Research Article

RP-HPLC Method Development and Validation of The Simultaneous Estimation of Glecaprevir and Pibrentasvr in Bulk and Dosage Forms

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ABSTRACT

A Simple, Accurate, Precise method was developed for the simultaneous estimation of the Glecaprevir and Pibrentasvir in Tablet dosage form. Chromatogram was run through Inertsil C18 150 x 4.6 mm, 5m. Mobile phase containing Buffer 0.01N KH_2PO_4: Acetonitrile taken in the ratio 60:40 was pumped through the column at a flow rate of 1 ml/min. The buffer used in this method was 0.01 NKH_2PO_4 buffer. The optimized wavelength selected was 260 nm. The retention time of Glecaprevir and Pibrentasvir was found to be 2.509 min and 3.111 min. %RSD of the Glecaprevir and Pibrentasvir were found to be 0.2 and 0.6 respectively. %Recovery was obtained as 99.47% and 99.57% for Glecaprevir and Pibrentasvir respectively. LOD and LOQ values of Glecaprevir and Pibrentasvir were 0.36, 1.0,8, and 0.16, 0.48 respectively. Retention times were decreased, so the method developed was simple and economical and can be adopted in regular Quality control tests in Industries.

Keywords: Glecaprevir, Pibrentasvir, RP-HPLC, method development, analytical validation.

INTRODUCTION

Glecaprevir is a direct-acting antiviral agent and Hepatitis C virus (HCV) NS3/4A protease inhibitor that targets viral RNA replication. In combination with Pibrentasvir, glecaprevir is a useful therapy for patients who experienced therapeutic failure from other NS3/4A protease inhibitors. The combinations of amino acid substitutions at NS3 position Y65H and D/Q168 also result in greater reductions in glecaprevir susceptibility, and NS3 Q80R in genotype 3a patients also leads to glecaprevir resistance.

Glecaprevir is available as an oral combination therapy with Pibrentasvir under the brand name Mavyret. This fixed-dose combination therapy was FDA-approved in August 2017 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 [2]. Mavyret is also indicated for HCV genotype 1-infected patients who have been previously treated with regimens either containing an NS5A inhibitor or an NS3/4A protease inhibitor, but not both [2].

MATERIALS AND METHODS

Materials:

Glecaprevir and Pibrentasvir, Combination of Glecaprevir and Pibrentasvir (**Mavyret**) tablet dosage forms, distilled water, Acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydro furan, tri ethyl amine, ortho-phosphoric acid etc.

Instrumentation:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto-Injector and PDA Detector. The software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance for Glecaprevir and Pibrentasvir solutions.

Chromatographic conditions:

The method was established using an Inertsil C18 measuring 150 x 4.6 mm with a particle size of 5µ. The mobile phase was composed of a mixture of Buffer 0.01N KH_2PO_4 and Acetonitrile taken in the ratio of 60:40v/v. The temperature was maintained at 30°C.

The optimized wavelength for detecting Glecaprevir and Pibrentasvir was set at 260nm. the mobile phase was delivered at a flow rate of 1ml/min, and the column temperature was held at 300c. under these specified conditions, the total run time for the chromatograms was consistently set at 6 minutes [3].

METHODS

Preparation of buffer:

Buffer: (0.1% OPA): Accurately weigh 1ml of OPA in a 1000ml clean and dried Volumetric flask. Add about 900ml of milli-Q water and degassed to sonicate and finally make up the volume with water.

0.01N KH_2PO_4 Buffer: Accurately weigh 1.36gm of Potassium dihydrogen Ortho phosphate into a 1000ml Volumetric flask. Add about 900ml of milli-Q water and sonicate for uniform mixing and make up the volume with water. Adjust the pH to 5.4 with a dilute Orthophosphoric acid solution.

Standard Preparation:

Accurately Weigh and transfer 25mg of Glecaprevir and 10mg of Pibrentasvir working Standards into a 50ml clean dry volumetric flask, add 3/4th volume of diluent, sonicate for 5 minutes, and makeup to the final volume with diluents.

1ml from the above stock solution was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation: 20 tablets were weighed accurately and the average weight was computed.

The weight equivalent to the tablet was transferred into a 100mL volumetric flask, and 50mL of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered.

From the filtered solution 0.5 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent.

METHOD VALIDATION

System Suitability Parameters

The system suitability parameters were determined by preparing standard solutions of Glecaprevir (50ppm) and Pibrentasvir (20ppm) the solutions were injected six times and the parameters like peak tailing, resolution, and USP plate count were determined.

The % RSD for the area of six standard injection results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard solutions: Accurately weigh 25mg of Glecaprevir and 10mg of Pibrentasvir and transfer to 50ml volumetric flasks 3/4 th of diluents was added to this flask and sonicated for 10 minutes. Flasks were made up of diluents and labeled as Standard stock solutions. (500µg/ml of Glecaprevir and 200µg/ml Pibrentasvir).

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with a diluent. (50µg/ml of Glecaprevir and 20µg/ml of Pibrentasvir)

Linearity: Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of Glecaprevir and Pibrentasvir are taken into 6 different volumetric flasks and diluted to 10ml with diluents to get 12.5ppm, 25ppm, 37.5ppm, 50ppm, 62.5ppm, 75ppm of Glecaprevir, and 5ppm, 10ppm, 15ppm, 20ppm, 25ppm,30ppm of Pibrentasvir.

Accuracy:

0.25ml, 0.5ml, **and** 0.75ml of sample stock solution were taken into a 10ml volumetric flask, and 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent to obtain 50%,100%, and 150% spiked solutions respectively.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102

Robustness:

Small deliberate changes in a method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized changes in the result, and they are within range as per ICH Guidelines.

All the robustness conditions were maintained and system suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Glecaprevir and Pibrentasvir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluent. From the above solutions 0.3ml each of Glecaprevir and Pibrentasvir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies:

Oxidation: To 1 ml of stock solution of Glecaprevir and Pibrentasvir, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 minutes at 60^0c. For the HPLC study, the resultant solution was diluted to obtain 50µg/ml&20µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Acid Degradation Studies: To 1 ml of stock solution of Glecaprevir and Pibrentasvir, 1 ml of 2N Hydrochloric acid was added and refluxed for 30 minutes at 60^0c. The resultant solution was diluted to obtain 50µg/ml&20µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation Studies: To 1 ml of stock solution of Glecaprevir and Pibrentasvir, 1 ml of 2N sodium hydroxide was added and refluxed for 30 minutes at 60^0c. The resultant solution was diluted to obtain 50µg/ml&20µg/ml solution 10 µl was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Dry Heat Degradation Studies: The standard drug solution was placed in an oven at 105°C for 6 h to study dry heat degradation. For the HPLC study, the resultant solution was diluted to 50µg/ml&20µg/ml solution and10µl was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 500µg/ml& and 200µg/ml solution to UV Light by keeping the beaker in a UV Chamber for 1 day or 200Watt hours/m^2 in a photostability chamber. For the HPLC study, the resultant solution was diluted to obtain 50µg/ml & 20µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hr at a temperature of 60º. For the HPLC study, the resultant solution was diluted to 50µg/ml&20µg/ml solution 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table: 1 System suitability parameters for Glecaprevir and Pibrentasvir

S no	Glecaprevir			Pibrentasvir			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	USP Resolution
1	2.497	7701	1.31	3.105	8059	1.34	4.7
2	2.499	7943	1.29	3.109	8087	1.31	4.7
3	2.499	8095	1.30	3.113	8154	1.29	4.7
4	2.501	8207	1.30	3.114	7772	1.29	4.7
5	2.502	8143	1.30	3.115	7856	1.29	4.6
6	2.503	8083	1.35	3.117	7393	1.29	4.6

Specificity:

Retention times of Glecaprevir and Pibrentasvir were 2.499 min and 3.105 min respectively. We did not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

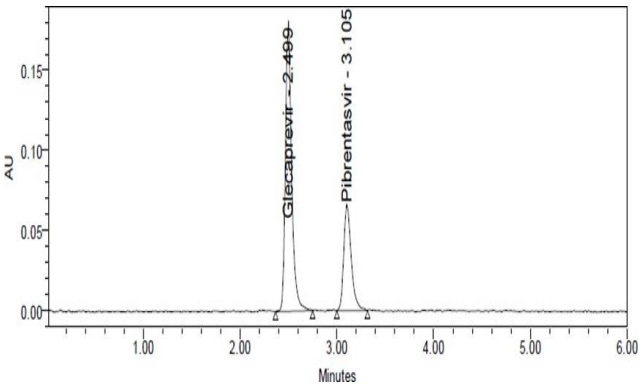

Figure 1: Optimized chromatogram

Linearity:

Six linear concentrations of Glecaprevir (125-75µg/ml) and Pibrentasvir (5-30µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Glecaprevir was y = 16343x + 4225.4 and of Pibrentasvir was y = 17496.x + 1373 The Correlation coefficient obtained was 0.999 for the two drugs.

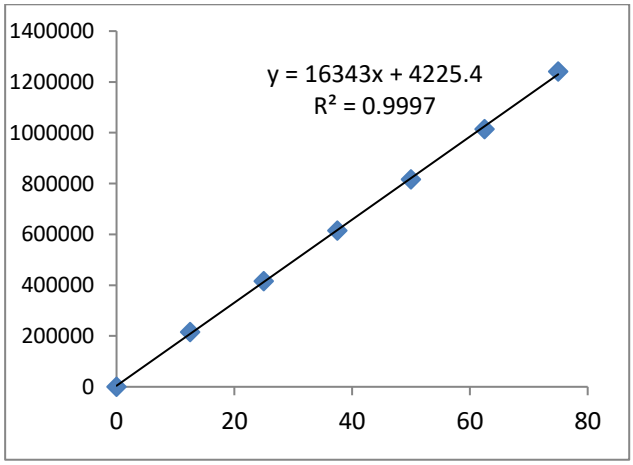

Figure 2: Calibration curve of Glecaprevir

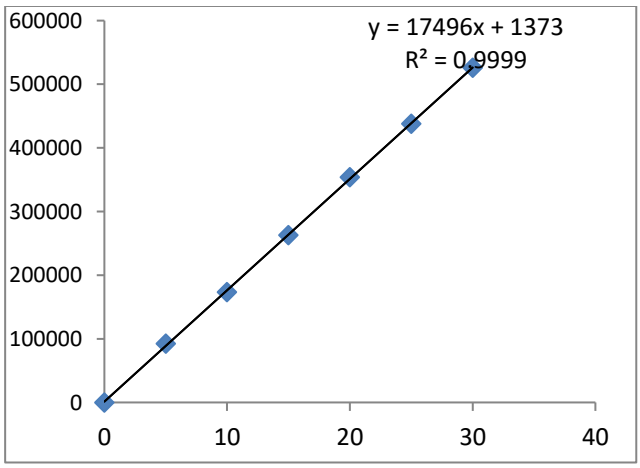

Figure 3: Calibration curve of Pibrentasvir

Precision:

System Precision: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation, and % RSD were calculated for two drugs. % RSD obtained as 0.8% and 0.7% respectively for Glecaprevir and Pibrentasvir. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day-Day Precision): Multiple sampling from a sample stock solution was done and six working sample solutions of the same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and the obtained areas were mentioned in the above table. Average area, standard deviation, and % RSD were calculated for two drugs and obtained as 0.4% and 0.9% respectively for Glecaprevir and Pibrentasvir. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy:

Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 98.47% and 99.57% for Glecaprevir and Pibrentasvir respectively.

Table 2: Accuracy table of Glecaprevir and Pibrentasvir

% Level	Amount Spiked (µg/mL)		Amount recovered (µg/mL)		% Recovery		% Recovery Mean	
	GLE	PIB	GLE	PIB	GLE	PIB	GLE	PIB
50%	25	10	25.156642	10.003	100.63	100.03	100.24	99.38
			25.061372	9.927	100.25	99.27		
			24.961329	9.884	99.85	98.84		
100%	50	20	49.400477	19.926	98.80	99.63	99.25	99.79
			49.519427	19.934	99.04	99.67		
			49.952946	20.015	99.91	100.07		
150%	75	30	73.787065	29.928	98.38	99.76	98.92	99.5
			74.369516	29.694	99.16	98.98		
			74.418589	29.957	99.22	99.86		

Robustness:

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in a duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 3: Robustness data for Glecaprevir and Pibrentasvir.

S.no	Condition	%RSD of Glecaprevir	%RSD of Pibrentasvir
1	Flow rate (-) 1.1ml/min	1.2	0.7
2	Flow rate (+) 1.3ml/min	0.5	1.8
3	Mobile phase (-) 35B:65A	0.9	1.2
4	Mobile phase (+) 45B:55A	1.5	0.3
5	Temperature (-) 25°C	1.5	0.1
6	Temperature (+) 35°C	0.4	0.8

Sensitivity:

Table 4: Sensitivity table of Glecaprevir and Pibrentasvir

Molecule	LOD	LOQ
Glecaprevir	0.36	1.08
Pibrentasvir	0.16	0.48

Assay:

Rhodes Pharmaceuticals claims to have GLE 7.5 mg and PIB 325 mg on their label. The assay was conducted using the formulation mentioned above, and the average percentage of assay obtained for PIB and GLE was 99.63% and 100.1%, respectively.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Glecaprevir and Pibrentasvir in Tablet dosage form. The retention time of Glecaprevir and Pibrentasvir was found to be 2.509 min and 3.111 min. %RSD of the Glecaprevir and Pibrentasvir were found to be 0.2 and 0.6 respectively. %Recovery was obtained as 99.47% and 99.57% for Glecaprevir and Pibrentasvir respectively. LOD and LOQ values obtained from regression equations of Glecaprevir and Pibrentasvir were 0.36, 1.08, and 0.16, 0.48 respectively. The regression equation of Glecaprevir is y = 16343.x + 4225, y = 17496.x + 1373 of Pibrentasvir. Retention times were decreased and that run time was decreased, so the method developed was simple and economical and can be adopted in regular Quality control tests in Industries.

Table 5: Assay Data of Glecaprevir and Pibrentasvir

S.no	Standard Area		Sample area		% Assay	
	Glecaprevir	Pibrentasvir	Glecaprevir	Pibrentasvir	Glecaprevir	Pibrentasvir
1	819660	357522	817240	359069	99.96	100.76
2	802447	358068	815816	354556	99.79	99.49
3	817777	353334	815109	354727	99.70	99.54
4	818341	358544	818350	353104	100.10	99.08
5	810782	353754	819426	353696	100.23	99.25
6	816781	352707	819848	355047	100.28	99.63
Avg	814298	355655	817632	355033	100.01	99.63
Standard deviation	6575.3	2658.8	1921.5	2103.3	0.24	0.6
%RSD	0.8	0.7	0.2	0.6	0.2	0.6

Degradation:

Table 6: Degradation Data of Glecaprevir

S.NO	Degradation Condition	%Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.38	1.565	2.215
2	Alkali	4.17	1.373	1.627
3	Oxidation	3.74	1.221	1.465
4	Thermal	2.92	1.619	2.361
5	UV	1.01	1.176	1.433
6	Water	1.01	1.143	1.404

Table 7: Degradation Data of Pibrentasvir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.38	2.902	4.043
2	Alkali	3.84	2.310	2.587
3	Oxidation	3.51	3.191	2.694
4	Thermal	2.25	3.400	4.561
5	UV	1.11	2.333	2.657
6	Water	0.76	3.139	2.517

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