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STABILITY INDICATING RP-UPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GLECAPREVIR AND PIBRENTASVIR IN BULK AND TABLET DOSAGE FORMS

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Abstract:
A novel, simple, accurate and rapid method has been developed and validated for and in bulk and in tablet dosage form. In RP-UPLC method, elution was achieved in isocratic mode using combination of Glecaprevir and Pibrentasvir in the ratio of 0.1% OPA: Acetonitrile (60:40) using a HH C18 (100×2.1mm, 1.8µm) column. The flow rate was 1ml/min and detection was done at 260nm.The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form.

Keywords: *Glecaprevir, Pibrentasvir, RP-UPLC, Validation.*

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INTRODUCTION:

Glecaprevir:

Glecaprevir (INN[1]) is direct acting antiviral drug and Hepatitis C virus (HCV) NS3/4A protease inhibitor and mainly targets viral RNA replication. (3aR, 7S, 10S, 12R, 21E, 24aR)-7-tert-Butyl-N-{(1R,2R)-2-(difluoromethyl)-1-[(1-methylcyclopropane-1-sulfonyl) carbamoyl] cyclopropyl} - 20, 20 – difluoro -5,8-dioxo2,3,3a,5,6,7,8,11,12,20,23,24a-dodecahydro-1H,10H-9,12-methanocyclopenta [18,19] [1,10,17,3,6] trioxadiazacyclonon adecino [11,12-b] quinoxaline-10-carboxamide. It is practically insoluble in water and sparingly soluble in ethanol and molecular weight of Glecaprevir 838.87g/mol[2,3] and chemical structure is given below figure no 1.

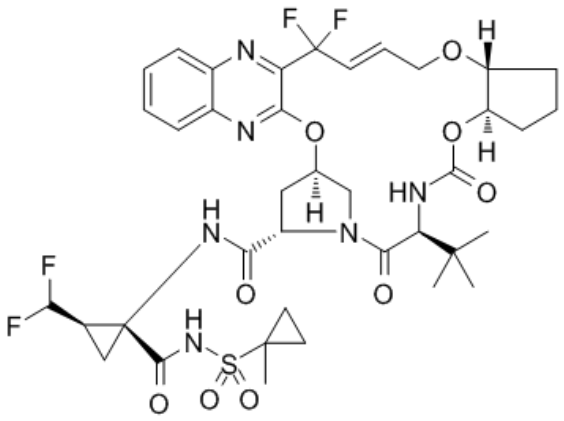

Figure no 1: Chemical Structure of Glecaprevir

Pibrentasvir[4,5]:

Pibrentasvir, an NS5A inhibitor antiviral agent is used along with Glecaprevir under the trade name Mavyret for the treatment of hepatitis-C. Mavyret is sold by Abbvie[6]. Methyl {(2S,3R)-1-[(2S)-2-{5-[(2R,5R)-1-{3,5-difluoro-4-[4-(4-fluorophenyl)-1-piperidinyl] phenyl}-5-(6-fluoro-2-{(2S)-1-[N-(methoxycarbonyl)-O-methyl-L-threonyl]-2-pyrrolidinyl}-1H-benzimidazol-5-yl)-2-pyrrolidinyl]-6-fluoro-1H-benzimidazol-2-yl}-1-pyrrolidinyl]-3-methoxy-1-oxo-2-butanyl} carbamate. It is practically insoluble in water and freely soluble in ethanol and molecular weight of Pibrentasvir 1113.201g/mol and chemical structure is given below figure no 2.

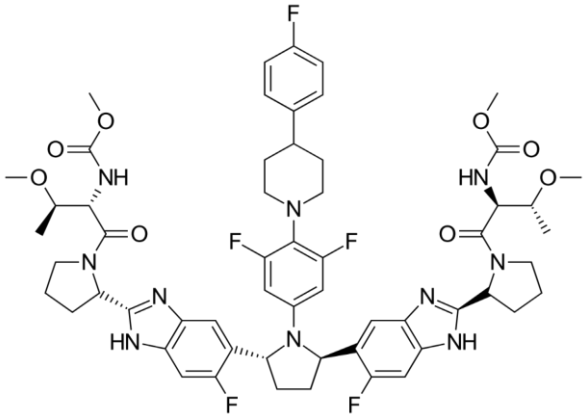

Figure no 2: Chemical structure of Pibrentasvir

A very few spectroscopic and liquid chromatographic procedures have been reported for the simultaneous estimation of Glecaprevir and Pibrentasvir. Therefore there is need to develop a rapid, sensitive, robust and selective RP-UPLC method for simultaneous estimation of Glecaprevir and Pibrentasvir in bulk and tablet dosage form[7-9].

MATERIALS AND METHODS:

Chemicals and Solvents:

UPLC grade Acetonitrile was purchased from Ramkem, Haryana, India. Ortho Phosphoric Acid was purchased from Fischer Scientific, Mumbai, and India. UPLC-grade water was prepared by using Millipore Milli- Q water purification system used throughout the process.

Instrumentation and Chromatographic Conditions:

The Present assay was carried out on Waters UPLC system equipped with photo diode array detector, auto sample injector and column HHS C18 (100×2.1mm, 1.8µm) respectively. The output signal was monitored and integrated using Waters Empower 2 software. Electronic balance was used for weighing the materials. Ultrasonicator was used for sonicating the solvents; Hot air oven was used for forced degradation studies. The mobile phase consisted of 0.1% ortho phosphoric acid and Acetonitrile (60:40), flowing through the column at a constant flow rate of 1mL/min at ambient temperature with a sample injection volume of 1µL. Detection of the analytes were carried out at a wavelength of 260 nm

Preparation of Mobile Phase:

Mixture of 0.1% OPA and Acetonitrile in the ratio 60:40 filtered through 0.45µm filter under vacuum.

Preparation of Working Standard Solutions:
Accurately Weighed and transferred 25mg of Glecaprevir and 10mg of Pibrentasvir working Standards into a 25ml clean dry volumetric flask, add 3/4 th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. (1000ppm of Glecaprevir and 400ppm of Pibrentasvir) 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (100ppm of Glecaprevir and 40ppm of Pibrentasvir)

Preparation of Sample Solutions:
5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 50mL of diluent added and sonicated for 25min, further the volume made up with diluent and filtered. (1000ppm of Glecaprevir and 400ppm of Pibrentasvir). From the filtered solution 1 ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent. (100ppm of Glecaprevir and 40ppm of Pibrentasvir)

RESULTS AND DISCUSSION:

Method development:
The present investigation reported is a new stability indicating RP-UPLC method development and validation of simultaneous estimation of Glecaprevir and Pibrentasvir. The method developed was proceeding within wavelength selection and the optimized wavelength as 260nm. In order to get the optimized RP-UPLC method various mobile phases and columns were used to get better resolution. Finally the analysis was performed by using 0.1% Ortho phosphoric acid and Acetonitrile in the ratio of 60:40 at a flow rate 1mL/min at an injection volume of 1µL and separation was carried out by using HHS C18,(100×2.1mm,1.8µm) and final optimized chromatogram are given in figure 3.

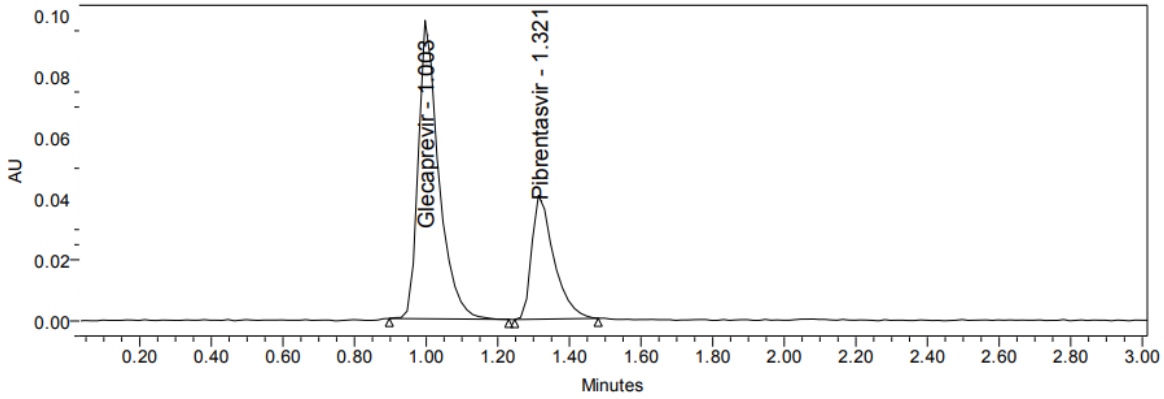

Figure no 3: Final Optimized Chromatogram

Method validation according to ICH[10]:

System suitability:
Six replicates of the working standard solution were prepared and injected 0.20µL of prepared solution to carry out system suitability parameters like Retention time, Peak area, USP Plate count, USP Resolution and USP Tailing. The theoretical plates were found to be not less than 5000 for the two drugs. The tailing factor was found to be less than 2.0 and USP resolution exceeds 1.5. The system suitability parameters values are given in table no 1.

Table no 1: System suitability test parameter

S. No	Peak Name	Retention Time	Peak Area	USP Plate Count	USP Resolution	USP Tailing
1	Glecaprevir	1.00	409997	3402		1.46
2	Pibrentasvir	1.32	181135	3058	2.7	1.51

Specificity:
A study to establish the interferences of blank and placebo was conducted. Analysis was performed on solution placebo and formulation as per test method. Chromatograms of blank, placebo and formulation solution showed no peaks at the retention time of Glecaprevir and Pibrentasvir peaks.

Linearity:
Aliquots of 0.25, 0.5, 0.75, 1, 1.25 and 1.5mL were taken from stock solution and diluted up to 10ml mark with diluent. Volume of 1µL of each sample solution was injected three times into the chromatographic system and the

chromatograms were recorded. Calibration curve was constructed by calculating the peak area values of three replicates and plotting the average peak area versus drug concentration. A linear relationship was observed in the concentration range on X axis and peak area response on Y axis. The calibration curves of Glecaprevir and Pibrentasvir are shown in figures no 4 to 5. The linearity studies and regression characteristics of the proposed method are presented in table no 2.

Table no 2: Linearity data of Glecaprevir and Pibrentasvir

Glecaprevir		**Pibrentasvir**	
Concentration(µg/mL)	***Peak Area**	**Concentration(µg/mL)**	***Peak Area**
25	109953	10	44426
50	212225	20	89144
75	325187	30	139624
100	428264	40	185043
125	526863	50	224465
150	626891	60	270872
Regression equation **y = 4186.5x+4497.5** **R^2 = 0.9996**		**Regression equation** **y = 4530.7x+304.3** **R^2 = 0.9994**	

*= Average peak area of 3 replicate injection for each concentration

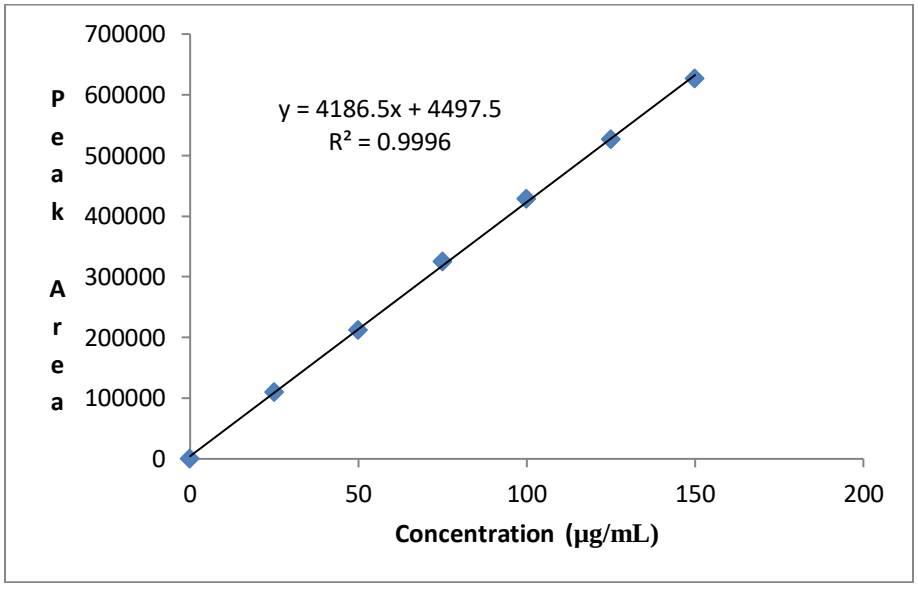

Figure no 4: Standard calibration graph of Glecaprevir

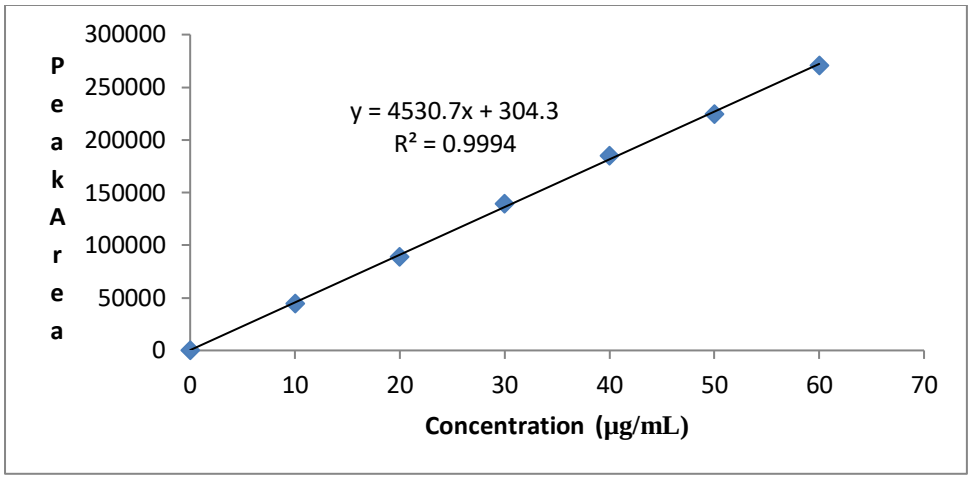

Figure no 5: Standard calibration graph of Pibrentasvir

Limit of Detection (LOD) and Limit of Quantification (LOQ):

A study to establish the LOD and LOQ for Glecaprevir and Pibrentasvir was conducted. Series of very dilute LOD and LOQ solutions were prepared as per the test method and injected triplicate into the UPLC system. The sensitivity results are shown in table no 3. Chromatograms of LOD and LOQ were given in figures no 6 to 7.

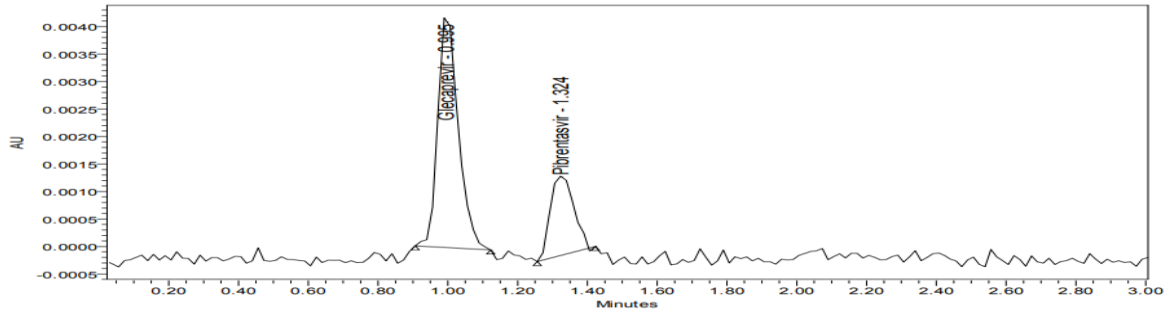

Figure no 6: Chromatogram of LOD

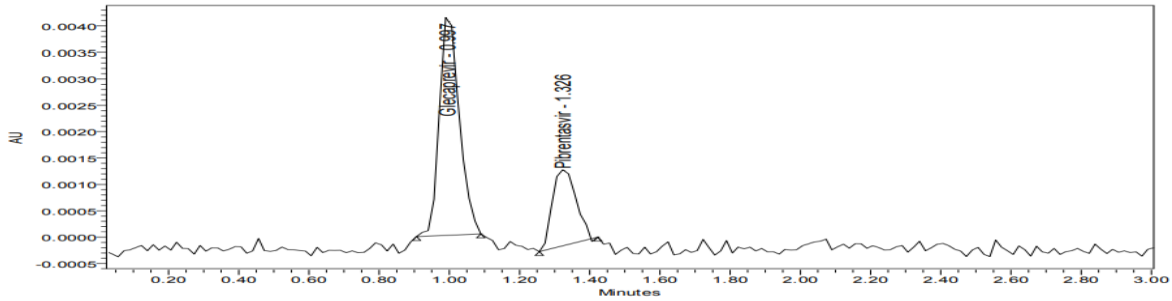

Figure no 7: Chromatogram of LOQ

Table no 3: Limit of Detection and Limit of Quantification data

S.No	Parameter	Measured values (µg/mL)	
		Glecaprevir	Pibrentasvir
1	LOD	2.0	0.08
2	LOQ	6.06	0.24

Method Precision:
Working sample solution of 1µL was injected six test preparations into the UPLC system and chromatograms were obtained. The % RSD of the assay result of six test preparations was calculated. The results obtained for method precision are presented in table no 4.

Table no 4: Method Precision data of Glecaprevir and Pibrentasvir

Sample No.	*Peak area response of drugs	
	Glecaprevir	Pibrentasvir
1	415405	181629
2	411433	179324
3	412008	180996
4	412721	180324
5	409271	180380
6	409212	181512
Mean	411675	180694
Std.Dev	2324.7	865.7
%RSD	0.6	0.5

*= Average assay of 3 replicates injection at each time interval
Results showed lower %RSD values. This reveals that the method is quite precise

Accuracy:
A known amount of Glecaprevir and Pibrentasvir at each three concentration levels 50%, 100% and 150% were added to a pre analyzed sample solution and injected in triplicate at each level into the chromatographic system and the chromatograms were recorded. The mean percent recovery and % RSD at each level was calculated. Results are presented in tables no 5 to 6 .

Table no 5: Accuracy studies of Glecaprevir

*Spiked level	Amount spiked (µg/mL)	Amount Recovery (µg/mL)	% Recovery	Mean Recovery ± % RSD
50%	50	50.2666	100.53	100.02±0.71
	50	49.47157	98.94	
	50	49.86622	99.73	
100%	100	101.3342	101.33	
	100	100.252	100.25	
	100	99.38772	99.39	
150%	150	149.8201	99.88	
	150	150.6959	100.46	
	150	149.447	99.63	

Table no 6: Accuracy studies of Pibrentasvir

*Spiked level	Amount spiked (µg/mL)	Amount Recovery (µg/mL)	% Recovery	Mean Recovery ± % RSD
50%	20	20.39728	101.99	100.06±1
	20	19.77786	98.89	
	20	20.13349	100.67	
100%	40	39.81406	99.54	
	40	39.46682	98.67	
	40	40.02046	100.05	
150%	60	60.29088	100.48	
	60	60.23415	100.39	
	60	59.91097	99.85	

*= Average results of 3 replicate injections

Forced Degradation Studies according to ICH[11]:

The degradation results of Glecaprevir and Pibrentasvir are reported in tables no 7 to 8.

Table no 7: Degradation studies of Glecaprevir

S.No	Degradation Conditions	Peak Area	% Degradation	Peak Purity
1	Acid	385955	6.15	Passes
2	Base	392645	4.52	Passes
3	Peroxide	392221	4.62	Passes
4	Thermal	401527	2.36	Passes
5	UV	403521	1.87	Passes
6	Water	408772	0.60	Passes

Table no 8: Degradation studies of Pibrentasvir

S.No	Degradation Conditions	% Peak Area	% Degradation	Peak Purity
1	Acid	173487	4.32	Passes
2	Base	173832	4.13	Passes
3	Peroxide	172002	5.14	Passes
4	Thermal	176851	2.46	Passes
5	UV	178568	1.52	Passes
6	Water	179532	0.98	Passes

CONCLUSION:

A new stability indicating RP-UPLC method was developed for the simultaneous estimation of Glecaprevir and Pibrentasvir in pharmaceutical dosage form and it was validated as per ICH guidelines. This method represents simple, economic, selective, and accurate with good precision and stability indicating analytical procedure for estimation of Glecaprevir and Pibrentasvir. Therefore, this method can be successfully applied to routine analytical purpose.

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