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Method Development and Validation of Glecaprevir and Pibrentasvir In Pure and Pharmaceutical Dosage Forms By RP-HPLC Method

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Keywords:

Glecaprevir, Pibrentasvir, RP-HPLC and Validation.

ABSTRACT

A simple, accurate, precise RP-HPLC method was developed for the simultaneous estimation of the glecaprevir and pibrentasvir in tablet dosage form. Chromatographic separation was performed on an HPLC system (Waters with Empower2596 Software) containing Xterra C18 column (4.6 x 150mm, 5 (m) column with UV- PDA detection using a mobile phase consisting of a mixture of buffer: methanol in the ratio of 30:70 v/v. The following system conditions were maintained throughout development and validation, i.e., flow rate 1mL/min, column was maintained at ambient temperature and the detected by a PDA detector at a wave length 244nm. The glecaprevir and pibrentasvir was well resolved on the stationary phase and the retention time was 2.205 min and 4.996 min. The method was validated in terms of linearity, precision, accuracy, limit of detection, limit of quantitation and robustness as per the International Conference on Harmonization (ICH) guidelines. The linearity of the method was found to be within the concentration range of 100-500 µg/ml solutions of glecaprevir and 40-200µg/ml solutions of pibrentasvir, the correlation coefficient (r2) for the drug was 0.999. Degradation products produced as a result of stress studies did not interfere with the detection of glecaprevir and pibrentasvir and the assay can thus be considered stability indicating. The developed method can be used for routine quality analysis of titled drugs in combination in tablet formulation.

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1. Introduction

Glecaprevir[1] is direct acting antiviral agent and hepatitis C virus (HCV) NS3/4A protease inhibitor that targets the viral RNA replication. In combination with pibrentasvir, glecaprevir is a useful therapy for patients who experienced therapeutic failure from other NS3/4A protease inhibitors. It demonstrates a high genetic barrier against resistance mutations of the virus. In cell cultures, the emergence of amino acid substitutions at NS3 resistance-associated positions A156 or D/Q168 in HCV genotype 1a, 2a or 3a replicons led to reduced susceptibility to glecaprevir[1, 2]. The combinations of amino acid substitutions at NS3 position Y65H and D/Q168 has also resulted in greater reductions in glecaprevir susceptibility, and NS3 Q80R in genotype 3a patients also leads to glecaprevir resistance[2].

Pibrentasvir[3] is a direct acting antiviral agent and hepatitis C virus (HCV) NS5A inhibitor that targets the viral RNA replication and viron assembly. In combination with glecaprevir, pibrentastiv is a useful therapy for patients who experienced therapeutic failure from other NS5A inhibitors. In cell cultures, the emergence of amino acid substitutions at known NS5A inhibitor resistance-associated positions in HCV genotype 1a, 2a or 3a replicons led to reduced susceptibility and resistance to pibrentasvir. These resistance-associated amino acid substitutions included Q30D/deletion, Y93D/H/N or H58D +Y93H in genotype 1a replicons, F28S + M31I or P29S + K30G in genotype 2a replicons, and Y93H in genotype 3a replicons. Individual NS5A amino acid substitutions that reduced susceptibility to pibrentasvir include M28G or Q30D in a genotype 1a replicon and P32-deletion in a genotype 1b replicon.

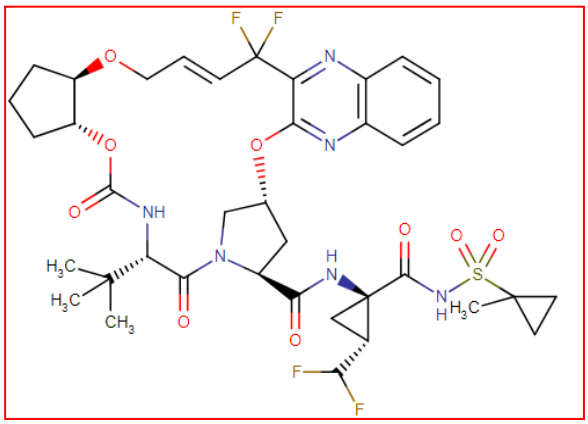

Fig 1: Chemical structure of glecaprevir

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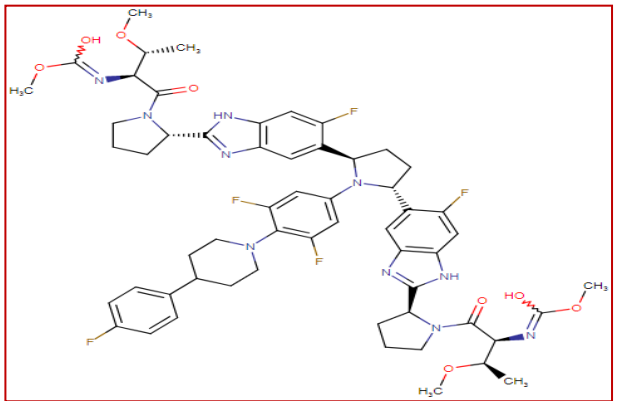

Fig 2:Chemical structure of pibrentasvir

Materials and Methods

Materials:

Glecaprevir, pibrentasvir (Pharmatrain Ltd), KH_2PO_4 (Finar chemical Ltd), ortho phosphoric acid (Merck), HPLC grade (water, methanol and acetonitrile) were used in the study.

Instrument:

HPLC-WATERS, software: Empower, 2695 separation module. 2487 UV detector, UV/VIS spectrophotometer-LABINDIA UV 3000^+, pH meter-Adwa – AD 1020 was used in the study.

Method Development

Wavelength Selection:

10 µg/ml of glecaprevir and 10 µg/ml of pibrentasvir in diluents (mobile phase composition) were recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 244 nm.

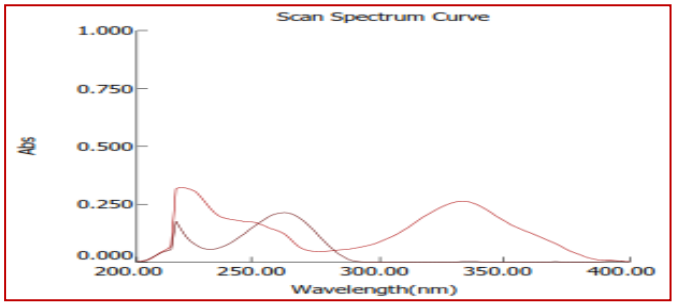

Fig 3: Scan spectrum curve of glecaprevir and pibrentasvir

Table 1: Optimized chromatographic conditions

An instrument used	:	Waters HPLC with auto sampler and PDA detector
Temperature	:	Ambient (25° C)
Mode of separation	:	Isocratic mode
Column	:	Xterra C18 column (4.6 x 150mm, 5µm)
Buffer	:	0.1% OPA
Mobile phase	:	30% buffer 70% Methanol
Flow rate	:	1ml per min
Wavelength	:	244 nm
Injection volume	:	20 µl
Run time	:	15 min.

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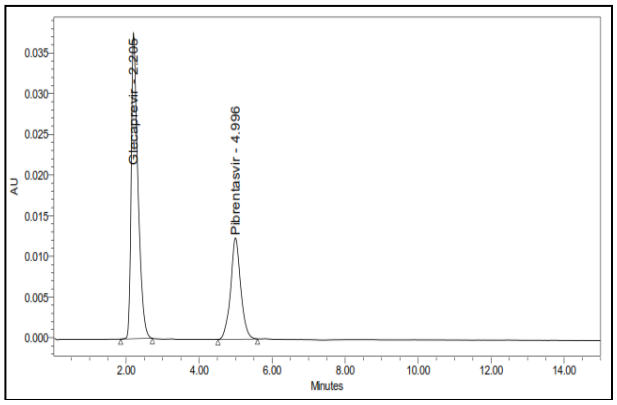

Fig 4: Optimized chromatographic conditions

Observation:

Peaks are separated and peak shapes are also good.

Preparation of Buffer and Mobile Phase:

Preparation of 0.1% OPA Buffer:

Take 1ml of orthophosphoric acid in 1000ml volumetric flask and make up to mark with water. Finally the solution was filtered through 0.45 µm membrane filter and sonicates it for 10 min.

Preparation of Mobile Phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml of methanol (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of Diluent:

The mobile phase was used as the diluent.

Preparation of Standard Solution:

Accurately weigh and transfer 25 mg of glecaprevir and 10 mg of pibrentasvir working standard into a 25 ml clean, dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample Solution:

Accurately weigh and transfer equivalent to 25 mg of glecaprevir and 10 mg of pibrentasvir working standard into a 25 ml clean, dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µL of the standard, sample into the chromatography system and measure the areas for glecaprevir and pibrentasvir peaks and calculate the % assay by using the formulae.

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Method of Validation[5, 6, 7]:

System Suitability:

The system suitability test was performed using five replicate injections of standards before analysis of samples. System suitability parameters were shown in table 2.

Table 2: Results of system suitability parameters

S.No	Name	RT (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Glecaprevir	2.205	478222	36550	5.59	1.58	3677.56
2	Pibrentasvir	4.996	239609	12483	6.76	1.04	4683.62

Linearity:

Accurately weigh and transfer 25 mg of glecaprevir and 10 mg of pibrentasvir working standard into a 25 ml clean, dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. The working standard solutions of glecaprevir and pibrentasvir were prepared by accurately transferring (1, 2, 3, 4 and 5 ml) aliquots to 10ml volumetric flask and were made up to mark with diluent to obtain a concentration of is 100-500 µg/ml for glecaprevir and 40-200 µg/ml for pibrentasvir respectively. The linear regression equations were y = 1605. x + 2605 (for glecaprevir) and y = 2021x + 1237 (for pibrentasvir) Linearity values were shown in table 3.

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Table 3: Linearity of glecaprevir and pibrentasvir

S. No	Glecaprevir		Pibrentasvir	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	100	165076	40	80057
2	200	323694	80	166200
3	300	480198	120	241067
4	400	645116	160	328200
5	500	807077	200	403253
Regression equation	**y = 1605.x + 2605**		**y = 2021x + 1237**	

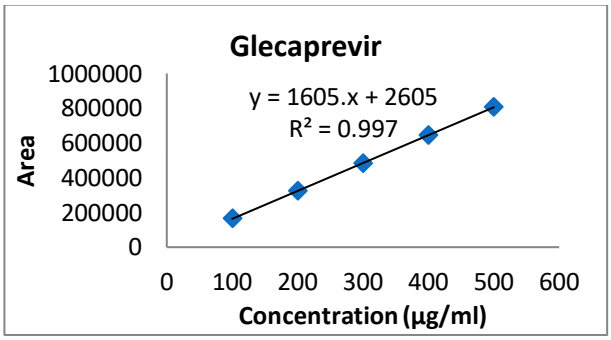

Fig 5: Calibration curve for glecaprevir

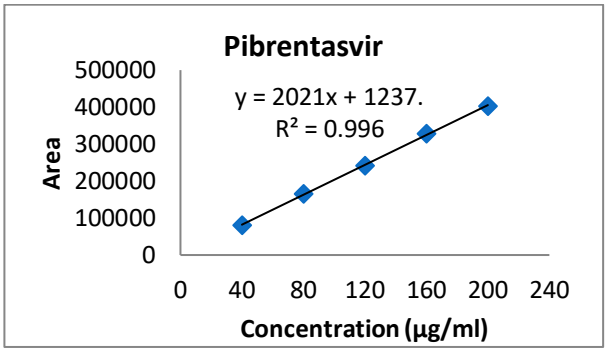

Fig 6: Calibration curve for pibrentasvir

Precision:

Preparation of Stock Solution:

Accurately weigh and transfer 25 mg of glecaprevir and 10 mg of pibrentasvir standard drug into a 25 ml clean, dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and make the volume up to the mark with the same diluent. Further pipette out 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, precision was performed on different days.

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Preparation of Stock Solution:

Accurately weigh and transfer 25 mg of glecaprevir and 10 mg of pibrentasvir standard drug into a 25 ml clean, dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solutions were prepared with the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Table 4: Results of Precision for Glecaprevir and Pibrentasvir

Injection	Precision		Intermediate precision	
	Area		Area	
	Glecaprevir	Pibrentasvir	Glecaprevir	Pibrentasvir
Injection1	483912	242261	482579	241793
Injection2	479899	241331	489171	241873
Injection3	487806	244327	482292	241291
Injection4	486352	243371	483377	241423
Injection5	482426	242500	483324	241328
Injection6	484893	241079	480775	242453
Mean±SD	**484214.7±2822.8**	**242478.2±1227.7**	**483586.3±2894.6**	**241693.5±444.5**
%RSD	**0.6**	**0.5**	**0.6**	**0.2**

Specificity:

For specificity blank and standard is injected into the system. There is no any interference of any peak in the blank with the retention time of the analytical peaks.

Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by the method to the assay value. Accuracy must be established across the specified range of the analytical procedure. Accuracy was

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determined over the range of 50%, 100% and 150% of the sample concentration.

Preparation of 50%, 100% and 150 % solution:

Accurately weigh and transfer 50%, 100%, 150% of glecaprevir and 50%, 100%, 150% of pibrentasvir working standard into a 25 ml clean, dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Calculate the Amount found and Amount added for glecaprevir & pibrentasvir and calculate the individual recovery and mean recovery values.

Table 5: Accuracy (recovery) data for glecaprevir and pibrentasvir

Drug	% Concentration (Level)	Sample Area	Sample weight added (mg)	Amount Found (mg) *	% Recovery*	% Mean Recovery
Glecaprevir	50%	242024.3	12.50	12.57	100.54	100.40
	100%	484977.0	25.00	25.18	100.73	
	150%	721772.3	37.50	37.48	99.94	
Pibrentasvir	50%	120660.7	5.00	5.01	100.23	100.25
	100%	241976.0	10.00	10.05	100.50	
	150%	361205.0	15.00	15.00	100.02	

*Average of three determinations

Acceptance Criteria

The mean% recovery of the glecaprevir and pibrentasvir at eached spiked level should not be less than 98 % and not more than 102 %.

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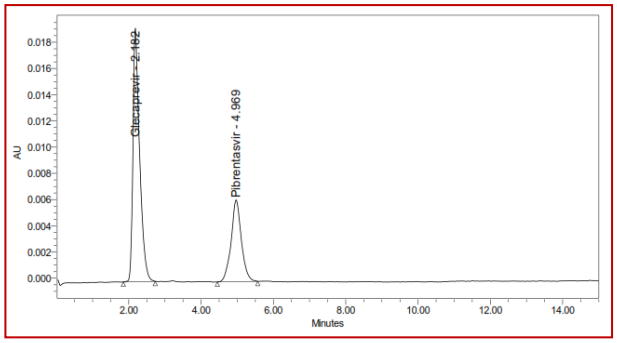

Figure 7: Chromatogram for Accuracy 50%

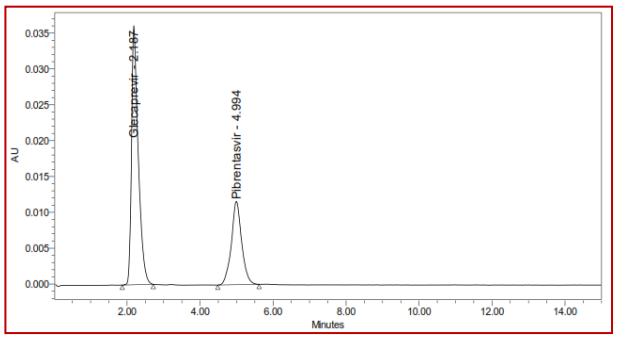

Figure 8: Chromatogram for Accuracy 100%

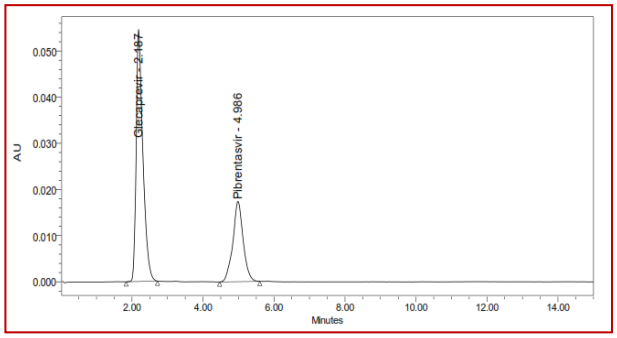

Figure 9: Chromatogram for Accuracy 150%

ASSAY:

Standard and sample solution injected under experimental work. The results are shown below.

Calculation:

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT= average area counts of sample preparation, AS = average area counts of standard preparation, WS= Weight of working standard taken in mg, P= Percentage purity of working standard, LC = Label Claim mg/ml.

Table 6: Results of assay for glecaprevir and pibrentasvir

Drug	Label Claim (mg)	% Assay
Glecaprevir	100	100.83
Pibrentasvir	40	100.23

Limit of detection and limit of quantification

Limit of detection (LOD):

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels.

The LOD for glecaprevir and pibrentasvir was found to be 2.98µg/ml and 3.00µg/ml respectively. The LOQ is the smallest

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concentration of the analyte, which gives a response that can be accurately quantified. The LOQ was 10.00µg/ml and 9.981µg/ml for glecaprevir and pibrentasvir respectively.

Preparation of 1.41µg/ml of glecaprevir and 1.68 µg/ml pibrentasvir solution:

Accurately weigh and transfer 25 mg of glecaprevir and 10 mg of pibrentasvir working standard into a 25 ml clean dry volumetric flask add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette out 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette out 0.47 ml and 1.4 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of quantification (LOQ):

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision, and variability.

Preparation of 4.74 µg/ml (glecaprevir) and 5.56 µg/ml (pibrentasvir) solution:

Accurately weigh and transfer 25 mg of glecaprevir and 10 mg of pibrentasvir working standard into a 25 ml clean, dry volumetric flask, add about 7 ml of diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette out 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette out 1.58 ml and 4.63 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Table 7: Results of LOD and LOQ

Drug	LOD			LOQ		
	Baseline noise (µV)	Signal obtained (µV)	S/N ratio	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Glecaprevir	58	173	2.98	58	580	10.00
Pibrentasvir	58	174	3.00	58	579	9.98

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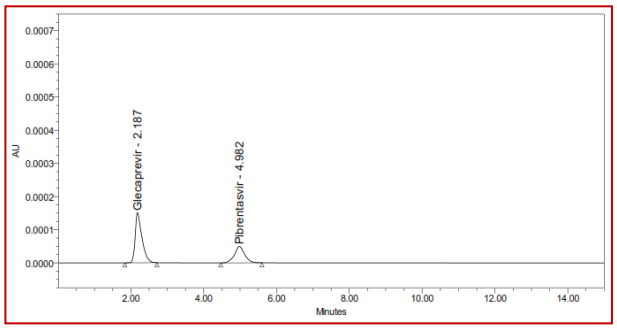

Figure 10: Chromatogram of Glecaprevir, Pibrentasvir showing LOD

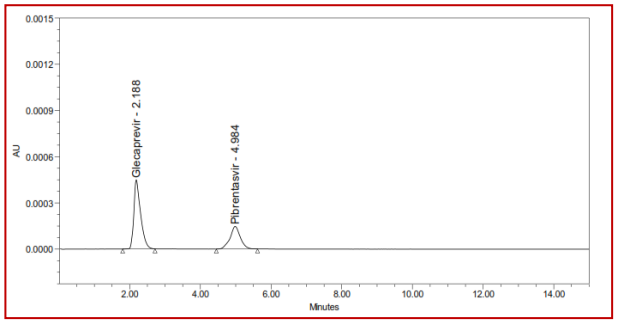

Figure 11: Chromatogram of Glecaprevir, Pibrentasvir showing LOQ

Robustness:

The method was found to be robust as the results were not significantly affected by slight variations in extraction time, composition of the mobile phase, wavelength and flow rate of the mobile phase.

As part of the robustness, deliberate change in the flow rate, mobile phase composition, temperature variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.9 ml/min to 1.1ml/min.

Standard solution 300 ppm of glecaprevir & 120 ppm of pibrentasvir was prepared and analyzed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by a change in the flow rate ±10%.

Table 8: Results for variation in flow for glecaprevir and pibrentasvir

S. No	Flow Rate (ml/min)	Glecaprevir		Pibrentasvir	
		System Suitability		System Suitability	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.9(Low)	3672.96	1.59	4701.86	1.04
2	1.0(accepted)	3678.77	1.57	4652.35	1.04
3	1.1(High)	3574.36	1.46	4388.51	1.01

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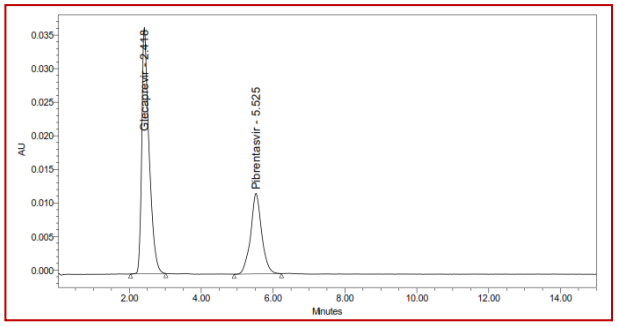

Figure 12: Chromatogram showing less flow rate

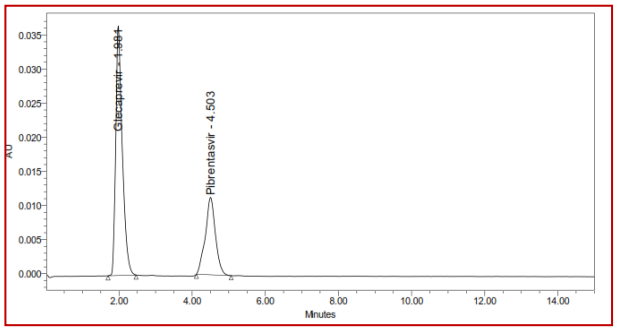

Figure 13: Chromatogram showing more flow rate

B. The Organic composition in the mobile phase was varied from ±10%.

Standard solution 300 ppm of glecaprevir & 120 ppm of pibrentasvir was prepared and analyzed using the varied mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±10.

Table 9: Results of mobile phase composition for glecaprevir and pibrentasvir

S. No.	Change in Organic Composition in the Mobile Phase	Glecaprevir		Pibrentasvir	
		System Suitability Results		System Suitability	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	10% less	3668.63	1.45	4446.54	0.83
2	Actual	3678.77	1.57	4652.35	1.04
3	10% more	3575.02	1.51	4051.10	1.20

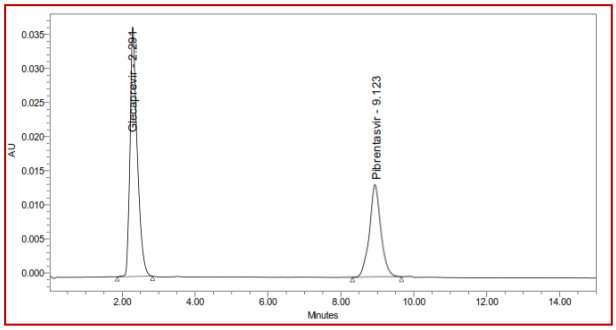

Figure 14: Chromatogram showing less organic composition

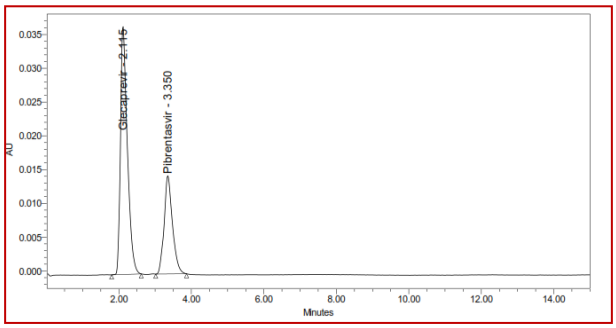

Figure 15: Chromatogram showing more organic composition

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Degradation Studies:

The stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The stress degradation studies on the glecaprevir and pibrentasvir using the proposed method carried out as per ICH guidelines.

Hydrolytic degradation under acidic condition

Pipette out 3 ml of above solution into a 10ml volumetric flask and add 3 ml of 0.1N HCl into 10 ml volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 micron syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette out 3 ml of the above solution into a 10ml volumetric and add 3ml of 0.1N NaOH into 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 micron syringe filters and place in vials.

Oxidative Degradation

Pipette out 3 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 micron syringe filters and place in vials.

Thermally induced degradation

Glecaprevir and Pibrentasvire sample was taken in a petri dish and kept in Hot air oven at 110^0 C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

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Table 10: Results for stability of glecaprevir and pibrentasvir

Sample Name	Glecaprevir		Pibrentasvir	
	Area	% Degraded	Area	% Degraded
Standard	480497		240280	
Acid	446911	6.99	230160	4.21
Base	453105	5.70	222491	7.40
Peroxide	427335	11.06	213084	11.32
Thermal	421312	12.32	207446	13.66

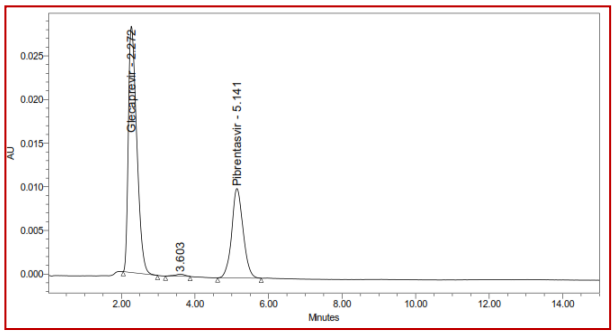

Figure 16: Chromatogram showing Acid degradation

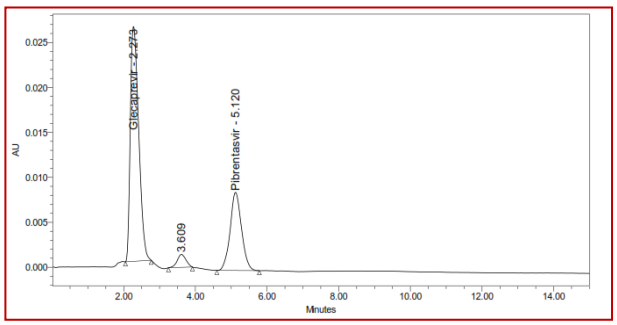

Figure 17: Chromatogram showing Base degradation

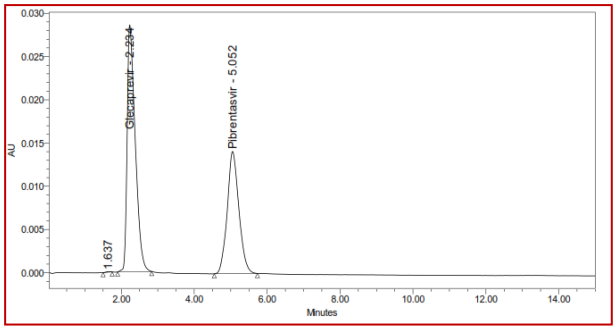

Figure 18: Chromatogram showing Peroxide degradation

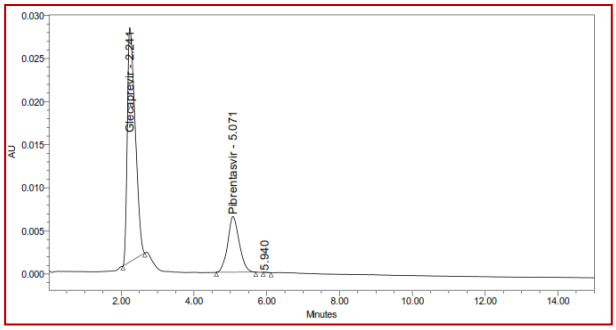

Figure 19: Chromatogram showing Thermal degradation

Results and Discussion

The HPLC method developed is a sensitive, precise and accurate for the analysis of glecaprevir and pibrentasvir in bulk drug and in pharmaceutical dosage forms. In order to affect the analysis of the component peaks, mixtures of acetonitrile, water, potassium dihydrogen orthophosphate, ammonium acetate buffer, methanol and mixed phosphate buffer in different combinations were tested as mobile phase on an Xterra C18 column (4.6 x

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150mm, 5 (m) stationary phase. A buffer and methanol in a proportion of 30:70 v/v was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention time obtained for glecaprevir and pibrentasvir was 2.205 min and 4.996 min respectively. Each of the samples was injected six times and the same retention times were observed in all cases. A good linear relationship (r^2=0.999) was observed between the concentration of glecaprevir and the respective peak areas and r^2=0.999 for concentration of pibrentasvir and the respective peak areas, which shows that the method is capable of producing good sensitivity. The regression curve was constructed by linear regression fitting and its mathematical expression was y = 1605.x + 2605 for glecaprevir, y = 2021x + 1237 for pibrentasvir. The regression characteristics are given in Table 3. When glecaprevir and pibrentasvir was analyzed by the proposed method for finding out intra and inter-day variations, low coefficient of variation was observed. The RSD is less than 2.0%. The precision values found 0.6 and 0.5 for glecaprevir and pibrentasvir, which shows that the method is precise.

The RSD values for intermediate precision is less than 2.0% and the values found 0.6 and 0.2 for glecaprevir and pibrentasvir, which shows that the method is repeatable when performed on different days also.

High recovery values obtained from the different dosage forms by the proposed method indicate the method is accurate. The absence of additional peaks indicates non-interference of common excipients used in the tablets. The total recovery was found to be 100.40% and 100.25% for glecaprevir and pibrentasvir. The validation of the developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The drug content in tablets was quantified using the proposed analytical method. The tablets were found to contain an average of 100.83 and 100.23 % of the labeled amount of the drug showed in table 6. The low coefficient of variation indicates the reproducibility of the assay of glecaprevir and pibrentasvir in dosage forms.

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The deliberate changes in the method did not much affect the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust. The lowest values of limits of detection (LOD) and limit of quantification (LOQ) for glecaprevir and pibrentasvir were found to be 2.98 and 10µg/ml and 3µg/ml and 9.98µg/ml respectively obtained by the proposed method indicate the method is sensitive. The values showed in table 7. The standard solution of the drug was stable up to 24 hours as the difference in percent assay is within limits. The LOD and LOQ for glecaprevir was found to be 2.98 and 10.00 and LOD and LOQ for pibrentasvir was found to be 3.00 and 9.98. The robustness limit for mobile phase variation and flow rate variation are well within the limit, the % degradation results are in limits. System suitability parameters were in the acceptable limits. Hence, the author concludes that the proposed HPLC method is sensitive and reproducible for the analysis of glecaprevir and pibrentasvir in pharmaceutical dosage forms with short analysis time.

Conclusion: From this study, it is concluded that the proposed method development and validation of glecaprevir and pibrentasvir by RP-HPLC method was found to be simple, sensitive, rapid, economical and useful for routine analysis of glecaprevir and pibrentasvir in bulk and its pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

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References

1) International Nonproprietary Names for Pharmaceutical Substances. Recommended International Nonproprietary Names: List 76. W.H.O. p. 503. Retrieved 25 February 2017.
2) Lawitz EJ, O'Riordan WD, Asatryan A, Freilich BL, Box TD\, Overcash JS, Lovell S, Ng TI, Liu W, Campbell\ A, Lin CW, Yao B and Kort J. Potent Antiviral Activities of the direct-acting antivirals ABT-493 and ABT-530 with three-day monotherapy for hepatitis C Virus Genotype 1 Infection. Antimicrobial Agents and Chemotherapy. 60 (3): 1546–55, 2015.
3) Forns, X, Lee, SS, Valdes, J. Glecaprevir plus Pibrentasvir for chronic hepatitis C virus genotype 1, 2, 4, 5, or 6 infection in adults with compensated cirrhosis (Expedition-1): a single-arm, open-label, multicentre phase 3 trial. Lancet Infect Dis.; 17: 1062–1068, 2017.
4) K. Hemalatha, C.Kistayya, N.D.Nizamuddhin, D. Dastiagiriamma. Simultaneous estimation of new analytical method development and validation of Glecaprevir and pibrentasvir by high performance liquid chromatography. Innovat international Journal of Med & Pharma Sci, vol 3 (supply1):5-8, 2018.
5) Mohammad T Zzaman. HPLC method development and validation for pharmaceutical analysis- A Review; Int pharma Scienica, 2(3): 13-21, 2012.
6) Hokanson GC. A life cycle approach to the validation of analytical methods during pharmaceutical product development; Int Pharm Tech, 118-30, 1994.
7) Hokanson GC. A life cycle approach to the validation of analytical methods during pharmaceutical product development; Int Pharm Tech, 92-100. 1994.