOD | 0.12 µg/ml | 0.008 µg/ml |
| 4 | LOQ | 0.43 µg/ml | 0.027 µg/ml |

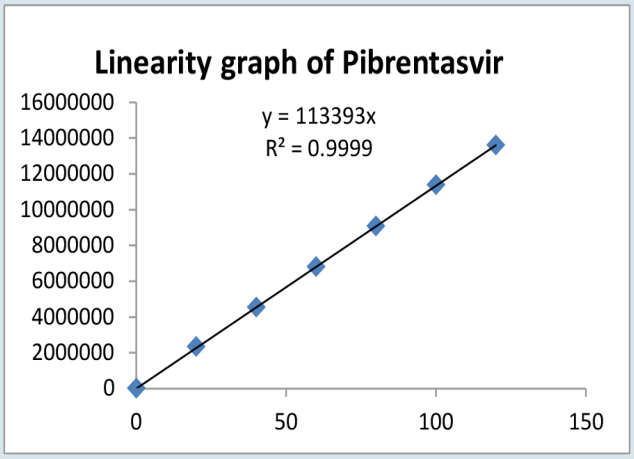


**Figure 6:** Linearity graph for Pibrentasvir.

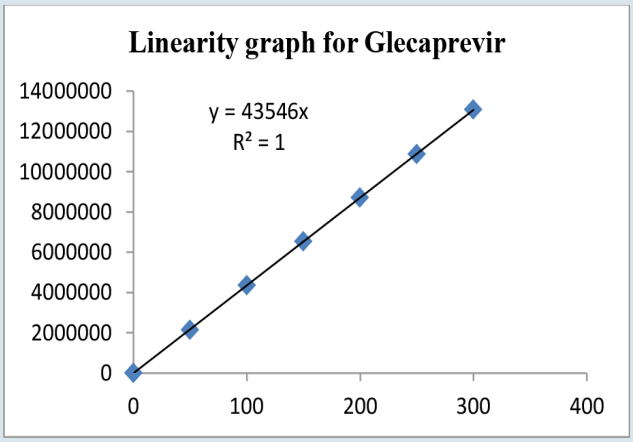


**Figure 7:** Linearity graph for Glecaprevir.

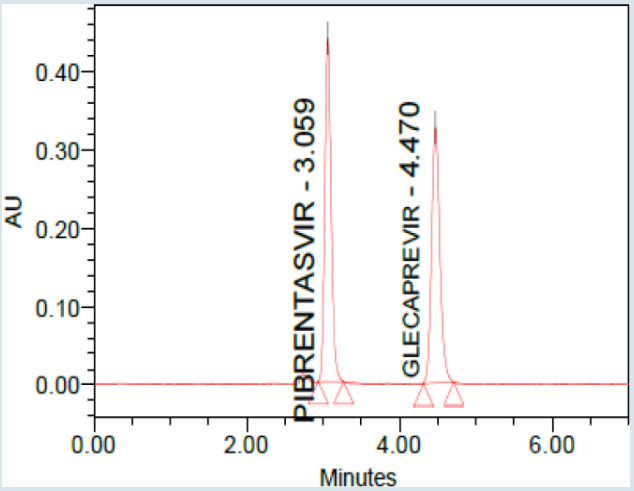


**Figure 8:** Chromatogram for LOD.

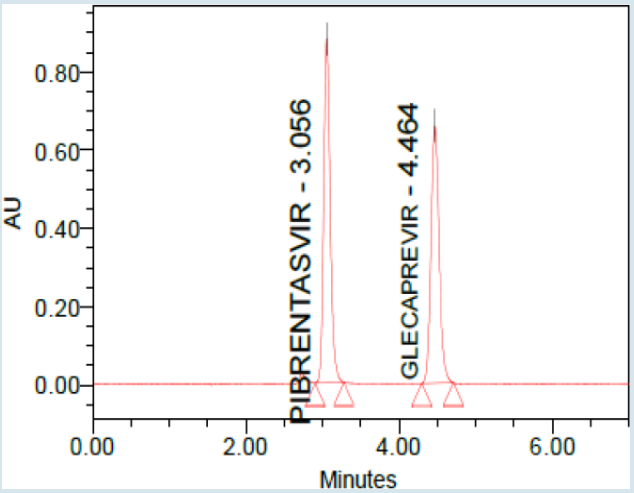


**Figure 9:** Chromatogram for LOQ.

150%. The recovery studies were performed under optimized conditions in replicate. The results were showed in [Table 3]. The accuracy should between 98%-102%. The % RSD value should not more than 2.0.

## Limit of detection and Limit of quantification

The detection limit and quantification limit were determined through signal to noise ratio 3:1 and 10:1 ratio. The limit of detection and limit of quantification were estimated 0.12 µg/mL-0.43 µg/mL for Pibrentasvir and 0.08 µg/mL-0.027 µg/mL for Glecaprevir. The results were tabulated in [Table 4]. The chromatograms of LOD and LOQ were showed in Figure 8 and 9.

## Robustness

The method was unaffected with deliberate changes with respected to flow rate (± 2), temperature of column, (± 5°C) mobile phase composition (± 3mL of organic phase) were performed at 100% test concentration. The method was robust to the above mentioned conditions. The results tabulated in [Table 5].

## Effect of flow rate

The effect of flow rate was studied with the variation of ± 0.2 ml/min from the normal conditions of the method. The mixture of both drugs containing solution analyzed by HPLC method as three independent samples.

## Effect of temperature

The effect of temperature was assessed with the variation of ±5.0°C from the normal conditions of the method. The mixture of both drugs containing solution analyzed by HPLC method as three independent samples.

## Effect of wavelength

The effect of wavelength was evaluated with the variation of the wave length ±2.0 nm from the normal conditions of the method. The mixture

**Table 5: Robustness results of Pibrentasvir & Glecaprevir.**

| S. No | Parameter | Condition | Pibrentasvir | | | Glecaprevir | | |
|---|---|---|---|---|---|---|---|---|
| | | | RT | Peak Area | % Assay | RT | Peak Area | % Assay |
| 1 | | 0.8 ml/min | 2.54 | 8968090 | 99.05 | 3.67 | 8691727 | 99.82 |
| 2 | Flow | 1 ml/min | 3.04 | 9052951 | 99.95 | 4.45 | 8704296 | 99.96 |
| 3 | | 1.2 ml/min | 3.80 | 9152951 | 101.05 | 5.50 | 8811130 | 101.19 |
| 4 | | 25 °C | 3.05 | 9003965 | 99.41 | 4.34 | 8693378 | 99.84 |
| 5 | Temp | 30 °C | 3.04 | 9052951 | 99.95 | 4.45 | 8704296 | 99.96 |
| 6 | | 35 °C | 3.05 | 9162567 | 101.16 | 4.46 | 8813283 | 101.21 |
| 7 | | B:A 75:22 v/v | 2.81 | 8989157 | 99.24 | 4.46 | 8688798 | 99.23 |
| 8 | Mobile Phase | B:A 75:25 v/v | 3.04 | 9057740 | 100.00 | 4.45 | 8704296 | 99.96 |
| 9 | | B:A 75:28 v/v | 2.68 | 9231110 | 101.91 | 4.49 | 8824536 | 101.24 |

**Table 6: Assay results of Pibrentasvir and Glecaprevir.**

| S.NO | PNR | | GPR | |
|---|---|---|---|---|
| | Peak Area | % Assay | Peak Area | % Assay |
| 1 | 9110742 | 100.40 | 8707008 | 99.90 |
| 2 | 9061995 | 99.95 | 8706154 | 99.89 |
| 3 | 9054193 | 99.86 | 8705139 | 99.88 |
| 4 | 9038029 | 99.19 | 8701982 | 99.84 |
| 5 | 8994188 | 99.20 | 8700572 | 99.82 |
| 6 | 9058562 | 99.90 | 8703774 | 99.86 |
| Average | 9052951.50 | 99.75 | 8704104.83 | 99.87 |
| STDEV | 37763.41 | 0.47 | 2481.68 | 0.03 |
| % RSD | 0.42 | 0.47 | 0.03 | 0.03 |

**Table 7: Degradation studies of Pibrentasvir & Glecaprevir.**

| Stress conditions | % Assay of active moiety | | | |
|---|---|---|---|---|
| | PNR | % degradation | GPR | % degradation |
| Acid (0.1 N HCl, refluxed for 3 H at 60°C) | 90.75 | -9.20 | 91.57 | -8.43 |
| Base (0.1 N NaOH refluxed for 4H at 60°C) | 91.43 | -8.57 | 90.35 | -9.65 |
| $H_2O_2$ (3% $H_2O_2$ Stored at room temperature for 5 H) | 90.76 | -9.24 | 91.49 | -8.51 |
| Hydrolytic (80°C refluxed for 8 H) | 92.34 | -7.66 | 92.99 | -7.08 |
| UV light (256 nm for 7 days) | 90.46 | -9.54 | 92.66 | -7.34 |

of both drugs containing solution analyzed by HPLC method as three independent samples.

## Assay of Pibrentasvir and Glecaprevir in Solid dosage form

Twenty tablets of marketed formulation (Mavyret) were weighed and finely powdered. Accurately weighed 10 mg equivalent quantity of tablet powder of Pibrentasvir and transfered into 10 mL volumetric flask. The volume was made with diluents. The final concentration of Pibrentasvir was 80 µg/ml and Glecaprevir was 200 µg/mL. Results were tabulated in [Table 6].

## Force degradation studies

The force degradation studies were performed on the PNR and GPR. There was no interference of degradants and blank, the developed RP-HPLC method proves the capability of stability indicating method for the analysis of Pibrentasvir and Glecaprevir. Different stress indicating studies were conducted like acid (0.1 N HCl, refluxed for 1 H at 60°C), Base (0.1 N NaOH refluxed for 4H at 60°C), $H_2O_2$ (3% $H_2O_2$ Stored at room temperature for 2 H), hydrolytic at 80°C and UV light (near UV ≥200 for 10 days). The degradation conditions were optimized to obtain target degradation between 10 to 30% as per ICH guidelines. The results were summarized in [Table 7]. Figures 10-14 shows chromatograms of different stress degradation conditions.

## RESULTS AND DISCUSSION

The optimization of reversed phase high performance liquid chromatographic method[9], different columns like (Waters $C_{18}$; 250×4.6 mm, 5µm and Zorbax C18; 250×4.6 mm, 5 µm ), organic solvents (methanol and acetonitrile), buffers ( acetates, citrates and phosphates) at different pH (4 and 4.5) were tested. The method was optimized with water's $C_{18}$ column capacitate (250X4.6 mm, 5 µm particle size), The mobile phase was composed with mixture of 0.5 mM Ortho phosphoric acid buffer: Acetonitrile in the ratio of 75:25 v/v, pH 4.3 was adjusted with hydrochloric acid and flow of mobile phase through the column was maintained 1mL/min. The column temperature was maintained at 30°C. At all these conditions got excellent peak area, theoretical plates, good resolution between the peaks, low retention times and low tailing factor for Pibrentasvir and Glecaprevir. A flow rate of mobile phase was 1 mL/min gave an optimal signal to noise ratio with a reasonable separation time. The re-

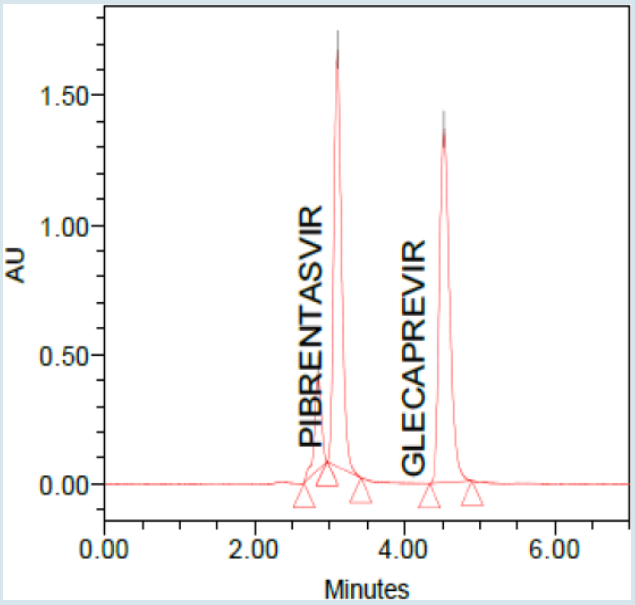


**Figure 10:** Chromatogram for acid degradation.

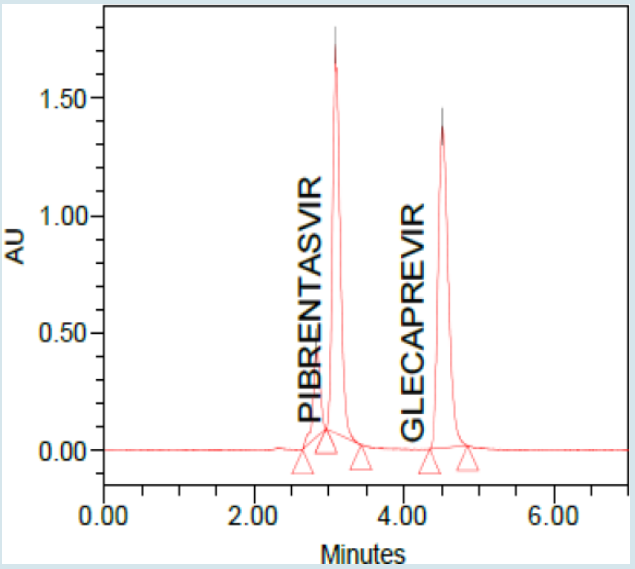


**Figure 12:** Chromatogram of peroxide degradation.

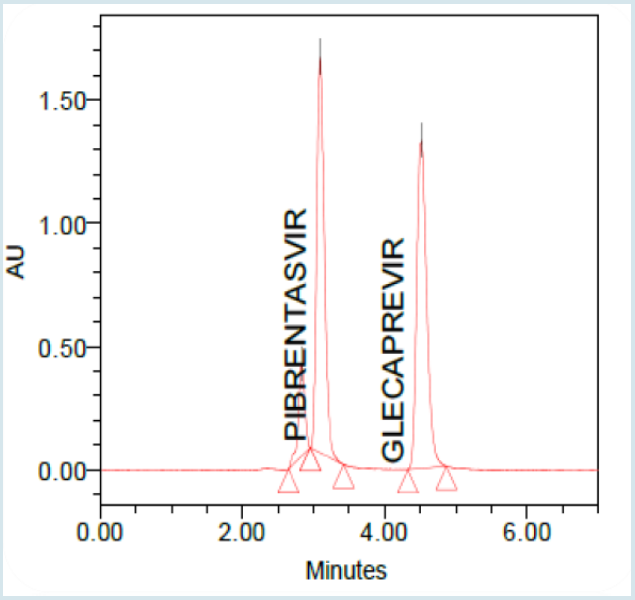


**Figure 11:** Chromatogram for base degradation.

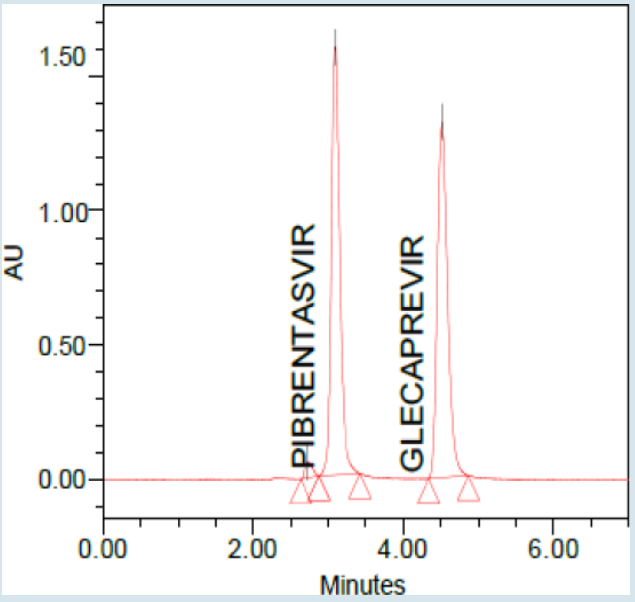


**Figure 13:** Chromatogram of hydrolytic degradation.

tention times of Pibrentasvir and Glecaprevir were found to be 3.04 and 4.45 min. Total run time of analysis was 7 min. The complete resolution of both chromatographic peaks were observed at 225 nm.

The validation of analytical method and resolution between peaks were ensured by system suitability parameter. Both peaks were eluted by forming symmetrical single peaks well separated as showed in chromatogram.

The precision of the analytical method was found to be reliable based on % Relative Standard Deviation (< 2%) corresponding to the peak areas and retention times and tabulated in 2. The % Relative Standard Deviation values were less than 2, thus the method was found to be precise for Pibrentasvir and Glecaprevir. Excellent linearity was obtained in the range of 20-120 µg/mL for Pibrentasvir and 50-300 µg/mL for Glecaprevir. The correlation coefficient ($R^2$) value greater than 0.999 (n=6) in all instances for both Pibrentasvir and Glecaprevir. The results of the calibration curve showed in Figures 7 and 8. The developed method was afforded to high recoveries of Pibrentasvir and Glecaprevir in tablet. The results of recovery studies showed in Table 3, indicated that the method was showed good accuracy. The detection limit and quantization limit of the method were found 0.12 and 0.43 µg/mL and 0.008 and

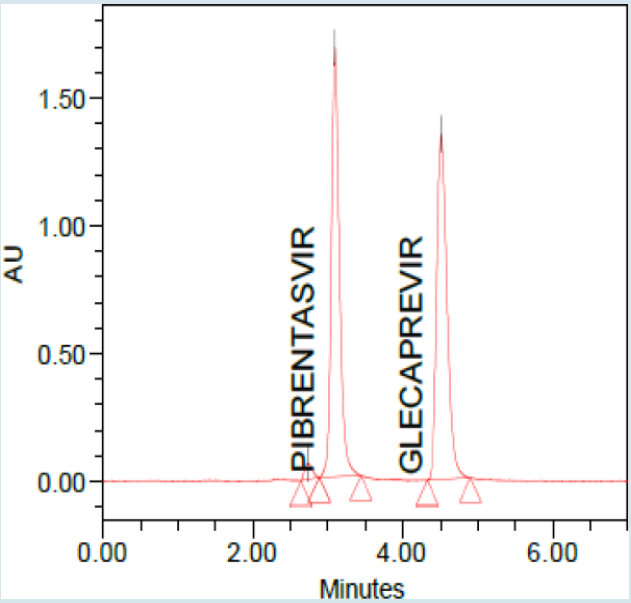


**Figure 14:** Chromatogram of UV light degradation.

0.027 µg/mL for Pibrentasvir and Glecaprevir. Results were tabulated in Table 4. Deliberate changes were performed from the normal conditions and got the % assay values within the range for both Pibrentasvir and Glecaprevir and results were tabulated in Table 5. The assay method was performed to Pibrentasvir and Glecaprevir, % assay values are 99.75% for Pibrentasvir and 99.87% for Glecaprevir. The results were showed in Table 6. The developed method was routinely used for qualitative analysis of combined dosage form of Pibrentasvir and Glecaprevir in tablet and its bulk dosage form.

The forced degradation studies were performed with acid (0.1 N HCl, refluxed for 1 H at 60ºC), base (0.1 N NaOH refluxed for 4H at 60ºC), $H_2O_2$ (3% $H_2O_2$ Stored at room temperature for 2 H), water at 60$^0$C and UV light (near UV ≥200 for 10 days). Analytes were degraded significantly.Both the drugs were found to be sensitive to acid, base, peroxide and UV-light effect where as they found to resistance to hydrolytic degradation.

## CONCLUSION

The developed RP-HPLC method was simple, sensitive, specific, accurate and precise, stability indicating simultaneous estimation of PNR and GPR in tablet dosage form. The developed method showed excellent resolution between PNR and GPR. The method was effectively validated in terms of system suitability, precision, linearity, range, accuracy, LOD, LOQ and robustness and stress indicating studies in agreement with ICH guidelines. Hence the method is routinely used for estimation and quality control, stability indicating samples of combined market formulation of Pibrentasvir and Glecaprevir.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

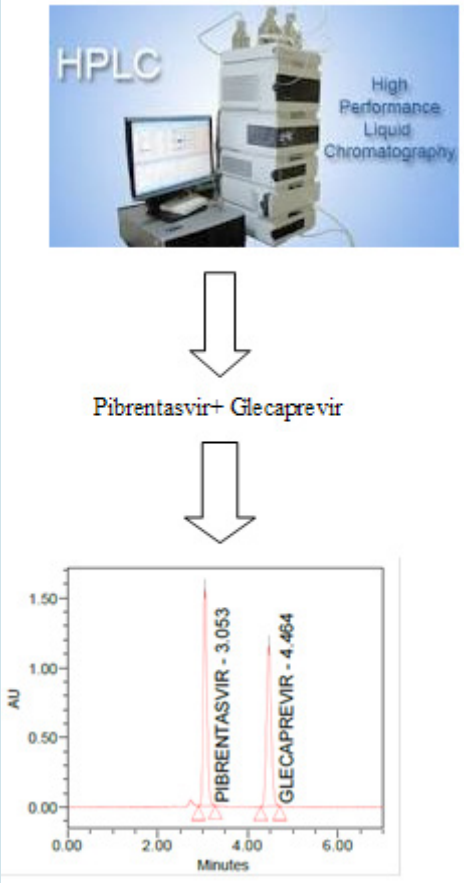
## ABBREVIATIONS

**PNR:** Pibrentasvir; **GPR:** Glecaprevir; **mL:** Milli liters; (µg) Microgram; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **HCI:** Hydrochloric acid; **NaOH:** Sodium hydroxide; **$H_2O_2$:** Hydrogen peroxide; **UV:** Ultraviolet; **µL:** Microliters; **mg:** milligrams; **nm:** Nanometers; **HCV:** Hepatitis C Virus; **NS5A:** Non structural 5A Protein; **NS3/4A:** Non structural 3,4A Protein; **Min:** Minutes; **RP-HPLC:** Reversed phase-High performance liquid chromatography.

## REFERENCES

1. Fathi H, Clark A, Hill NR, Dusheiko G. Effectiveness of current and future regimens for treating genotype 3 hepatitis C virus infection: a large-scale systematic review. BMC Infect Dis. 2017;17(1):722.
2. Hemalatha K, Kistayya C, Nizamuddhin ND, Dastagiramma D. Simultaneous Estimation of New Analytical Method Development and Validation of Glecaprevir and Pibrentasvir by High performance Liquid chromatography. Innovat International Journal of Medical and Pharmaceutical Sciences. 2018;3(1):5-8.
3. Poordad F, Felizarta F, Asatryan A, Sulkowski MS, Reindollar RW.Glecaprevir and pibrentasvir for 12 weeks for hepatitis C virus genotype 1 infection and prior direct-acting antiviral treatment. Hepatology. 2017;66(2):389-97.
4. Teresa I, Rakesh Tripathi Ng , Reisch T, Liangjun Lu, Middleton T, *et al. In vitro* Antiviral Activity and Resistance Profile of the Next-Generation Hepatitis C Virus NS3/4A Protease Inhibitor Glecaprevir. Antimicrobial Agents and Chemotherapy. 2017;62(1):1-50.
5. Krishnan P, Schnell G, Tripathi R, Beyer J, Reisch T, *et al.* Integrated Resistance Analysis of CERTAIN-1 and CERTAIN-2 Studies in Hepatitis C Virus-Infected Patients Receiving Glecaprevir and Pibrentasvir in Japan. Antimicrobial Agents and Chemotherapy. 2018; 62(2):1-16.
6. Yvette N. Lamb. Glecaprevir/Pibrentasvir: First Global Approval Drugs. 2017; 77(16):1797-1804. 6. Lin C, Dutta S, Ding B, Wang T, Zadeikis N, *et al.* Pharmacokinetics, Safety, and Tolerability of Glecaprevir and Pibrentasvir in Healthy White, Chinese, and Japanese Adult Subjects. The Journal of Clinical Pharmacology. 2017;57(12):1616-24.
7. International Conference on Harmonization of Technical Requirement of Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology, Q2B. Geneva, Switzerland. 1996.
8. Snyder LIyod R, Joseph J, Krikland JJ, Glajch LJ. Practical HPLC Method Development. 2nd Ed. New York: John Wiley and Sons Inc. 1997:709.
9. ICH, Q2 (R1) Validation of analytical procedures: Text and methodology, Proceedings of the International Conference on Harmonization, November. 2005.

## PICTORIAL ABSTRACT




## SUMMARY


- The developed RP-HPLC method for the simultaneous estimation of Pibrentasvir and Glecaprevir showed good theoretical plates and low tailing factor. The method was showed excellent linearity and $R^2$ were found to be 0999 for PNR and 1 for GPR and also showed wider concentration range. The method was showed good precision, accuracy and robustness, the values were within the limit as per ICH guidelines. The LOD and LOQ values were found to be 0.12-0.43 µg/mL for PNR and 0.008-0.027 µg/mL for GPR. The results were showed highly sensitivity of the method. The stress indicated studies were performed with different conditions of acid, alkaline, peroxide, hydrolytic and UV-light conditions, the results of the method were showed high stability of the method.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