STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF GLECAPREVIR AND PIBRENTASVIR IN API FORM AND COMBINED TABLET DOSAGE FORM

Datla Spandhana[1*], K.S.L.Harika[2]

[1]Department of Pharmaceutical Sciences, (Pharmaceutical Analysis), CPS - Centre for pharmaceutical sciences, IST (Institute of Science and Technology), Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Telangana 500085

[2]Assistant Professor, Department of Pharmaceutical Sciences, (Pharmaceutical Analysis), CPS - Centre for pharmaceutical sciences, IST (Institute of Science and Technology), Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Telangana 500085

ABSTRACT : A new analytical simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the simultaneous determination of Glecaprevir and Pibrentasvir in bulk and pharmaceutical dosage form dosage form. The chromatographic method was standardized using Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. i.d. column with UV detection at 255nm and Methanol: Phosphate buffer (0.02M) with 55:45 (pH-2.6) ratios at a flow rate of 1.0 ml/ min. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines. The method was linear over the range of 0-14µg/ml for Glecaprevir and 0-28µg/ml for Pibrentasvir. The recovery was in the range of 98% to 102%. The LOD was found to be 0.06 µg/ml and 0.09 µg/ml for Glecaprevir and Pibrentasvir respectively. The LOQ was found to be 0.18 µg/ml and 0.27 µg/ml for Glecaprevir and Pibrentasvir. The proposed method was successfully applied to the simultaneous determination of Glecaprevir and Pibrentasvir in bulk and pharmaceutical dosage form.

Keywords: RP-HPLC, Glecaprevir and Pibrentasvir, ICH Guidelines, Accuracy, Precision.

I.INTRODUCTION

Glecaprevir is a direct acting antiviral agent and Hepatitis C virus (HCV) NS3/4A protease inhibitor that targets the viral RNA replication. In combination with Pibrentasvir, glecaprevir[1] 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. 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. Glecaprevir[2] is a Hepatitis C Virus NS3/4A Protease Inhibitor. The mechanism of action of glecaprevir is as a HCV NS3/4A Protease Inhibitor, and P-Glycoprotein Inhibitor, and Breast Cancer Resistance Protein Inhibitor, and Organic Anion Transporting Polypeptide 1B1 Inhibitor, and Organic Anion Transporting Polypeptide 1B3 Inhibitor, and Cytochrome P450 3A Inhibitor, and Cytochrome P450 1A2 Inhibitor, and UGT1A1 Inhibitor. The IUPAC Name of Glecaprevir[3] is (1R,14E,18R,22R,26S,29S)-26-tert-butyl-N-[(1R,2R)-2-(difluoro methyl)-1-[(1-methyl cyclo propyl) sulfonyl carbamoyl] cyclo propyl]-13,13-difluoro-24, 27-dioxo-2, 17, 23-trioxa-4, 11, 25, 28-tetraza penta cyclo [26.2.1.03,12.05,10.018,22] hentriaconta-3, 5, 7, 9, 11, 14-hexaene-29-carboxamide. The Chemical Structure of Glecaprevir is as follows

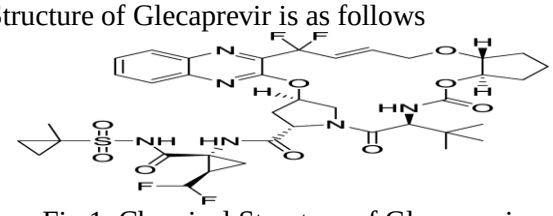

Fig.1. Chemical Structure of Glecaprevir

Pibrentasvir 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 [DB13879], 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[4]. 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. Pibrentasvir[5] is available as an oral combination therapy with [DB13879] 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. 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. Hepatitis C viral infection often leads to decreased liver function and subsequent liver failure, causing a significantly negative impact on the patients' quality of life. The ultimate goal of the combination treatment is to achieve sustained virologic response (SVR) and cure the patients from the infection. In clinical trials, this combination therapy achieved SVR12 rate or undetectable Hepatitis C for twelve or more weeks after the end of treatment, of ≥93% across genotypes 1a, 2a, 3a, 4, 5 and 6. The IUPAC Name of Pibrentasvir[6] is Methyl N-[(2S,3R)-1-[(2S)-2-[6-[(2R,5R)-1-[3,5-difluoro-4-[4-(4-fluorophenyl) piperidin-1-yl] phenyl]-5-[6-fluoro-2-[(2S)-1-[(2S,3R)-3-methoxy-2-(methoxy carbonylamino) butanoyl] pyrrolidin-2-yl]-3H-benzimidazol-5-yl] pyrrolidin-2-yl]-5-fluoro-1H-benzimidazol-2-yl] pyrrolidin-1-yl]-3-methoxy-1-oxobutan-2-yl] Carbamate. The Chemical Structure of Pibrentasvir[7] is as follows

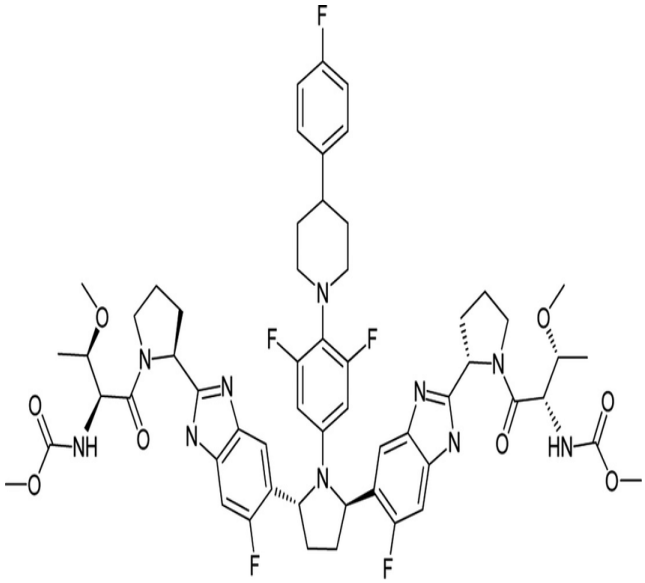

Fig.2. Chemical Structure of Pibrentasvir

Therefore, it was thought of interest to develop simple, accurate, fast and cost effective method for the analysis of Glecaprevir and Pibrentasvir in bulk form and in its marketed tablet formulation[22-24]. This paper describes development and validation of simple, specific, sensitive, accurate and precise Chromatographic method for the simultaneous estimation of Glecaprevir and Pibrentasvir in bulk and its formulation.

II. MATERIALS AND METHODS

Table-1: List of Instrument used

S. No.	Instruments/Equipment/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	T60-LAB INDIA UV – Vis spectrophotometer

3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator(Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

Table-2: List of Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	SD fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	SD fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	SD fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	SD fine-Chem ltd; Mumbai

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase[8] diluting with the same solvent.(After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Glecaprevir and Pibrentasvir, so that the same wave number can be utilized in HPLC UV detector[9] for estimating the Glecaprevir and Pibrentasvir. The scanned UV spectrum is attached in the following page.

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Glecaprevir standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

25 mg of Pibrentasvir standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Preparation of Phosphate buffer (PH: 2.6):

Weighed 0.50 grams of di-sodium hydrogen phosphate and 0.301 grams of potassium dihydrogen phosphate was taken into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water, adjusted the pH to 2.6 with orthophosphoric acid.

Preparation of Mobile Phase:

The mobile phase was prepared with the combination of Methanol and Phosphate buffer (0.02M, pH-2.6) at the volume of 1000ml. 550ml of Methanol and 450ml of Phosphate buffer were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration[10].

Preparation of Standard Solutions:

10 mg of Glecaprevir and Pibrentasvir was weighed accurately and transferred into 10 ml volumetric flask. About 10 ml mobile phase was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 10µg/ml and 10µg/ml of Glecaprevir and Pibrentasvir respectively.

Method Validation

System Suitability

System suitability is defined by ICH as "the checking of a system, before or during the analysis of unknowns, to ensure system performance." System suitabilit[y11] criteria may include such factors as plate count, tailing, retention, and/or resolution. System suitability criteria should also include a determination of reproducibility[12] (%RSD) when a system suitability "sample" (a mixture of main components and expected by-products/interferences) is run.

Specificity

One of the significant features of HPLC is its ability to generate signals free from interference. Specificity[13] refers to the strength of the analytical method to differentiate and quantify the analyte in complex mixtures. An investigation of specificity is to be conducted during the determination of impurities and validation of identification tests. An ICH guideline[14] defines specificity as the ability to assess unequivocally the analyte in the presence of other compounds that may be likely to be present. Typically these might be impurities, degradants, matrix, etc.

Precision

The closeness of agreement between a series of measurements multiple samplings of the same homogeneous sample under prescribed condition. The precision[15] of test method is usually expressed as the standard deviation or relative standard deviation of a series of measurements. Precision may be considered at three levels: Repeatability, Intermediate Precision and reproducibility.

Accuracy

It is the closeness of agreement between the actual value and measured value. Accuracy[16] is calculated as the percentage of recovery by the assay of the known added amount of the analyte in the sample or the difference between the mean and accepted true value together with confidence intervals.

The ICH guidance recommended to take a minimum of 3 concentration levels covering the specified range and 3 replicates of each concentration are analyzed (totally 3* 3 = 9 determination).

Linearity and Range

Linearity

The linearity[17] of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Range

The range[18] of analytical procedure is the interval between the upper and lower concentrations of analyte in the analytical procedure has a suitable level of precision, accuracy, and linearity.

Detection Limit

Definition: It is the lowest amount of analyte in a sample that can be detected[19] but not necessarily quantitated under the stated experimental conditions.

Quantitation Limit

Definition: It is lowest amount of analyte in a sample, which can be quantitatively[20] determined with acceptable accuracy and precision.

Ruggedness

Definition: Ruggedness is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of test conditions such as different laboratories, analysis, instruments, reagent lots, elapsed assay times, temperature, days, etc. It can be expressed as a lack influence of the operation and environmental variable on the test results of the analytical method.

Robustness

Definition: It is a measure of the method's ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure.

Forced Degradation Studies

Forced degradation studies[21] provide the approach to analyse the stability of drug samples in pharmaceutical industries. Drug product safety and efficacy is affected by the chemical stability of the molecule. Stability of molecule information provides the data for selecting proper formulation, package, proper storage conditions and shelf life. These data also play a significant role which is required in the regulatory documentation. Before filling registration dossier it is obligatory to execute stability studies of new drug molecules.

III. RESULTS AND DISCUSSION

Wavelength Detection (Or) Selection of Wavelength

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of

10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Glecaprevir and Pibrentasvir was obtained and the isobestic point of Glecaprevir and Pibrentasvir showed absorbance's maxima at 255nm.

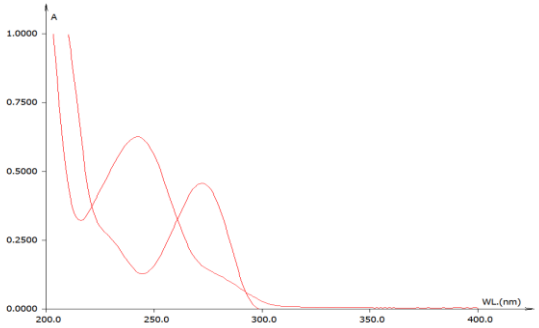

Fig.2.Overlay spectrum for *Glecaprevir* and Pibrentasvir

Optimized Chromatographic Method:

Table-3: Optimized Chromatographic Conditions

Mobile phase	Methanol : Phosphate buffer (0.02M, pH-2.6) = 55:45%v/v
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	255 nm
Flow rate	1.0 ml/ min.
Run time	07 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic

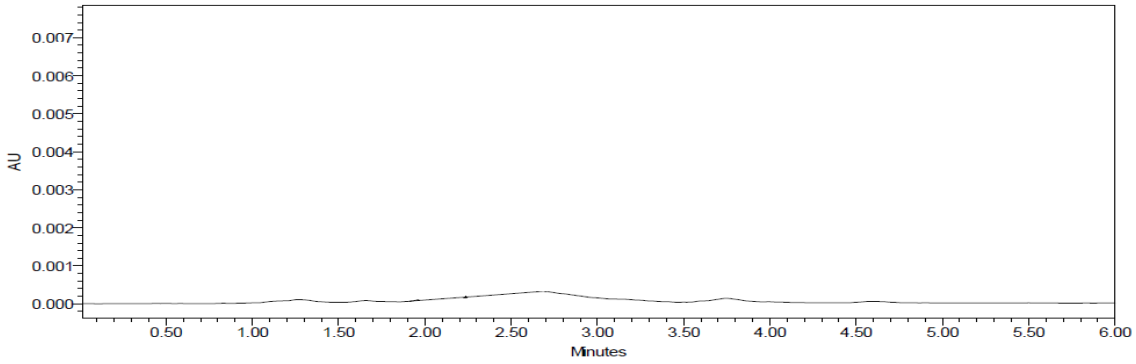

Fig.4. HPLC Spectrum of Glecaprevir and Pibrentasvir (Blank Solution)

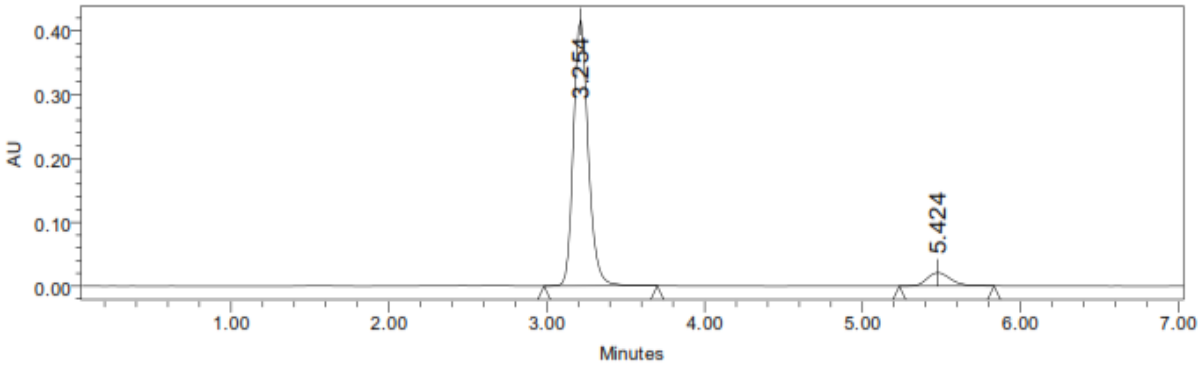

Fig.5. Chromatogram of Glecaprevir and Pibrentasvir in Optimized Chromatographic Condition

Validation of Method

1. System Suitability: System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-4.

Table-4: System suitability results for Glecaprevir and Pibrentasvir (Flow rate)

S.No.	Parameter	Limit	Result
1	Resolution	Rs> 2	3.57
2	Asymmetry	T ≤ 2	Glecaprevir = 0.12 Pibrentasvir = 0.24
3	Theoretical plate	N > 2000	Glecaprevir = 2987 Pibrentasvir = 3014

2. Linearity: To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 0-14µg/ml for Glecaprevir and concentration ranging from 0-28µg/ml for Pibrentasvir. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 10µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Plotting of Calibration Graphs:

The resultant areas of linearity peaks are plotted against Concentration.

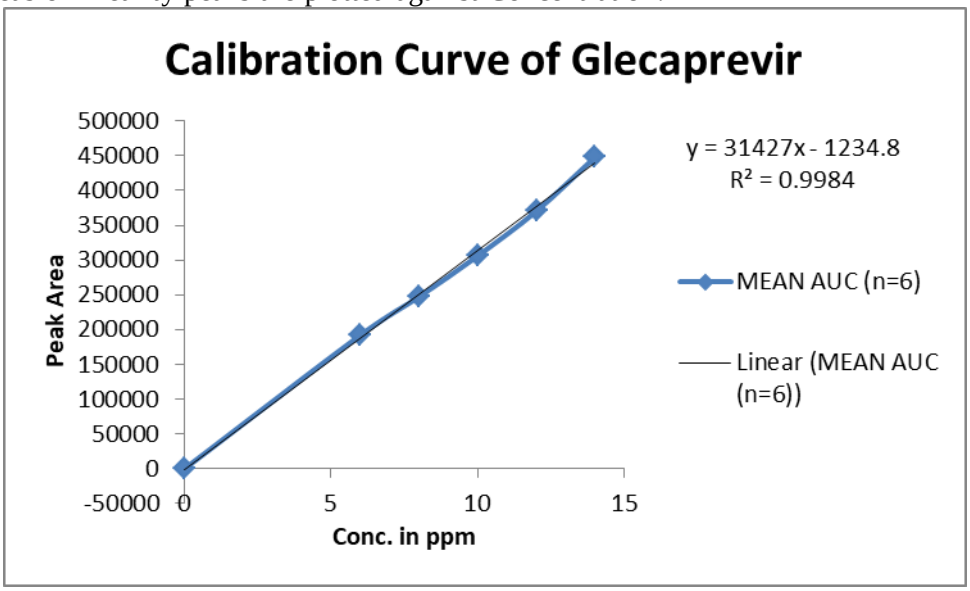

Fig.6. Standard curve for Glecaprevir

Table-5: Linearity Readings for Glecaprevir

CONC. (µg/ml)	MEAN AUC (n=6)
0	0
6	192164
8	247293

10	306089
12	370481
14	447930

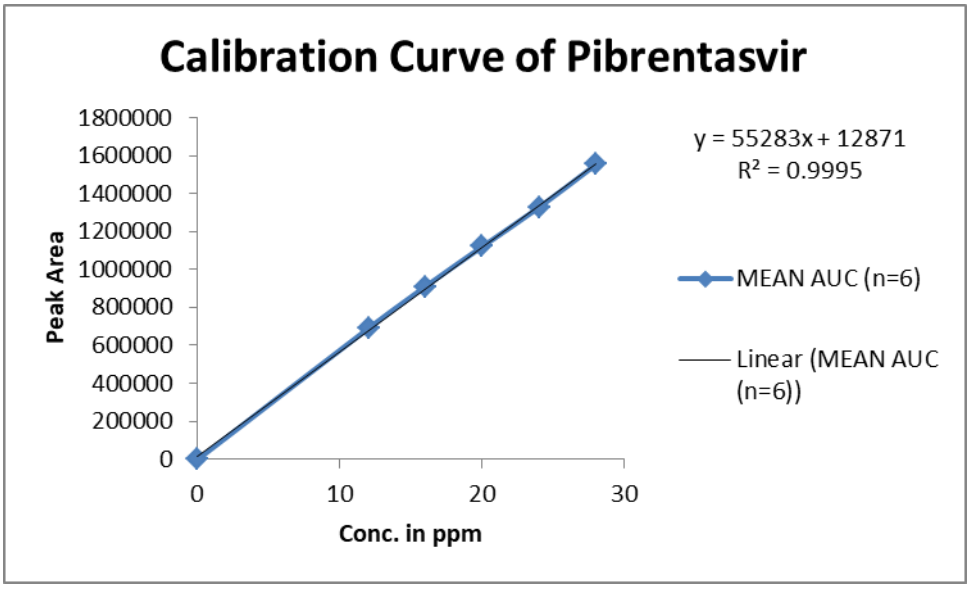

Fig.7. Standard curve for Pibrentasvir

Table-6: Linearity Readings for Pibrentasvir

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

Observation: Linearity range was found to be 0-14 µg/ml for Glecaprevir. The correlation coefficient was found to be 0.999, the slope was found to be 55283 and intercept was found to be 12871 for Glecaprevir. Linearity range was found to be 0-28 µg/ml for Pibrentasvir. The correlation coefficient was found to be 0.999, the slope was found to be 55283 and intercept was found to be 12871 for Pibrentasvir.

3. Accuracy:

Recovery Study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drugs of Glecaprevir and Pibrentasvir were taken and 3 replications of each has been injected to HPLC system. From that percentage recovery values were calculated from the linearity equations y = 31427x + 1234.8 and y = 55283x + 12871. The results were shown in table-7 and 8.

Table-7: Accuracy results of Glecaprevir

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.064107	458679	99.867	Mean= 100.4113% S.D. = 0.473694346 % R.S.D.= 0.471753
S_2 : 80 %	8	7.843532	446485	100.637	
S_3 : 80 %	8	8.19449	465887	100.73	
S_4 : 100 %	10	9.892661	559767	99.41	Mean= 100.6646667% S.D. = 1.166369295 R.S.D.= 1.158667
S_5 : 100 %	10	9.978655	564521	100.868	
S_6 : 100 %	10	10.19623	576549	101.716	
S_7 : 120 %	12	11.85907	668476	99.878	Mean= 100.4637% S.D. = 0.51154309 % R.S.D. = 0.509181
S_8 : 120 %	12	12.16785	685546	100.69	
S_9 : 120 %	12	12.18644	686574	100.823	

Table-8: Accuracy results of Pibrentasvir

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	16	15.71861	881843	98.24132	Mean= 98.66425667% S.D. = 0.558426265% R.S.D.= 0.565996
S_2 : 80 %	16	15.75267	883726	98.4542	
S_3 : 80 %	16	15.88756	891183	99.29725	
S_4 : 100 %	20	20.00427	1118767	100.0213	Mean= 100.8802% S.D. = 0.945972362 % R.S.D.= 0.9377182
S_5 : 100 %	20	20.37881	1139473	101.8941	
S_6 : 100 %	20	20.14504	1126549	100.7252	
S_7 : 120 %	24	23.69705	1322915	98.73771	Mean= 98.87614% S.D. = 0.137893172 % R.S.D. = 1.401528
S_8 : 120 %	24	23.73053	1324766	98.87722	
S_9 : 120 %	24	23.76324	1326574	99.01349	

Observation: The mean recoveries were found to be 100.411, 100.664 and 100.463% for Glecaprevir and 98.664, 100.880 and 98.876% for Pibrentasvir. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

4. Precision: The precision of each method was ascertained separately from the peak areas obtained by actual determination of five replicates of a fixed amount of drugs Glecaprevir & Pibrentasvir. The percent relative standard deviations were calculated for Glecaprevir & Pibrentasvir are presented in the Table-9.

i) Repeatability

Table-9: Repeatability Results of Glecaprevir and Pibrentasvir

HPLC Injection Replicates	AUC for Glecaprevir	AUC for Pibrentasvir
Replicate – 1	623568	1113214
Replicate – 2	613241	1105241
Replicate – 3	625408	1113424
Replicate – 4	617412	1105987
Replicate – 5	612541	1104216
Replicate – 6	622546	1113219
Average	**615786**	**1109216.833**
Standard Deviation	**5510.431332**	**4493.157884**
% RSD	**0.890043**	**0.405074**

Observation: The repeatability study which was conducted on the solution having the concentration of about 10µg/ml for Glecaprevir and 20µg/ml for Pibrentasvir (n =3) showed a RSD of 0.890043% for Glecaprevir and 0.405074% for Pibrentasvir. It was concluded that the analytical technique showed good repeatability.

ii) Intermediate precision / Ruggedness

Table-10: Ruggedness Results for Glecaprevir

Conc. of Glecaprevir (API) (µg/ml)	Observed Conc. of Glecaprevir (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
8	8.21	0.76	8.23	0.46
10	10.37	0.33	10.36	0.57
12	12.56	0.23	12.56	0.75

Table-11: Ruggedness Results for Pibrentasvir

Conc. Of Pibrentasvir (API) (µg/ml)	Observed Conc. of Pibrentasvir (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
16	16.12	0.65	16.34	0.55
20	20.43	0.54	20.67	0.27
24	24.33	0.76	24.37	0.51

Observation: Intraday and interday studies show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

5. Robustness: Robustness is defined as the capacity of that method to be unaffected by even small deliberate changes that occur in the method parameters. The evaluation of robustness of a method is done by varying the chromatographic parameters such as pH, temperature, flow rate, mobile phase proportions change, ionic strength etc., and determining any possible effect on the results obtained by that method.

Table-12: Result of Method Robustness Test for Glecaprevir

Change in parameter	% RSD
Flow (0.8 ml/min)	0.55
Flow (1.2 ml/min)	0.86
More Organic	0.88
Less Organic	0.81
Wavelength of Detection (261 nm)	0.81
Wavelength of detection (257 nm)	0.79

Observation: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & organic phase (±5%) studied to determine the robustness of the method are also in favour of (Table-12, % RSD < 2%) the developed RP-HPLC method for the analysis of Glecaprevir (API).

Table-13: Result of Method Robustness Test for Pibrentasvir

Change in parameter	% RSD
Flow (0.8 ml/min)	1.03
Flow (1.2 ml/min)	0.68
More Organic	0.77
Less Organic	0.63
Wavelength of Detection (233 nm)	1.09
Wavelength of detection (229 nm)	0.92

Observation: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & organic phase (±5%) studied to determine the robustness of the method are also in favour of (Table-13, % RSD < 2%) the developed RP-HPLC method for the analysis of Pibrentasvir (API).

6. LOD: The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected,

but not quantitated. LOD is a limit test that specifies whether an analyte is above or below a certain value. Signal-to-noise ratio of three-to-one is used to determine LOD.

L.O.D. = 3.3 (SD/S).

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Observation: The LOD was found to be 0.06 µg/ml and 0.09 µg/ml for Glecaprevir and Pibrentasvir respectively.

7. LOQ: The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ.

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Observation: The LOQ was found to be 0.18 µg/ml and 0.27 µg/ml for Glecaprevir and Pibrentasvir respectively.

8. Assay: – Assay refers to chromatography based purity assay where a compound of unknown activity or purity is compared to a reference standard with precisely determined bioactivity or purity.

$$\text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:

AT = Test Preparation Peak Area
AS = Standard preparation Peak Area
WS = Working standard weight taken in mg
WT = Sample weight taken in mg
DS = Standard solution dilution
DT = Sample solution dilution
P = Working standard percentage purity

The assay was performed as explained in the previous chapter. The results which are obtained are following:

Table-14: Assay of GLECAPREVIR & PIBRENTASVIR Tablets

Brand name of tablets	Labelled amount of Drug (mg) Glecaprevir & Pibrentasvir	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Maviret Tablets (Abbvie)	100/40	99.869 (±0.08)/39.687 (±0.05)	99.78(±0.48) /99.77(±0.12)

Results and Discussion: The assay of Maviret Tablets containing Glecaprevir was found to be 99.78 % and Pibrentasvir was found to be 99.77 %.

Forced Degradation Studies

The results of the forced degradation studies indicated the specificity of the developed method that has been developed. Glecaprevir and Pibrentasvir were stable only in basic and thermal, oxidation stress conditions and photolytic stress conditions. The results of stability studies are given in the following Table-15.

Table-15: Results of Force Degradation Studies of Glecaprevir and Pibrentasvir API.

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)

Acid Hydrolysis (0.1N HCl)	24Hrs.	95.62	4.38	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	97.13	2.87	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	96.24	3.76	100.00
UV (254nm)	24Hrs.	95.43	4.57	100.00
3% Hydrogen peroxide	24Hrs.	96.16	3.84	100.00

IV.SUMMARY AND CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis ofGlecaprevir and Pibrentasvir in bulk and pharmaceutical dosage form.Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Glecaprevir and Pibrentasvir indicated that the developed method is specific for the simultaneous estimation of Glecaprevir and Pibrentasvir in the bulk and pharmaceutical dosage forms.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The specific Retention times for Glecaprevir and Pibrentasvir are found to be 3.254 and 5.424. The tailing factors were found to be 1.14 and 1.22 with theoretical plates 2236 and 2762 for Glecaprevir and Pibrentasvir respectively. The %Recoveries was determined as 100.512% and 99.473% for Glecaprevir and Pibrentasvir in Accuracy. The %RSD in Repeatability is 0.89 and 0.40 with Intermediate Precision are 0.44 and 0.65 for Glecaprevir and Pibrentasvir in Precision. In Linearity, the correlation coefficient was found to be 0.999 and 0.999 for Glecaprevir and Pibrentasvir. The LOD for Glecaprevir and Pibrentasvir was 0.06 and 0.09 and LOQ for Glecaprevir and Pibrentasvir are 0.18 and 0.27.

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