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Original Research Article

RP-HPLC method development and validation for the simultaneous estimation of glecaprevir and pibrentasvir in bulk and tablet dosage form

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Abstract

Development and Validation of the RP-HPLC Method for Concurrent Estimation of Glecaprevir and Pibrentasvir in Tablet Dosage Forms. Pibrentasvir and glicaprevir's medicinal dosage forms were identified using the RP-HPLC analytical method. The chromatographic separation was performed using an Altima C18 150 x 4.6 mm, 5 µm and a mobile phase consisting of buffer 0.1% OPA: Acetonitrile (60:40) (v/v). 0.1% OPA was the buffer utilised in this procedure. Without derivatisation, detection was done at 260.0 nm with a flow rate of 1 ml/min. Both pibrentasvir and glicaprevir were shown to be linear over the 45–105 µg/mL and 125–325 µg/mL ranges, respectively. Using the linear regression equation y = 9124x + 494.7 (r^2 = 0.998) for Glecaprevir and y = 13172x + 8283 (r^2 = 0.998) for Pibrentasvir, it was shown that this linearity was -134 for both drugs. The calibration curve produced a linear area under the curve for Glecaprevir and Pibrentasvir. In accordance with the schedule, the computed retention time was 3.140 (2%) and 2.249 minutes. Glecaprevir and Pibrentasvir were found to have respective % RSDs of 0.3 and 0.4. The anticipated LOD and LOQ values of 0.040, 0.121, and 0.0791, 0.241%, respectively, were obtained from this validation. The range of recovery (%Recovery) was 99.63% to 99.53%. The recovery (%Recovery) ranged from 99.63% to 99.53%. Following statistical validation, the robustness of the proposed technique ranged from 1.236 to 2.636. The recovery range of 99.63 to 99.53% suggests that the strategy was suitable. Regular quality control testing in industries could make use of the suggested RP-HPLC technology due to its affordability, accuracy, precision, and ease of use.

Keywords: Glecaprevir, Pibrentasvir, HPLC, Retention time, Degradation studies.

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1. Introduction

For the treatment of HCV, glicaprevir and pibrentasvir are being investigated together. Grazoprevir is a second-generation inhibitor of HCV proteases that targets nonstructural proteins. Nonstructural protein 5A (NS5A) is inhibited by the antiviral drug pibrentasvir. The IUPAC names for glecaprevir are 1R, 14E, 18R, 22R, 26S, and 29S [(1R,2R)]. N-2-(difluoromethyl) Sulfonyl [(1-methylcyclopropyl) carbamoyl} -26-tert-butyl-N-2 -1-~Difluoro-24, 27-cyclopropyl-13, 13-Trioxa-4,11,25,28-tetraazapentacyclo-dioxo-2,17,23 [26.2.1]. 0.0{5, 10}. 0{18, 22.0{3,12}] Hetriaconta-3,5(10), 6,8,11,14-hexaene-29-carboxamide. PNV is a direct-acting antiviral that inhibits the Hepatitis C virus NS5A. Viral RNA replication and viron assembly are inhibited. The combination of pibrentasvir (PNV) and glecaprevir (GPV) offered an optimistic cure rate for patients who had not responded to previous NS5A inhibitors. (2S,3R) is the IUPAC name for pibrentasvir.-1-[(2S)]-2-{5-[(2R,5R)] The compound 4-(4-fluorophenyl)piperidin-1-yl 3,5-difluoro-4-phenyl-1--5 to 6-fluoro-2-[(2S)] (S,3R) [hydroxyl (methoxymethylidene) amino} -1-[Methoxy-butanoyl pyrrolidin -2-yl-3-Two-yl pyrrolidin [-1H-1,3-benzodiazole-5-yl}]1-yl pyrrolidin and 6-fluoroThe hydroxyl (methoxymethylidene) amino derivative -1H-1,3-benzodiazol-2-yl -2 3-methylbutan.Great rewording: The DAA agents glecaprevir and pibrentasvir have a broad pangenotypic coverage and a substantial resistance barrier. For eight and twelve weeks, we examined the safety and efficacy of 300 mg of glecaprevir plus 120 mg

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of pibrentasvir in HCV genotype 1 or 3 infected individuals without cirrhosis.

It inhibits the NS3/4A protease of the HCV virus. These proteins are inhibited by the substances glicaprevirr and P-glycoprotein, organic anion transporting polypeptide 1B1, organic anion transporting polypeptide 1B3, cytochrome P450 3A, cytochrome P450 1A2, UGT1A1, and HCV NS3/4A protease. Pibrentasvir is an HCV virus NS5A inhibitor. P-Glycoprotein, Breast Cancer Resistance Protein, Organic Anion Transporting Polypeptides 1B1 and 1B3, Cytochrome P450 3A and 1A2, and UGT1A1 are all inhibited by it.The creation of a single dosage form, including a combination of medications, must be accompanied by the establishment of an analytical method for consistent and trustworthy product testing. It's evident from reading the research that there aren't enough established techniques for quantifying grazoprevir and pibrentasvir either alone or in conjunction with other medications.[11-15] This study's main objective was to develop and validate grazoprevir and pibrentasvir in tablet formulation in compliance with International Council of Harmonisation (ICH) Q2 (R1) guidelines, as assessed by reverse-phase high-performance liquid chromatography (RP-HPLC). The ICH guidelines Q1A (R2) were also followed for conducting stress analyses.[1-19] No study on the analysis of the glecaprevir and pibrentasvir combination approach using RP-HPLC has been published in the literature, despite a thorough evaluation of the research on glecaprevir and pibrentasvir estimation and combinations.

2. Materials and Methods

2.1. Chemicals and reagents

Phosphate buffer, methanol, potassium dihydrogen ortho phosphate buffer, distilled water, acetonitrile, glicaprevir and pibrentasvir tablets in combination (Mavyret), glicaprevir and pibrentasvir tablets in pure form (API), and ortho-phosphoric acid. Rankem is the source of all the chemicals and solvents listed above.

3. Instruments and Chromatographic Conditions

The Waters HPLC-2695 system with quarternary pumps, photo diode array detector, auto sampler with Empower2 software, Denver Electronic Balance, BVK Enterprises' ultrasonicator, and BVK Enterprises' PH meter in India. PG Instruments T60, a UV-Vis spectrophotometer with a distinctive band width of 2 mm and 10 mm, matching quartz cells, and UV Win 6 software were used to test the absorbance of Glecaprevir and Pibrentasvir solutions. The mobile phase was composed of 60:40 acetonitrile and 0.1% orthophosphoric acid. For six minutes at 30°C, the experiment was carried out on a 150x4.6mm, 5µ column at a rate of 1 ml/min. The injection volume was 10µL, and the wave length was optimised at 270 nm.

3.1. Preparation of solvents and solutions

3.1.1. Diluent

Water and acetonitrile were taken in a 50:50 ratio, depending on how soluble the medicines were.

3.2. Preparation of buffer

3.2.1. Preparation of 0.1% Ortho phosphoric acid Buffer:

HPLC-grade water was used to dilute 1 millilitre of Ortho phosphoric acid to 1000 millilitres.

3.3. Preparation of mobile phase:

The mobile phase was made by combining acetonitrile and 0.1% orthophosphoric acid in a 60:40 ratio. Any remaining gas was then removed by sonicating the mixture in an ultrasonic bath, and it was vacuum-filtered through a 0.45µ Millipore nylon filter.

3.4. Preparation of standard stock solutions

An accurate weighing machine was used to transfer 10 mg of pibrentasvir and 25 mg of glecaprevir to two 25 ml volumetric flasks. After that, 3/4 diluents were added, and they spent five minutes being sonicated. Glecaprevir (1000 µg/ml) and pibrentasvir (400 µg/ml) standard stock solutions were prepared in each flask using diluents.

3.5. Preparation of standard working solutions (100% solution)

One millilitre of each stock solution was pipetted into a 10-millilitre volumetric flask, and diluents were subsequently added. Pibrentasvir at 40 µg/ml and glecaprevir at 100 µg/ml.

3.6. Preparation of sample stock solutions

Five tablets were weighed, and their average weight was determined. The weight of one pill was then transferred into a 100 ml volumetric flask. HPLC filters (400 µg/ml of Pibrentasvir and 1000 µg/ml of Glecaprevir) were used to dilute and filter the mixture after 5 ml of diluents were added and sonicated for 25 minutes.

3.7. Preparation of Sample working solutions (100% solution)

Each stock solution was pipetted out and diluted with diluent before being added to a 10 ml volumetric flask in 1 ml increments. (Pibrentasvir 40 µg/ml and glecaprevir 100 µg/ml)

3.8. Method validation

Characteristics including Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD), and Limit of Quantitation (LOQ) were assessed in compliance with ICH requirements once the method was validated.

3.9. Specificity

The ability of the analytical technique to evaluate the analyte's reaction without being influenced by unrelated chemicals in order to generate peaks that are well-resolved.

4. Linearity

25%. To make 10 ml, two standard stock solutions, each containing 0.25 ml, were pipetted out. (Glecaprevir 25µg/ml and Pirentasvir 10µg/ml).50% The conventional answer is: A total of 10 ml was obtained by pipetting out two standard stock solutions, each comprising 0.50 ml. Glecaprevir (50 µg/ml) with pibrentasvir (20 µg/ml).75% of the standard solution A total of 10 ml was obtained by pipetting out two standard stock solutions, each comprising 0.75 ml. (Glecaprevir 75 µg/ml and Pirentasvir 30 µg/ml).One millilitre of each of two standard stock solutions was pipetted out to create ten millilitres of the 100 percent standard solution. Glecaprevir 100 µg/ml and Pibrentasvir 40 µg/ml. The 125% standard solution was made by pipetting off 1.25 ml of each of the two standard stock solutions to create 10 ml. Glecaprevir (125 µg/ml) and Pibrentasvir (50 µg/ml). Standard solution: 150 millilitres of each of the two standard stock solutions were pipetted out and diluted to 10 millilitres. Glecaprevir at 150 µg/ml and Pibrentasvir at 60 µg/ml.

5. Accuracy

To create the mobile phase, 0.1% orthophosphoric acid and 60:40 acetonitrile was combined, the mixture was sonicated in an ultrasonic bath to liberate gas, and it was then vacuum-filtered through a 0.45-meter Millipore nylon filter.

5.1. Preparation of Standard stock solutions

Precisely weighed doses of Glecaprevir 25 mg and Pibrentasvir 10 mg were placed in two distinct 25 ml volumetric flasks. After transferring approximately three-fourths of the diluent to each flask, it was subjected to a 10-minute sonication. The flasks were designated the Standard Stock Solution, which contained 400 µg/mL of pibrentasvir and 1000 µg/mL of glecaprevir, after diluent was added until the mark was attained.

5.2 Preparation of standard working solutions (100% solution)

Pipetting one millilitre of each stock solution separately, moving it to a 10-milliliter volumetric flask, and diluting it. (100µg/ml of glecaprevir and 40µg/ml of pibrentasvir)

5.3. Preparation of 50% spiked solution

Diluent was mixed with 1.0 mL of each standard stock solution using a pipette. This was followed by adding 0.5 mL of the sample stock solution to a 10 mL volumetric flask.

5.4. Preparation of 100% spiked solution

A 10 ml volumetric flask was filled with 1.0 ml of the sample stock solution, and then 1.0 ml of each standard stock solution was pipetted and combined with the diluent.

5.5. Preparation of 150% spiked solution

After transferring 1.5 ml of the sample stock solution into a 10 ml volumetric flask, 1.0 ml of each standard stock solution was pipetted and combined with the diluent.

5.6 Acceptance criteria

The % Recovery for each level should be between 98.0 and 102

6. Robustness

Even with little deliberate modifications to the process, such as tweaks to the temperature, mobile phase ratio, and flow rate, no appreciable differences were seen in the outcomes, and the values stayed within the range given by ICH guidelines. Samples were injected in duplicate under the following circumstances to guarantee robustness: lower temperature (25°C) and higher temperature (35°C); lower and higher mobile phase ratios (65B:35A and 55B:45A); and lower and higher flow rates (0.9 ml/min and 1.1 ml/min). Every parameter complied with the necessary requirements, and the system suitability parameters had little effect. The percentage RSD stayed well within reasonable bounds.

6.1. Limit of detection (LOD)

Although it might not be measurable under the given experimental conditions, the lowest analyte concentration in a sample that can be detected is known as the limit of detection (LOD).

7. LOD Sample Preparation

Following pipetting and transfer to different 10 mL volumetric flasks, two standard stock solutions, each containing 0.25 mL, were diluted. Following preparation, 0.1 mL of the glecaprevir and pibrentasvir solutions were reconstituted using the same diluents in separate 10 mL volumetric flasks.

8. LOQ sample Preparation

Two standard stock solutions, each containing 0.25 ml, were pipetted and moved to different 10 ml volumetric flasks for dilution. Following this, 0.3 ml of each of the Glecaprevir and Pibrentasvir solutions were moved to separate 10 mL volumetric flasks and reconstituted with the same diluent.

8.1. Degradation studies

8.1.1. Oxidation

To 1 ml of the Glecaprevir and Pibrentasvir stock solutions, a different 1 ml quantity of 20% hydrogen peroxide (H2O2) was added. 30 minutes were spent incubating the mixes at

60°C. After diluting the resultant solution to concentrations of 100 µg/mL and 40 µg/mL for HPLC analysis, 10 µL was injected into the system to evaluate the sample's stability. After that, chromatograms were made.

9. Acid Degradation Studies

After adding 1 millilitre of 2N hydrochloric acid to the Glecaprevir and Pibrentasvir stock solutions, they were refluxed for 30 minutes at 60°C. Following the dilution of the resultant solution to 100 µg/ml and 40 µg/ml concentrations, 10 µL of each solution was introduced into the system. Chromatograms were taken in order to determine how stable the sample was.

10. Alkali Degradation Studies

Glecaprevir and Pibrentasvir stock solutions were combined with 1 ml of 2N sodium hydroxide, and the mixture was refluxed for 30 minutes at 60°C. The final solution was then diluted to 40 µg/mL and 100 µg/mL concentrations. To evaluate the stability of the material, chromatograms were acquired after 10 µL of each solution was injected into the apparatus.

11. Dry Heat Degradation Studies

The standard drug solution was heated to 105°C for an hour in order to investigate dry heat degradation. For HPLC analysis, the resultant solution was subsequently diluted to values of 40 µg/ml and 100 µg/ml. Chromatograms were taken after 10 µL of each solution was injected into the system in order to evaluate the sample's stability.

11.1. Photo stability studies

By subjecting a 1000 µg/mL solution of Glecaprevir and 400 µg/mL solution of Pibrentasvir to UV radiation for a day, or 200 Watt hours/m2, in a photostability chamber, the drug's photochemical stability was further evaluated. Following the dilution of the resultant solution to 100 µg/mL and 40 µg/mL for HPLC analysis, 10 µL was introduced into the system. In order to assess the stability of the material, chromatograms were then recorded.

12. Neutral Degradation Studies

To perform stress testing under neutral conditions, the drug was refluxed in water at 60°C for an hour. After diluting the resultant solution to concentrations of 40 µg/ml and 100 µg/ml, 10 µL of it was injected into the apparatus for the HPLC analysis. In order to evaluate the sample's stability, chromatograms were then captured.

12.1 System suitability

Six injections of standard Glecaprevir (100 ppm) and Pibrentasvir (40 ppm) solutions were made. The system appropriateness was assessed by measuring parameters such peak tailing, resolution, and USP plate count. The six standard injections should have an RSD of no more than 2%.

13. Assay of Glecaprevir and Pibrentasvir

A combination tablet of Glecaprevir and Pibrentasvir (Mavyret), containing 100 mg of Glecaprevir and 40 mg of Pibrentasvir, respectively, and pure Glecaprevir and Pibrentasvir (API) were used in the assay, which was carried out using the suggested method under ideal chromatographic circumstances. The sample solutions were injected into the HPLC apparatus and scanned at 260 nm to determine the drug percentage.

14. Results and Discussion

Optimized Chromatographic conditions:	
Mobile phase	60% OPA (0.1%): 40% Acetonitrile
Flow rate	1ml/min
Column	Altima C18 150 x 4.6 mm, 5⌈.
Detector wave length	260nm
Column temperature	30°C
Injection volume	10⌈L
Run time	6 min
Diluent	Water and Acetonitrile in the ratio 50:50

Pibrentasvir and Glecaprevir were determined simultaneously in tablet and bulk dosage forms using the RP-HPLC method, which was created and put into use. Following the assessment of numerous preparatory and chromatographic factors, the optimal chromatographic conditions are shown in (**Table 1**). Acetonitrile and orthophosphoric acid were used in a 60:40 ratio for the final analysis, which was carried out at a flow rate of 1 mL/min. An Altima C18 150x4.6mm, 5µm column, a detector wavelength of 260 nm, an injection volume of 10 µL, and a run time of 6 minutes were used to analyse the samples. The technique was adjusted to yield distinct peaks with little tailing and a respectable level of resolution. The optimised chromatogram is displayed in (**Figure 4**).

14.1. Validation

In duplicate injections of glicaprevir (25–150 µg/mL) and pibrentasvir (10–60 µg/mL) at six distinct doses each, linearity was observed. The average areas and linearity equations were as follows: - for Pibrentasvir: y = 9421x + 4947 - for Glecaprevir: y = 1312x + 8283. For both medications, the correlation value (R2) was 0.999. (**Figure 4**) and (**Figure 5**) display the linearity calibration curves for Glecaprevir and Pibrentasvir, respectively. Glecaprevir and Pibrentasvir had retention durations of 2.246 and 3.140 minutes, respectively. During these retention periods, neither the blank nor the placebo showed any interference peaks. As a result, this approach keeps its distinctiveness.

At three different accuracy levels—50%, 100%, and 150%—three injections were carried out in duplicate. (**Table 2**) displays the mean percentage recovery for Glecaprevir and Pibrentasvir, which were 99.63% and 99.53%, respectively. By injecting known amounts, the percentage RSD was determined; repeatability values for Glecaprevir and Pibrentasvir were 0.3% and 0.4%, respectively. The percentage RSD values for Glecaprevir and Pibrentasvir were 0.5% and 0.4%, respectively, for moderate precision. The method's accuracy is demonstrated by the low percentage RSD readings in (**Table 3**). Using the slope of their calibration curves and the response's RSD values, the LOD and LOQ values for glicaprevir and pibrentasvir were determined. Table 4 indicates that the quantitation limits for Glecaprevir and Pibrentasvir were 0.079 and 0.241, respectively, and the detection limits were 0.040 and 0.121.

Samples were injected in duplicate under robustness conditions, which included temperature changes (25°C for temperature minus and 35°C for temperature plus), mobile phase composition, and flow rate variations (0.9 ml/min for flow minus and 1.1 ml/min for flow plus). There was no discernible impact on the system suitability criteria because all of the requirements were satisfied. (**Table 5**) illustrates that the percentage RSD stayed within reasonable bounds. As shown in (**Table 6**), the data were evaluated to make sure they satisfied the system suitability criteria, which include retention time, resolution, USP plate count, and peak asymmetry. Pure Glecaprevir and Pibrentasvir (API) and a combination of Glecaprevir and Pibrentasvir (Mavyret), respectively, corresponding to 100 mg and 40 mg, were used in the experiment. As indicated in (**Table 7**), the average percentage of the medication was 99.31% for Glecaprevir and 99.53% for Pibrentasvir. (**Figure 6**) and (**Figure 7**), respectively, show the chromatograms for the tablet dosage forms and the conventional medications pibrentasvir and glecaprevir. Assay and degradation results are shown in (**Table 7**) & (**Table 8**).

15. Conclusion

A new technique that is accurate, exact, easy to use, and dependable has been created for the simultaneous estimate of glicaprevir and pibrentasvir in tablet dosage forms. The technique was proven to be sensitive, accurate, precise, and reliable when used to analyse Glecaprevir and Pibrentasvir in both bulk and tablet form. The outcomes validated the suggested method's precision and accuracy, which conforms to the ICH Guidelines. Because of its shorter run time and shorter retention durations, the procedure is economical and appropriate for standard industry quality control testing. Ultimately, this suggested approach yields straightforward, accurate solutions with extra advantages like robustness and predictability.

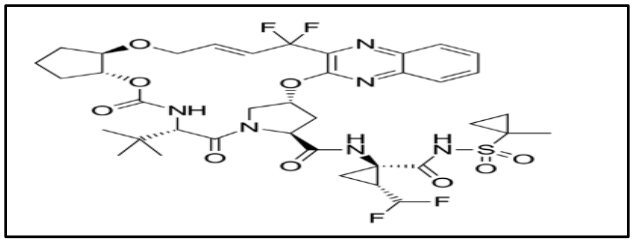

Figure 1: Chemical structure of glecaprevir

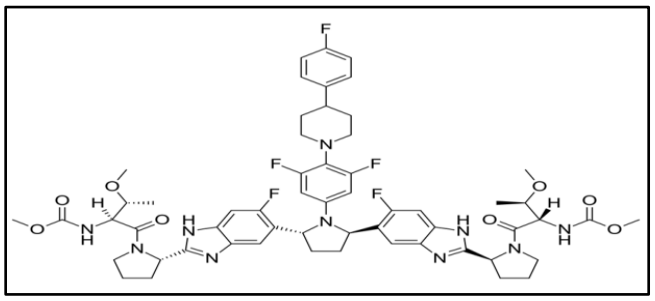

Figure 2: Chemical structure of pibrentasvir

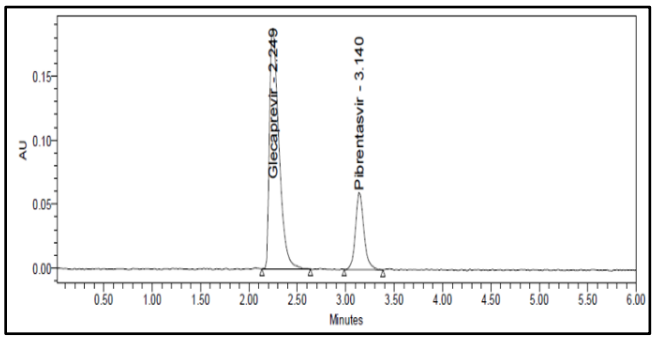

Figure 3: Optimized chromatogram of glecaprevir and pibrentasvir

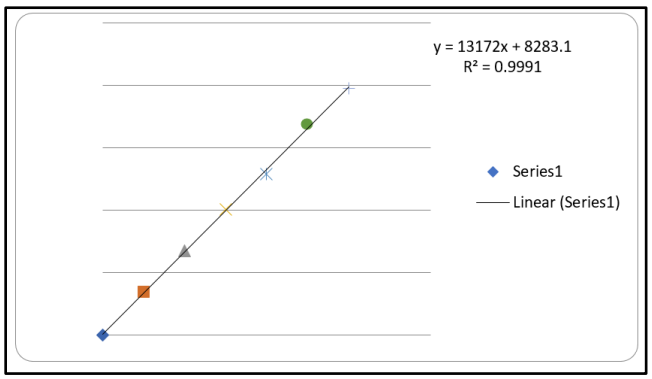

Figure 4: Linearity curve of glecaprevir

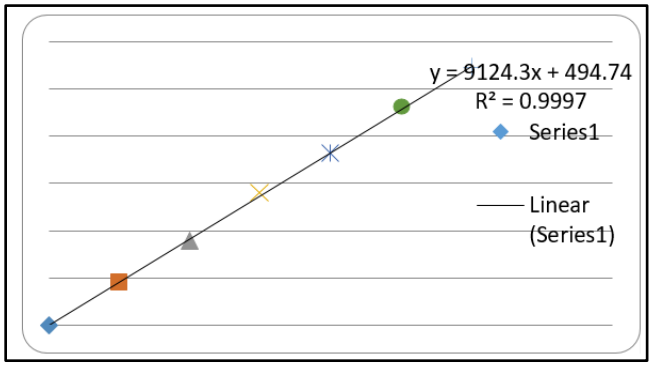

Figure 5: Calibration curve of pibrentasvir

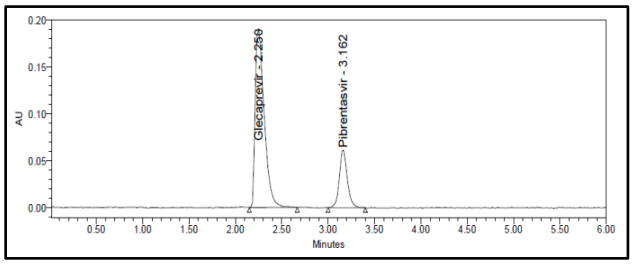

Figure 6: Standard chromatogram of glecaprevir and pibrentasvir

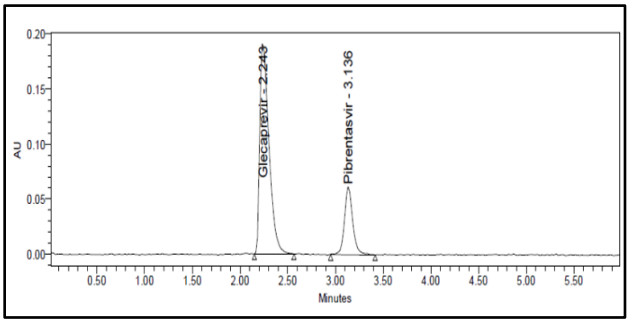

Figure 7: A Sample chromatogram of glecaprevir and pibrentasvir tablet dosage form

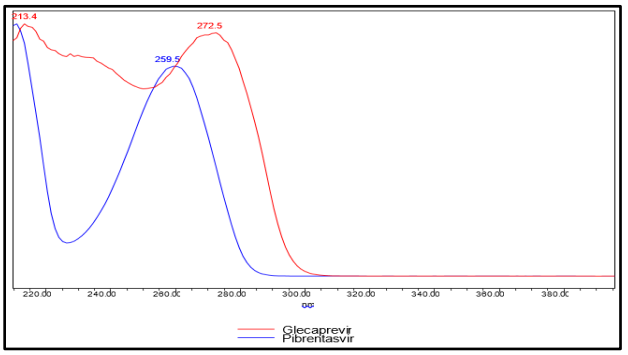

Figure 8: Overlay UV spectra of glecaprevir and **pibrentasv**

Table 1: Optimized chromatographic conditions

Parameter	**Condition**
RP-HPLC	Waters HPLC 2695 SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	Buffer and ACN: taken in the ratio 60:40
Flow rate	1ml/min
Column	Altima 150 x 4.6 mm, 5⌈.
Detector wave length	260nm
Column temperature	30°C
Injection volume	10⌈L
Run time	6 min
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Glecaprevirr2.249min and Pibrentasvir3.140min
Theoretical Plates	Glecaprevirr2580 and Pibrentasvir6791

Table 2: Accuracy results of glecaprevir and pibrentasvir

Conc.	**Glecaprevirr**			**Pibrentasvir**		
	Amount added (µg/ml)	**Amount recovered (µg/ml)**	**% Recovery**	**Amount added (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**
50%	50	49.73406	99.47	20	19.92507	99.63
	50	49.65943	99.32	20	19.9837	99.92
	50	49.55489	99.11	20	19.91663	99.58
100%	100	99.69162	99.69	40	39.63912	99.10
	100	99.65191	99.65	40	39.93559	99.84
	100	99.83632	99.84	40	39.88517	99.71
150%	150	149.9443	99.96	60	59.46913	99.12
	150	149.8239	99.88	60	59.61248	99.35
	150	149.6281	99.75	60	59.71923	99.53
Mean % Recovery			99.63%	Mean % Recovery		99.53%

Table 3: Precision results of glecaprevir and pibrentasvir

S. No	Repeatability		Intermediate precision	
	Area of Glecaprevirr	Area of Pibrentasvir	Area of Glecaprevirr	Area of Pibrentasvir
1.	1283511	365344	1238004	360976
2.	1287205	362287	1249350	362605
3.	1279435	364974	1235230	362023
4.	1278284	364232	1242417	360369
5.	1280579	364889	1249855	363901
6.	1280640	362558	1247542	361240
Mean	1281609	364047	1243733	361852
S.D	3247.0	1311.4	6170.8	1275.6
%RSD	0.3	0.4	0.5	0.4

Table 4: LOD and LOQ values of glecaprevir and pibrentasvir

Molecule	LOD	LOQ
Glecaprevir	0.040	0.121
Pibrentasvir	0.079	0.241

Table 5: Robustness data of glecaprevir and pibrentasvir

S.no.	Condition	% RSD of Glecaprevir	%RSD of Pibrentasvir
1	Flow rate (-) 0.9ml/min	0.9	1.0
2	Flow rate (+) 1.1ml/min	0.5	0.9
3	Mobile phase (-) 35B:65A	0.5	0.8
4	Mobile phase (+) 45B:55A	0.7	1.2
5	Temperature (-) 25°C	0.8	1.8
6	Temperature (+) 35°C	0.8	1.1

Table 6: System suitability parameters results of glecaprevir and pibrentasvir

S no	Glecaprevir			Pibrentasvir			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.242	2574	1.70	3.127	7357	1.12	5.2
2	2.249	2580	1.77	3.140	6791	1.17	5.2
3	2.249	2580	1.74	3.151	6866	1.11	5.5
4	2.250	2597	1.73	3.153	7044	1.11	5.4
5	2.254	2500	1.69	3.162	7097	1.13	5.4
6	2.254	2492	1.70	3.162	6970	1.09	5.4

Table 7: Assay results of glecaprevir and pibrentasvir

S. no	Glecaprevir			Pibrentasvir		
	Standard Area	Sample area	% of Drug	Standard Area	Sample area	% of Drug
1.	1290220	1283511	99.54	367412	365344	99.89

2.	1295363	1287205	99.82	366400	362287	99.05
3.	1274363	1279435	99.22	365590	364974	99.79
4.	1286429	1278284	99.13	362311	364232	99.58
5.	1289021	1280579	99.31	363160	364889	99.76
6.	1293886	1280640	99.31	367485	362558	99.12
Mean	1288214	1281609	99.39	364720	364047	99.53
S.D	7523.2	3247.0	0.25	2190.5	1311.4	0.36
%RSD	0.6	0.3	0.3	0.6	0.4	0.4

Degradation Studies: After the formulation underwent degradation tests, the deteriorated samples were administered. After calculating the assay of the injected samples, every sample passed the degradation limitations.

Table 8: Degradation data of glecaprevir

S. no	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.91	1.12	1.401
2	Alkali	4.00	0.52	1.046
3	Oxidation	3.29	0.527	0.747
4	Thermal	2.82	0.503	0.711
5	UV	1.89	0.498	0.722
6	Water	0.96	0.686	0.760

Table 9: Degradation data of pibrentasvir

S. no	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	5.00	3.125	3.509
2	Alkali	3.48	1.500	2.608
3	Oxidation	3.23	1.139	1.376
4	Thermal	2.19	1.197	1.455
5	UV	1.65	1.255	1.654
6	Water	0.69	1.201	1.630

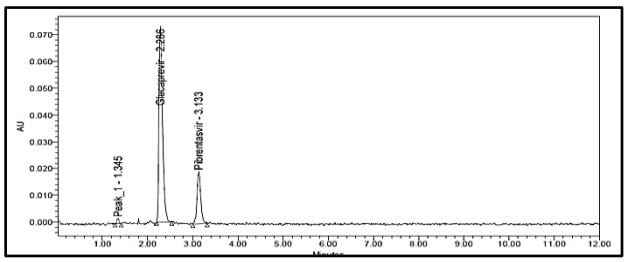

Figure 9: Acid chromatogram of glecaprevir and pibrentasvir

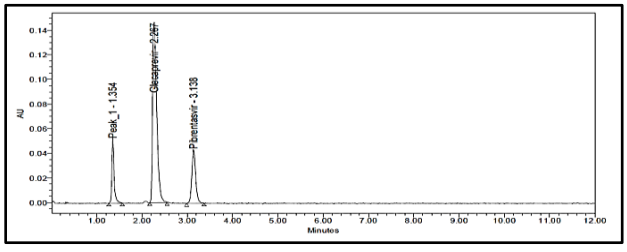

Figure 10: Base chromatogram of glecaprevir and pibrentasvir

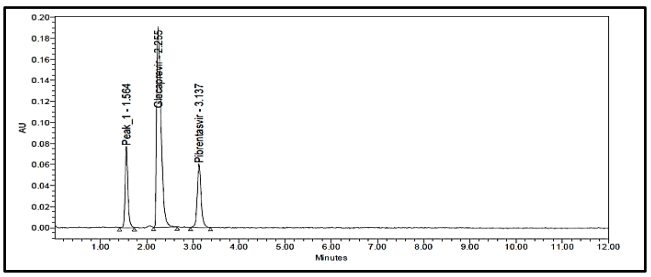

Figure 11: Peroxide chromatogram of glecaprevir and pibrentasvir

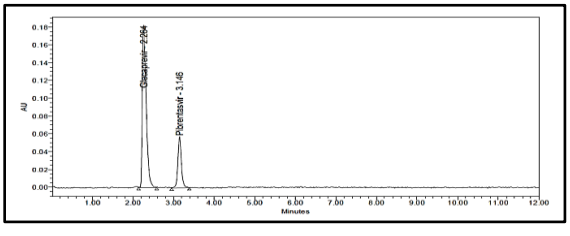

Figure 12: Thermal chromatogram of glecaprevir and pibrentasvir

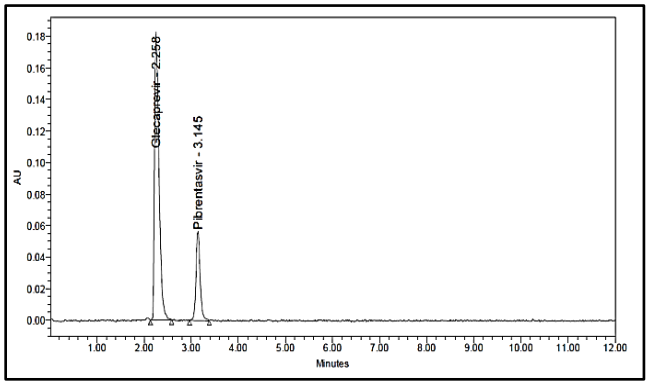

Figure 13: UV chromatogram of glecaprevir and pibrentasvir

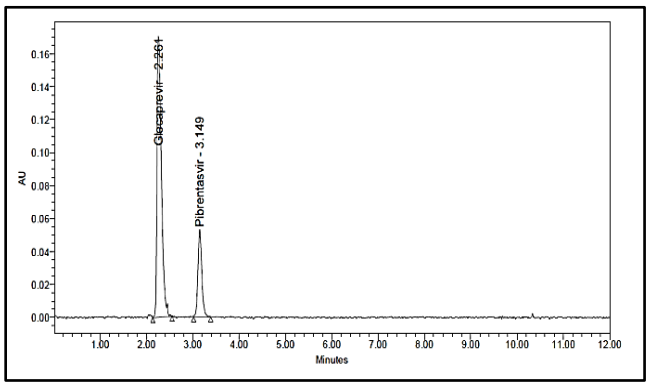

Figure 14: Water chromatogram of glecaprevir and pibrentasvi.

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18. Conflict of Interest

Authors have no conflict of interest with respect to this production.

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