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A NOVEL STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PIBRENTASVIR AND GLECAPREVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Development and validation of RP-HPLC method for the simultaneous determination of Glecaprevir & Pibrentasvir in pure form and their combined tablet dosage forms. The current separation was achieved on a Chromasil ODS Column (4.6 x 250mm, 5.0 µ) using the composition of the mobile phase of (0.1 M KH_2PO_4 buffer: methanol) in the ratio of (60:40) at pH 4.5. The pump flow rate was adjusted after several one milliliter per minute and both the samples were separated at 230nm. The retention times are 1.7 min and 2.3 min, for Glecaprevir and Pibrentasvir respectively with linear ranges 20 µg/ml- 60 µg/ml and 50 µg/ml-150 µg/ml. The method was statistically validated for linearity, accuracy, precision and selectivity as per ICH guidelines. The drugs were subjected to stress conditions of hydrolysis, oxidation, photolysis of the RP-HPLC method. Both medications were tested under various stress forced conditions, including alkali, acid, oxidative, photodegradation, & hydrolysis degradation.

The accuracy limit is the percentage recovery should be in the range of 98.0% - 101.0%. The total recovery was found to be 99.69% and 99.39% for Glecaprevir and Pibrentasvir. The validation of 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 robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Keywords: Glecaprevir, Pibrentasvir, RP-HPLC Method, Isocratic Elution, ICH Guidelines, Photo Diode Array (PDA) Detection, Degradation Studies;

RASĀYAN *J. Chem.*, Vol. 16, No. 3, 2023

INTRODUCTION

Glecaprevir[1]

It is used as an antiviral drug and that specifically targets viral RNA replication. Glecaprevir is an effective treatment for patients who fail to respond to other NS3/4A protease inhibitors when combined with pibrentasvir. It shows that there is a strong genetic barrier to virus-resistance mutations. In HCV genotype 1a, 2a, or 3a replicons, the development of amino acid changes at NS3 resistance-associated sites A156 or D/Q168 resulted in decreased sensitivity to glecaprevir.[1,2] Greater reductions in glecaprevir susceptibility have also been shown with the combination of amino acid changes at NS3 position Y65H and D/Q168, and NS3 Q80R in genotype 3a patients also results in glecaprevir resistance2. Hepatitis C is treated with the direct-acting antiviral drug pibrentasvir.[3]

According to the literature, a few spectroscopic,[5–9] chromatographic methods[10–12] forced degradation/stability indicators, bio-analytical techniques, and plasma extraction methods. It has been reported for the determination of these drugs alone or in combination with other drugs in pharmaceutical dosage forms. With the combination of the aforementioned medications, a few HPLC[13-15] techniques are available, some of which have a narrower linearity range or longer retention durations. The author developed and validated a cost-effective RP-HPLC test technique for estimating glecaprevir and pibrentasvir from formulated dosage forms. The current research method was validated as per guidelines

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of USP & ICH[16–22] for a wider linearity range than other methods currently on the market and with faster retention times.

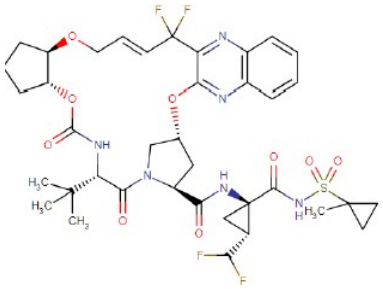


Fig.-1A: Glecaprevir

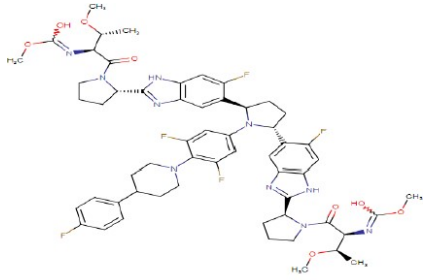


Fig.-1B: Pibrentasvir

EXPERIMENTAL

Material and Methods

Reference samples of Glecaprevir & Pibrentasvir procured from MSN Laboratories, Hyderabad. Organic solvents (HPLC grade acetonitrile and methanol) and Milli-Q –Q water were purchased from Merck Chemicals Group, Mumbai, India. MAVYRET fixed dosage forms containing 100 and 40 mg of Glecaprevir and Pibrentasvir were procured from the local market.

Instrumentation

Waters HPLC of 2996 series and PDA detector attached which have autosampler system and Hamilton syringe chosen for the current separation method. An ultrasonicator removes dissolved gases & is connected to the column oven to maintain the optimum temperature. The composition of Mobile phase 0.23 M phosphate buffer (pH5.0): acetonitrile: 60:40% ratio of solvent system adjusted flow with 1.00 ml per minute flow rate and chromatic ODS Column, 4.6 mm ×250 mm, 5µicron particle size as a column and 10 ppm of nominal concentration of sample solution was injected into the HPLC system and both the molecules were separated at 230 nanometers.

General Procedures

Preparation of Standard Stock Solution: Weighed accurately 100 and 40 milligrams of Glecaprevir and Pibrentasvir and transferred them into the 100 mL standard volumetric flasks respectively, made up with diluent. The above stock solutions were degassed for 15 min.

Preparation of Calibration Curve Standards: The linearity of both drugs performed by spiked the sample solutions made from the stock solutions in the concentration ranges from 50 - 150 ppm for Glecaprevir and 20-60 ppm for Pibrentasvir respectively as mentioned in Table-1.

Table-1: Linearity detail of Glecaprevir & Pibrentasvir

S.No.	Conc. (ppm) of Glecaprevir	Conc. (ppm) of Pibrentasvir	Peak area of Glecaprevir	Peak area of Pibrentasvir
1	50	20	450530	264089
2	80	32	730415	440362
3	100	40	919374	548514
4	120	48	1094213	665825
5	150	60	1389541	836521
Slope			9349	14281
Intercept			18125	20160
Correlation coefficient			0.9997	0.9999

Preparation of Sample Solution

Weighed accurately 20 tablets of MAVYRET containing 100 milligrams of Glecaprevir and 40 milligrams of Pibrentasvir and grounded into the fine powder form using mortar and pestle. Weighed an accurately equivalent amount of 100 mg of tablet powder and transferred into the 100 mL standard vol.

flask and the volume was adjusted with the diluents. thoroughly mixed and sonicated and this solution was membrane-filtered paper using 0.45µ membrane & pipetted out 2.0 ml of the above solution was transferred into the 10 ml standard volumetric flask and made the volume with diluent.

RESULTS AND DISCUSSION

Development of the Method

The selected combination drugs were soluble in the desired diluent to get a clear solution. Few analytical techniques were reported based on the literature review for the current research method. Optimization of the mobile phase was done by several changes in the ratio of the solvent system (buffer solution and organic solvents). The pH of the buffer solution is optimized based on the pKa values of both drugs. The good resolution of the peak resolution and shape obtained using the composition of mobile phase possess acetonitrile: 0.2 M KH_2PO_4 buffer: 60: 40% & pump flow rate adjusted 1.0 mL per minute and analyzed at 230 nanometers. Observed retention times for both drugs (3.1 for GPV and 4.94.6 for PNV). it allowed a rapid analysis of these drugs. The represented graph is shown below in Fig.-2.0

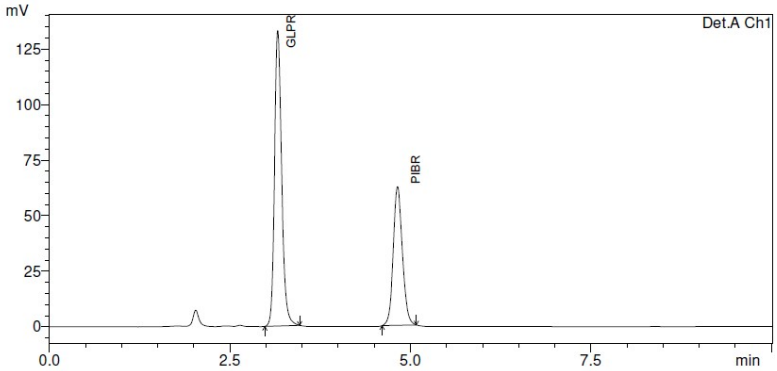


Fig.-2: Standard Chromatogram of Glecaprevir and Pibrentasvir

Validation

The system suitability parameters HETP (no. of theoretical plates), Peak area and chromatogram tailing factor, and capacity factor were validated and all the parameters were within the limits as per the ICH guidelines and mentioned tabulated data below Table-2.

Table-2: Data of System Suitability

System suitability	Glecaprevir	Pibrentasvir
Asymmetric factor /Tailing factor)	1.37	1.35
HETP	10282	35224

Specificity

The proposed research work was performed by injecting spiked and unspiked samples into the system. The results showed no impurity at the retention time of Glecaprevir and Pibrentasvir. The specificity values of the Glecaprevir and Pibrentasvir chromatograms mentioned below in Fig.-3.

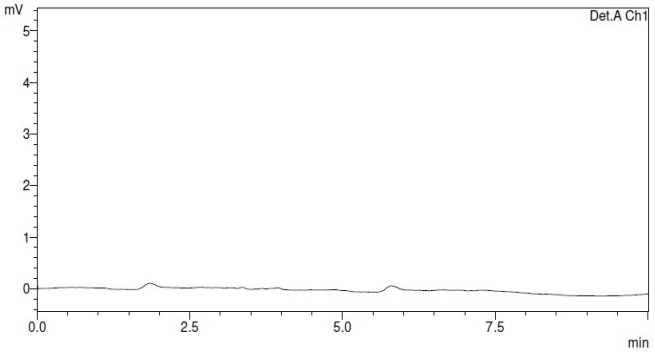


Fig.-3: Placebo Chromatogram

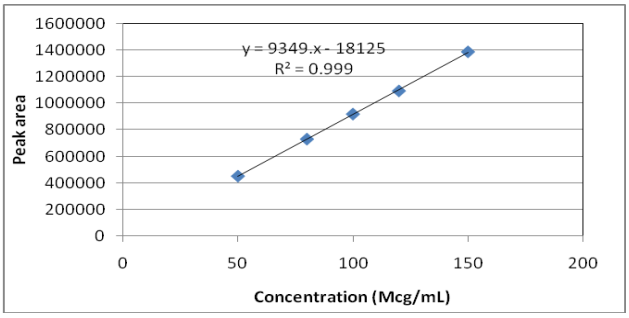


Fig.-4: Calibration Curve of Glecaprevir

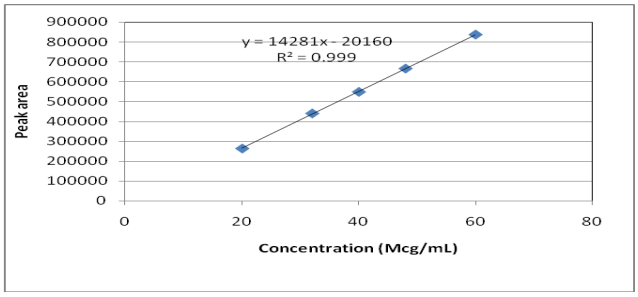


Fig.-5: Calibration Curve of Pibrentasvir

Linearity

The standard solutions of Glecaprevir (50-150 ppm) and Pibrentasvir (20-60 ppm) were prepared and injected into the HPLC system under the optimized chromatographic conditions as mentioned above. Plotted the linearity between drug concentrations vs. corresponding areas of peak achieved at 230nm and both standard drugs showed a linear response.

Accuracy Study

Drug accuracy was done by three different levels as per the test method for each spike level sample solution which consists of Glecaprevir and Pibrentasvir concentrations ranging from 50, 100, and 150 percentage levels of the label amount. The average percentage recovery of Glecaprevir and Pibrentasvir was calculated from each chromatogram and the tabulated data mentioned in Table-3.

Table-3: Data of Accuracy Studies of Glecaprevir and Pibrentasvir

	Area of peak		% Recovery	
Level (%)	GPV	PNV	GPV	PNV
50	461018	277733	100.60	99.39
	456169	271508	101.60	101.78
	454159	261903	100.05	100.84
100	907152	552544	99.89	101.39
	909748	554904	100.42	100.52
	899426	537044	101.18	100.66
150	1398530	855433	99.58	99.85
	1348013	857891	100.47	99.86
	1400913	837447	99.59	99.94
		Average	96.24	98.97
		Standard deviation	1.09	1.07
		Percentage of relative std. deviation	1.03	1.08

Repeatability Studies

Repeatability was performed by precision studies (intraday precision and interday precision). The percentage relative standard deviation values were calculated and obtained within the acceptable limits (percentage of Relative standard deviation less than 2 value) and shown in Table-4.

Robustness Study

Deliberated changes in chromatographic conditions like increasing /decreasing flow rate, temperature of column, and ratio of solvent system (composition of mobile phase). The data on the robustness of results are shown in Table-5.

Table-4: Data of Precision Study Details of Glecaprevir and Pibrentasvir

Validation Parameter	Intra-Day		Inter-Day	
	GPV	PNV	GNV	PNV
Percentage of Mean	98.79	99.99	100.01	98.79
Standard deviation (S.D)	0.51	0.23	0.87	0.33
% Relative std. deviation	0.34	0.42	0.98	0.43

Table-5: Data of Robustness

Column Temp.	Deliberated value	Rt.		Asymmetric values		Percentage of assay	
		GPV	PNV	GPV	PNV	GPV	PNV
	25	3.179	4.909	1.76	1.33	98.70	98.30
	35	3.013	4.06	1.31	1.34	99.10	99.50
Flow Rate	0.8	2.97	3.98	1.55	1.36	99.93	101.20
	1.2	4.1	4.2	1.59	1.35	100.80	100.90
Mobilephase	55:45	1.64	3.06	1.35	1.36	100.20	100.50
	65:35	1.68	4.69	1.49	1.35	99.30	99.10
Mean /value of Average						98.85	98.42
Standard deviation (S.D)						1.24	1.36
% Relative std. deviation.						1.53	1.27

Detection Limit and Quantification Limit

The current proposed research study of low conc. detection limit and limitation of quantification of GPV and PNV were estimated & calculated mentioned below.

Detection Limit and Quantification Limit Data

S.No.	GPV (ppm)	PNV (Ppm)
1.	3.23	9.03
2.	8.06	24.03

Stability Study

It was performed by the mobile phase after twenty-four hours at room temperature using the optimized chromatographic conditions. These confirmed both drugs were stable up to 2 hours and results are given inTable-6.

Table-6: Data of Stability Studies

	% values of Assay	
Drug	Percentage of Assay @ Zero hour	Percentage Assay @ 24 hours.
GPV	100.23	98.25
PNV	98.54	98.78

Assay of Marketed Formulation

The assay of the formulation was performed by quantification studies for current research work and was applied for the determination of Glecaprevir & Pibrentasvir in combined tablet dosage forms & data given below in Table-7.

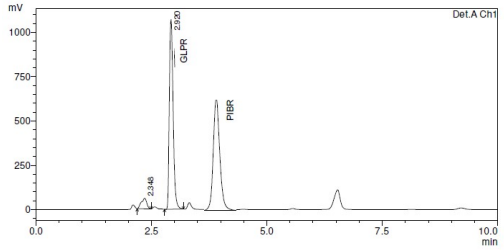
Table-7: Quantification of Tablet Dosage Form

Sample No	Percentage	
	GPV	PNV
i.	99.24	99.59
ii.	98.04	99.28
iii.	101.01	98.64
iv.	101.41	98.46
v.	99.91	100.38

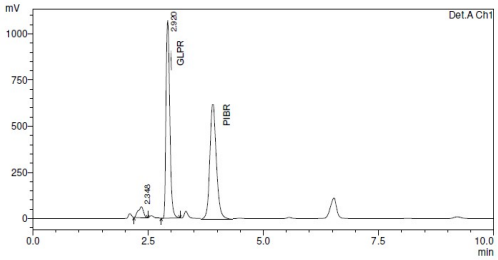
vi.	98.58	98.15
AVG	100.12	97.43
SD	0.43	0.27
%RSD	0.12	0.28

Degradation Study

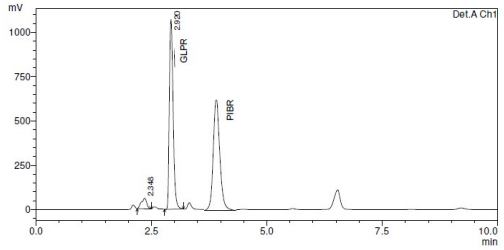
The degradation of a sample solution (Glecaprevir and Pibrentasvir) was generated by accurately weighing 100 mg of powder and transferring it into a 100 ml flask. The sample solution was then treated under acidic, alkaline, thermal, oxidizing, and photolytic conditions. After the process of degradation was finished, the solution was allowed to equilibrate at room temperature and was diluted with mobile phase to produce solutions containing 30 ng/mL of glecaprevir and pibrentasvir. The details of the individual's deteriorating conditions are provided in Table-8.



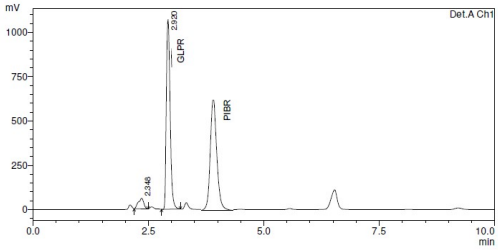
Degradation by Acid



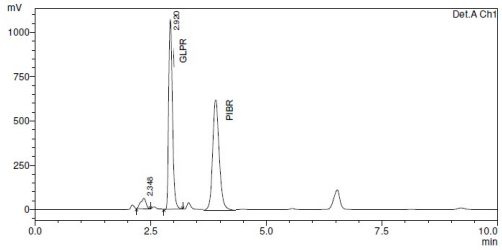
Base Degradation Studies



Degradation of Peroxide



Photolytic Degradation



Thermal Degradation

Table-8: Degradation Data

Drug Name		Acid degradation	Alkali degradation	Oxidative degradation	Photolytic Degradation	Thermal Degradation
Glecaprevir	Standard solution area of peak	907152				
	Sample solution peak area	908562	905241	908564	908641	908563
	% of Degradation	+0.7%	+4.1%	+4.6%	+4.7%	+4.1%
Pibrentasvir	Std. peak area	562987				
	Sample peak area	562897	567841	562894	568244	562871
	% of Degradation	+0.6%	+7.5%	+7.0%	+7.5%	+7.2%
% Average of Degradation		0.65%	5.8%	5.8%	6.1%	5.65%

CONCLUSION

The current novel stability indicating RP-HPLC method for simultaneous quantification of Glecaprevir and Pibrentasvir in active pharmaceutical ingredient and their combined tablet dosage form was as per guidelines of ICH & USP. The linearity values were discovered to be in the distinct concentration ranges of 50 to 150 ppm & 20 to 60 ppm, GPV and PNV respectively. It was discovered that the linearity values were greater than 0.999 and that they could be successfully applied to the quality control department. This demonstrates that the existing study methodology is accurate, exact, and cost-effective. Compared to previously described approaches, the proposed method has a number of advantages. The current research approach very accurately measured the retention periods of both medications.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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