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A New RP-hplc method for Simultaneous Estimation of Glecaprevir and Pibrentasvir in Its Pure and Pharmaceutical Dosage Form

Ayesha begum K*, Syeda Naseem Sultana

Department of Pharmaceutical Analysis, Shadan Women's College of Pharmacy, Khairatabad, Hyderabad, India

***Corresponding author: Ayesha begum K**
Email: ayeshabegumk@mail.com

ABSTRACT

An efficient and simple HPLC method has been developed and validated for the simultaneous determination of glecaprevir and pibrentasvir in bulk and was applied on marketed glecaprevir and pibrentasvir products. The mobile phase used for the chromatographic runs consisted of phosphate buffer (pH 3.0) and acetonitrile (80:20, v/v) The separation was achieved on an Inertsil ODS (4.6 x 250mm, 5µ) column using isocratic mode. Drug peaks were well separated and were detected by a UV detector at 230 nm. The method was linear at the concentration range 50–125 µg/ml for glecaprevir and 10–50 µg/ml for pibrentasvir respectively. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness.

Keywords: Glecaprevir and Pibrentasvir, Validation, stability indicating method, degradation products.

INTRODUCTION

Glecaprevir is a direct acting antiviral agent and Hepatitis C virus (HCV) NS3/4A protease inhibitor that targets the viral RNA replication[1, 2]. 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.[2] 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.[3-5] The combinations of amino acid substitutions at NS3 position Y65H and D/Q168 also results in greater reductions in glecaprevir susceptibility, and NS3 Q80R in genotype 3a patients also leads to glecaprevir resistance.[6,7] Glecaprevir is a white to off-white crystalline powder with a solubility of less than 0.1 to 0.3 mg/mL across a pH range of 2–7 at 37°C and is practically insoluble in water, but is sparingly soluble in ethanol.[12]

Pibrentasvir is a direct acting antiviral agent and Hepatitis C virus (HCV) NS5A inhibitor that targets the viral RNA replication and viron assembly. Pibrentasvir is available as an oral combination therapy with Glecaprevir under the brand name Mavyret.[7,8] 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. NS5A is a phosphoprotein that plays an essential role in replication, assembly and maturation of infectious viral proteins.[9,10] The basal phosphorylated form of NS5A, which is maintained by C-terminal serine cluster, is key in ensuring its interaction with the viral capsid protein, or the core protein. By blocking this interaction, pibrentasvir inhibits the assembly of proteins and production

of mature HCV particles.[11] NS5A also interacts with viral and cellular proteins to form the HCV replicase complex, and supports the RNA replication of HCV. Pibrentasvir is a white to off-white to light yellow crystalline powder with a solubility of less than 0.1 mg/mL across a pH range of 1–7 at 37°C and is practically insoluble in water, but is freely soluble in ethanol.[12]

Only few methods were reported for the simultaneous estimation of glecaprevir and pibrentasvir by HPLC.[13-15] Hence we had made an attempt to develop a simple, accurate and precise RP-HPLC method for the simultaneous estimation of glecaprevir and pibrentasvir.(Fig1&2)

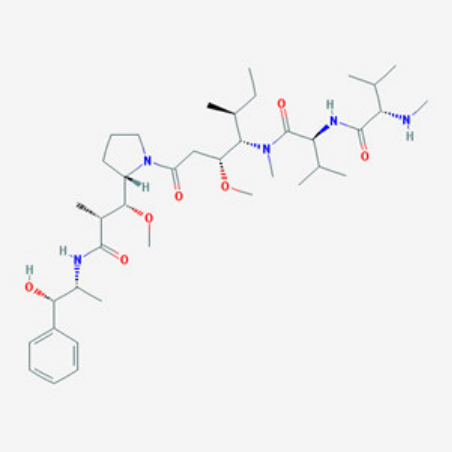

Fig.1 Glecaprevir

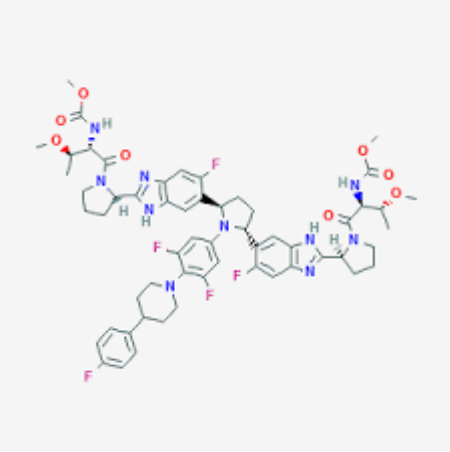

Fig.2 Pibrentasvir

MATERIALS AND METHODS

Gift samples of glecaprevir and pibrentasvir were received from pharmatrain lab, Hyderabad, whereas water, methanol for HPLC, acetonitrilefor HPLCand ortho phosphoric acid were purchased from LICHROSOLV, Merck.

Instrumentation: Waters HPLC (2695 separation module) was used for the separation of tolcapone and quinapril. UV/VIS spectrophotometer (LABINDIA UV 12.500^{+}) was used for detection. Instruments such as; pH meter used was of Adwa — AD 10100 and weighing machine was of Afcoset ER-1000A.

Preparation of buffer and mobile phase:
Pipette 1ml OPA in 1000 ml of HPLC water Ph was balanced up to 3.0.final arrangement was sifted through 0.44 µm Layer channel and sonicates it for 10 mins.

Preparation of versatile phase: Accurately measured 800 ml (80%) of over buffer and 200 ml of Acetonitrile HPLC (20%) were blended and degassed in an ultrasonic water shower for 10 minutes and after that sifted through 0.45 µ channel beneath vacuum filtration. Diluent Preparation: The Portable stage was utilized as the diluent.

Standard Solution Preparation: Precisely weigh and exchange 25mg of Glecaprevir and 10 mg of Pibrentasvir working standard into a 100 ml clean dry volumetric jar include almost 7 mL of Diluent and sonicate to break down it totally and make volume up to the check with the same dissolvable. (Stock solution) Further pipette 3 ml of the over stock arrangements into a 10ml volumetric carafe and weaken up to the stamp with diluent.

Sample Solution Preparation: Precisely weigh 10 tablets smash in mortar and pestle and exchange proportionate to 25 mg of Glecaprevir and 10 mg Pibrentasvir test into a 100 mL clean dry volumetric carafe include approximately 7 mL of Diluent and sonicate it up to 15 mins to break up it totally and make volume up to the check with the same dissolvable. At that point it is sifted through 0.45 micron Infusion channel. (Stock solution) Further pipette 3ml of glecaprevir and pibrentasvir from the over stock arrangement into a 10ml volumetric jar and weaken up to the stamp with diluent.

Method development and optimisation: Due to the significant difference in the physical and chemical properties of glecaprevir and pibrentasvir, several mobile phases and columns were initially trailed in order to have both eluents on the same chromatogram. The suitability of the column and the mobile phase used in the optimized method have been decided based upon the basis of the selectivity, sensitivity as well as acceptable chromatographic parameters of the produced peaks in terms of peak sharpness, peak symmetry, tailing factor and resolution between the two peaks. We used the mobile phase as a solvent for all samples to ensure minimum noise and to eliminate any unwanted solvent peaks.

RESULTS AND DISCUSSION

Method Validation: The optimized method for simultaneous determination of glecaprevir and pibrentasvir has been validated as per International Conference of Harmonisation (ICH) guidelines Q2 (R1) [26] for evaluating system suitability, specificity, precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.[16]

Optimized chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient
Column	:	Inertsil ODS (4.6 x 250mm, 5µm)
Buffer	:	0.1% OPA
pH	:	3.0
Mobile phase	:	80% buffer 20% Acetonitrile
Flow rate	:	1 ml per min
Wavelength	:	230 nm
Injection volume	:	20 µl
Run time	:	10 min.

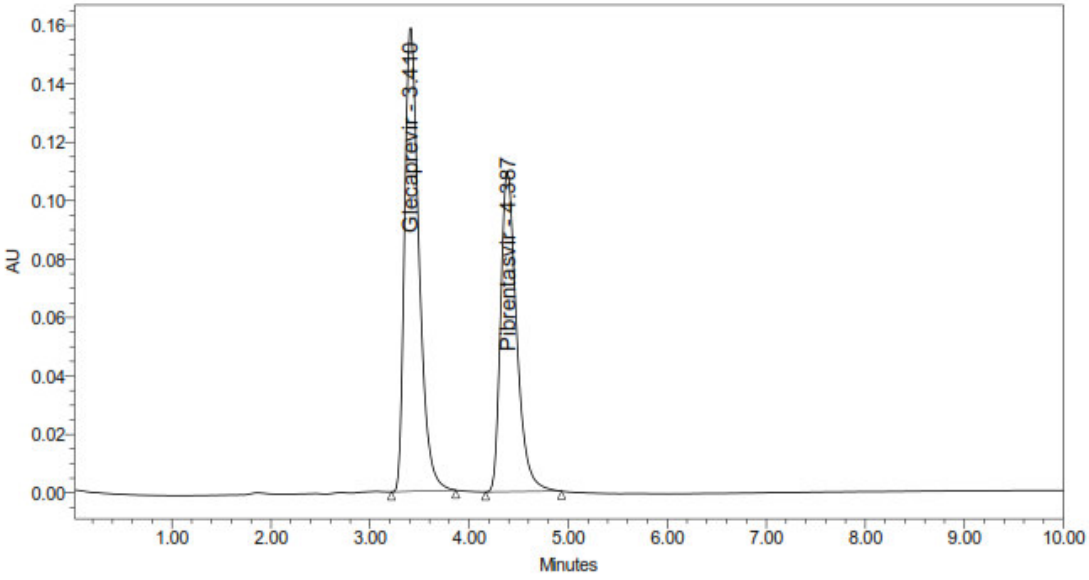

Fig. 3: Standard Chromatogram of Glecaprevir and Pibrentasvir

System Suitability: Following figure 3 for the crests due to Glecaprevir and Pibrentasvir in Standard arrangement ought to not be less than 2000. Resolution for the Glecaprevir and Pibrentasvir crests in standard arrangement ought to not be less than 2. **(**Table 1)

Table 1: Results of system suitability parameters

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Glecaprevir	3.410	1608392	159104		1.42	2657.20
2	Pibrentasvir	4.387	1226854	109856	3.52	1.40	3669.74

Linearity: The standard stock solution of Glecaprevir is diluted in the concentration range of (25–125 µg/ml). Triplicates of such concentration range were prepared and plotted on a metformin calibration curve.The standard stock solution of Pibrentasvir is diluted in the concentration range of (10–50 µg/ml). Triplicates of such concentration range were prepared and plotted on a gliclazide calibration curve. Slope, intercept and correlation coefficient of the calibration curves (peak area versus concentration) were determined to ensure linearity of the analytical method. (Table 2)

Table 2: Results of Linearity of Glecaprevir and Pibrentasvir

S. No.	Glecaprevir		Pibrentasvir	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	25	524876	10	380761
2	50	1059982	20	782401
3	75	1574201	30	1164038
4	100	2068062	40	1549472
5	125	2604868	50	1965315

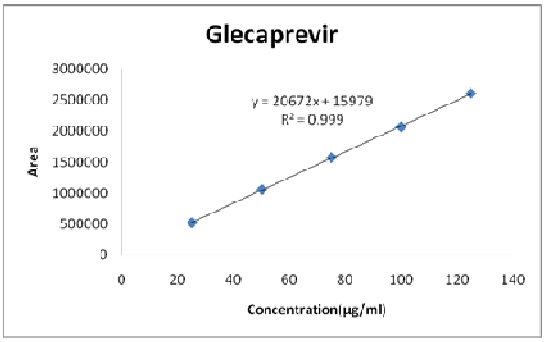

Fig 4: Calibration graph for Glecaprevir

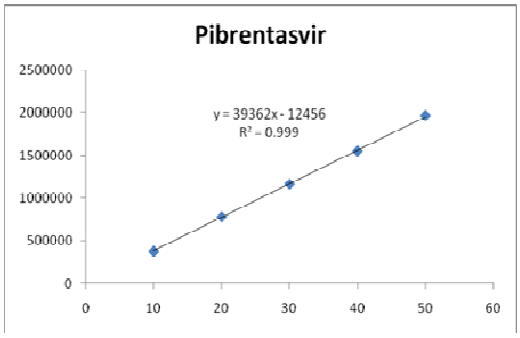

Fig 5: Calibration graph for Pibrentasvir

Table 3: Results of Precision for Glecaprevir

Injection	Area
Injection-1	1610934
Injection-2	1609985
Injection-3	1619309
Injection-4	1608645
Injection-5	1610885
Injection-6	1618951
Average	1613118.2
Standard Deviation	4731.4
%RSD	0.3

Table 4: Results of Precision for Pibrentasvir

Injection	Area
Injection-1	1228406
Injection-2	1223300
Injection-3	1213803
Injection-4	1201667
Injection-5	1228897
Injection-6	1220372
Average	1219407.5
Standard Deviation	10327.1
%RSD	0.8

Accuracy: Accuracy of the proposed method was confirmed with glecaprevir and pibrentasvir separately at 3 different levels 50%, 100% and 150%, the determinations of these 3 levels have been recorded to obtain the mean and % recovery.

Table 5: Accuracy (recovery) data for Glecaprevir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	809552.3	12.5	12.60	100.82	
100%	1611682	25	25.09	100.36	100.39
150%	2408440.7	37.5	37.49	99.98	

*Average of three determinations

Table 6: Accuracy (recovery) data for Pibrentasvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	617877.7	5	5.04	100.75	
100%	1224225.3	10	9.98	99.81	100.04
150%	1831657.7	15	14.93	99.55	

*Average of three determinations

LOD and LOQ

The LOD and LOQ arrangements was arranged infused, for three times and measured the region for all three infusions in HPLC. The %RSD for the zone of six reproduce infusions was found to be inside the required limits.

Table 7: Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Glecaprevir	52	160	3.08
Pibrentasvir	52	156	3.00

Table 8: Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Glecaprevir	52	525	10.10
Pibrentasvir	52	525	10.02

Degradation Studies

The ICH entitled solidness testing of modern sedate substances and items requires that stretch testing be carried out to illustrate the characteristic soundness characteristics of the dynamic substance. The point of this work was to perform the stretch debasement considers on the Glecaprevir and Pibrentasvir utilizing the proposed strategy. (Fig6, 7, 8, 9&10)

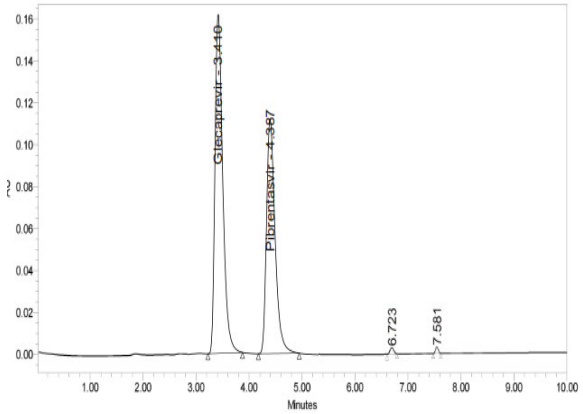

Fig 6: Acid degradation Chromatogram

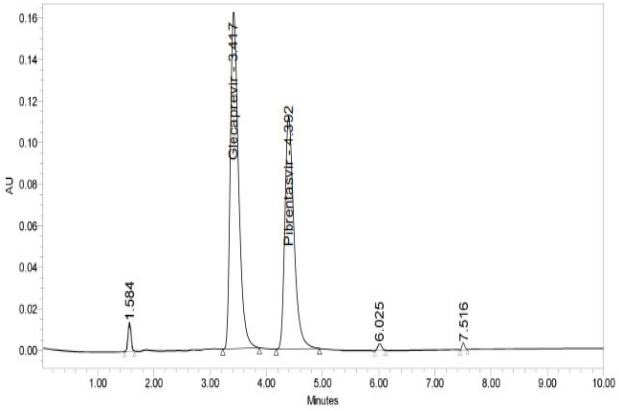

Fig 7: Base degradation Chromatogram

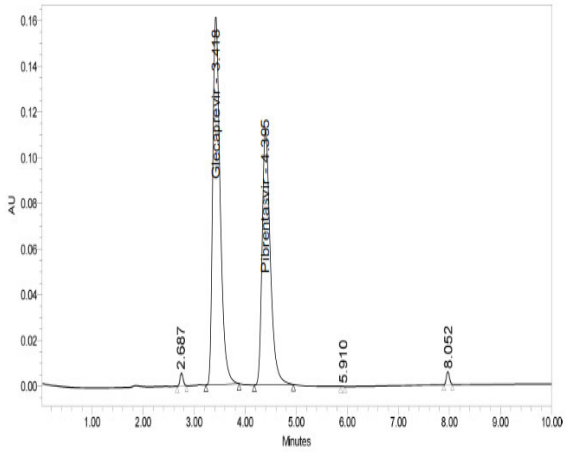

Fig8: Peroxide degradation Chromatogram

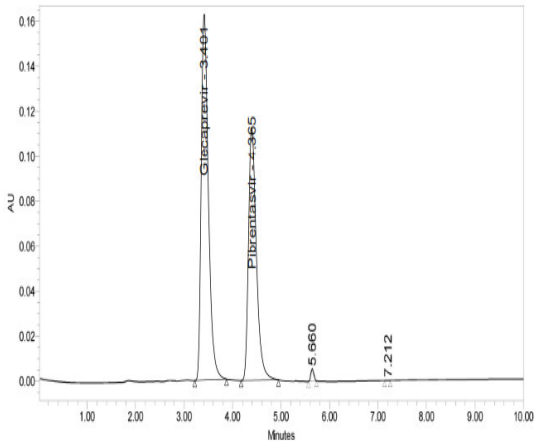

Fig 9: Thermal degradation Chromatogram

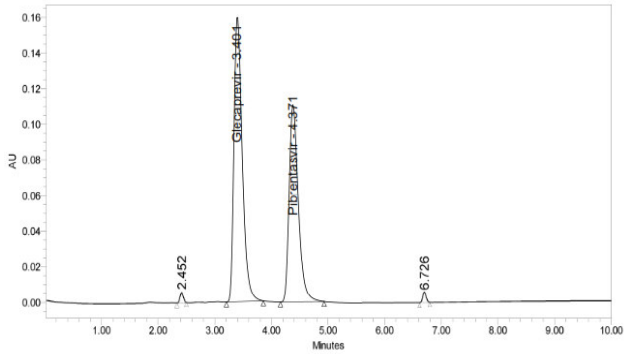

Fig10: Photo degradationChromatogram

Table 9: Results for Stability of Glecaprevir andPibrentasvir

Sample Name	Glecaprevir		Pibrentasvir	
	Area	% Degraded	Area	% Degraded
Standard	1602702		1224118	
Acid	1583722	1.18	1207822	1.33
Base	1528333	4.64	1173832	4.11
Peroxide	1558673	2.75	1146223	6.36
Thermal	1492533	6.87	1196732	2.24
Photo	1509356	5.82	1127897	7.86

Table 10: Results of Assay for Glecaprevir and Pibrentasvir

	Label Claim (mg)	% Assay
Glecaprevir	100	100.08
Pibrentasvir	40	99.94

CONCLUSION

The presented validated method is rapid, economic, simple, accurate, sensitive, robust, specific and linear. It can be used for routine analysis of glecaprevir and pibrentasvir in combination products.

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