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A NEW TECHNICAL METHOD FOR GLECAPREVIR AND PIBRENTASVIR IN COMBINED DOSAGE FORMS USING NON POLAR HPLC

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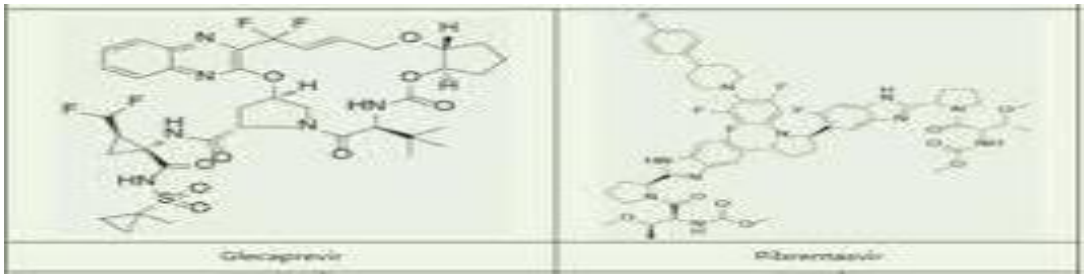
ABSTRACT

Antiviral drugs are in combined dosage forms may effectively used to treat hepatitis infections caused by the virus by competitive binding with NS3/4a and NS5 part of virions and make relief from the infection. The method was developed by using Methanol, TEA and Acetonitrile in 55:25:20 v/v ratios as mobile phase and Altima C18 stationary phase having 150*4.6 mm 5µ particle sizes. The HPLC is waters - 2695 with PDA equipped and empowered with soft ware for integration of data. The Glecaprevir (GLEC) and Pibrentasvir (PIBR) were observed at 225 nm with retention of 3.53 and 2.10 min respectively. This technical procedure was evaluated as per the guidelines of the ICH for its sensitivity, selectivity, and Linearity correlation was found to be in 0.999. The accuracy and LOD, LOQ were found in 0.2, 2.3 mcg/ml and 0.8, 7.04 mcg/ ml respectively. Robustness and ruggedness are also approved. The study is continued for its stability testing to know about the drug with various corrosive environmental factors.

KEYWORDS: Glecaprevir, Pibrentasvir, antiviral, Non Polar HPLC.

INTRODUCTION

Hepatitis C is a liver infection caused by virus and make inflammation to the part. Unlike Hepatitis A & B there is no vaccine for prevention only cure is using antiviral drugs which inhibit the replication of virus by protecting from Ns3/4a and NS5 part of the virions. As on today more than 71 millions of peoples in the world suffering with HCV for every year. It is contagious infection spread by blood to blood contact. The Glecaprevir and Pibrentasvir are belonging to antiviral category having effective in curing Hepatitis Infection. The study is carried by testing solubility of both drugs in various solvents and there by selecting the solvent, finding the isobestic wave length point for both drugs in UV spectrometer and method development in HPLC using non polar stationary phase and polar solvent as mobile phase and minimizing the conditions to make optimized method and the developed method is validated as per the guidelines are done by step by step process. The Chemical structure represented in Fig: 1.



METHODOLOGY AND MATERIALS

Glecaprevir, Pibrentasvir was purchased from the sigma Aldrich having analytical Grade declaring the purity 99.8 & 99.9% purity respectively. Acetonitrile, Tri Ethanolamine Acetate, water, methanol all were HPLC grade having not less than 99%. Purity The instrument used HPLC **WATERS** make and Model 2695 powered with auto sampler and PDA detector. The peaks are integrated with Software **Empower 2.0**. The column is Altima C18 column having 150*4.6mm having 5µ particle size.

Preparation of Solvent

The regents used for this purpose were HPLC grade. 0.1% ortho phosphoric acid (OPA) prepared by

dissolving 0.1 cc in 100 cc of water. Take 3 portions of OPA and combine with 7 portions of HPLC grade Acetonitrile. Mix the solution vigorously and filter through the 0.25µ membrane filter for degassing. This is a necessary step to avoid void peaks in the chromatogram. Now onwards this solution will be termed as solvent used for dilutions and as a running mobile phase.

Standard Solution Preparation

Accurately measure out 25 mg of HPLC grade Glecaprevir having NLT 99.8% and 10 mg of Pibrentasvir having NLT 99.9% will be included in the pre calibrated 25 ml capacity of standard flask, add few volume of solvent and subjected for sonication and fill the flask up to the line with solvent. Filter the solution if necessary through 0.25µ membrane filter. The resultant solution would become 1000 and 400 mcg per ml. This is termed as primary stock solution. Store this flask in a dark place and wrapped with aluminum foil.

Pipette out 3 cc of primary stock solution and diluted with 10 cc of solvent in an another 10 ml capacity volumetric flask. This is a working standard solution having concentration of 300 and 120mcg/ml. from this take out 1 ml and further diluted to 10 ml with same diluent.

Sample solution Preparation

Mavyret is a composition of Glecaprevir 100 mg and Pibrentasvir 40 mg from the manufacturer AbbVie Pharmaceuticals, North Chicago, USA and Imported to India by GNH India, Mumbai. The tablet strip packed with 21 tablets. Take 10 tablets and weighed for its average crush with pestle in a mortar.

Take equivalent weight 25 mg and 10 mg of Glecaprevir and Pibrentasvir in a 25 ml capacity of standard flask. Add few volume of solvent for sonication and wait for complete dissolution of drug in the solvent fill the flask with solvent and filter through 0.25µ membrane filter for the removal of insoluble particles from the solvent. This is primary sample stock solution.

Take out 3 ml of sample stock and added into 10 ml volumetric flask made dilution for filling the flask up to the line with same solvent. Pipette out 1 ml of this and dilute to 10 ml with solvent in a standard flask of 10 ml.

Method Development

The Method developed were started by using various sizes of columns starting 100 mm and 300 mm in length with diameter 4.6 µ and Non polar stationary Phase is filled into the column under pressurized condition. Based on the literature various dimensions of the columns were used in the optimization of the method.

The Altima column 150*4.6*5 dimension is used for the analysis of Glecaprevir and Pibrentasvir in the standard and sample solutions simultaneously. The solution is pumped continuously with a pressure pump maintaining 62 MPa The eluent liquid from column is connected to the PDA detector for the identification of their retention time in the column which is measurable and for ease of integration.

With use of Hamilton Syringe inject 10µl into the system and find the peak area of each drug in the chromatogram.

The development scheme is as follows:

Trail	Solvent Mix	Stationary Phase	Flow rate	Detection WL	Retention	Comment
01	ACN: Water 30:70	Symmetry C18 150*4.6*5	1.1	225	PIB: 2.25	Two drugs are not separated
02	Water: Methanol 15:85	Zodiac C18 250*4.6*5	1.2	225	PIB: 1.38 GLE: 1.75	Improper separation and resolution is not good
03	Methanol: TEA: ACN 55:35:10	Zodiac C18 250*4.6*5	0.8	225	PIB: 2.49 GLE: 5.38	Base line and symmetry not good, void peaks are seen
04	Methanol : TEA: ACN 55:30:15	Symmetry C18 250*4.6*5	1.0	225	PIB: 2.198 GLE: 3.80	separations of two peaks, base line, peak symmetry, resolution are proper
05	Methanol : TEA: ACN 55:25:20	Altima C18 150*4.6*5	1.0	225	PIB: 2.10 GLE: 3.53	proper retention time, resolution, peak tail and plate count

The trials chromatograms were pictured from 2 – 7.

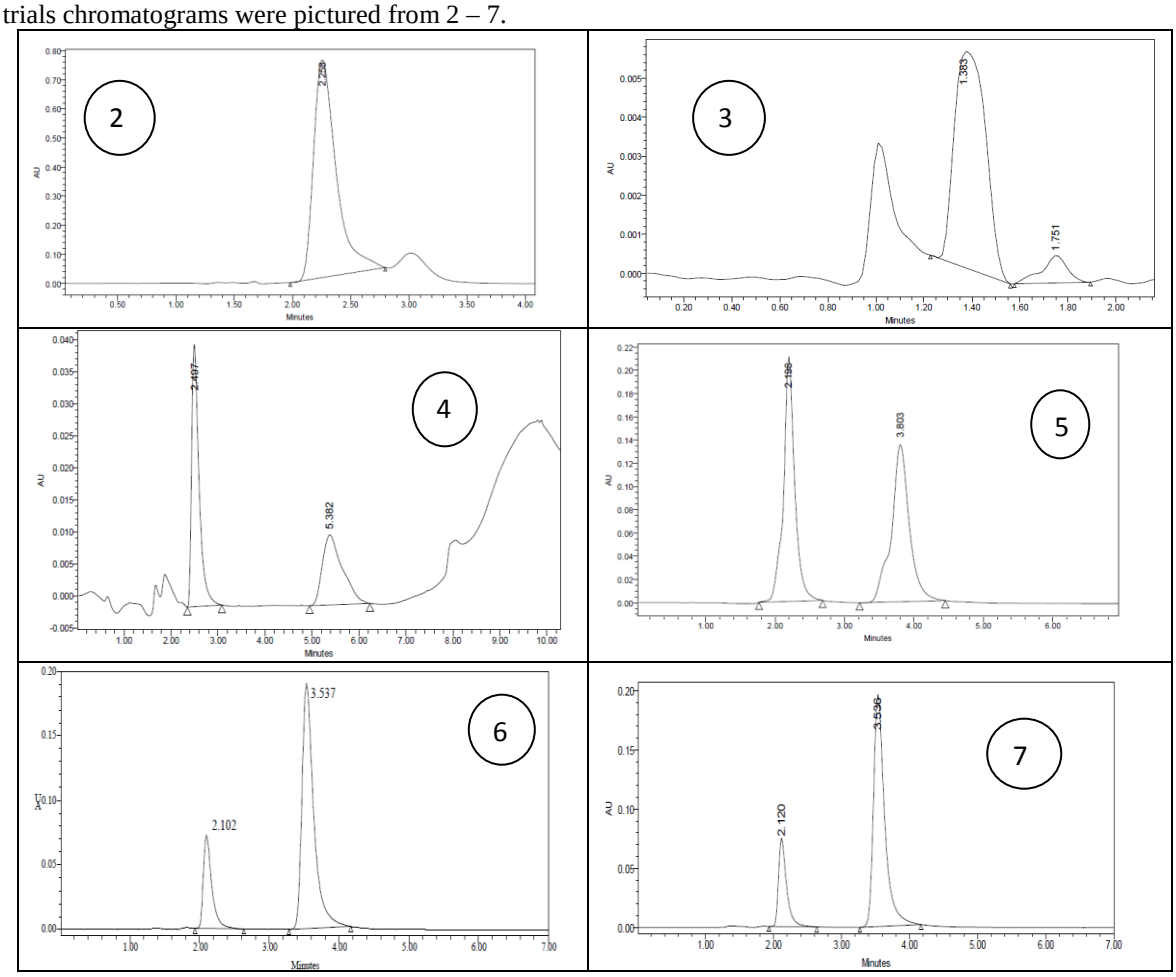


Table No. 1: Result of sample chromatogram.

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Pibrentasvir	2.120	605610	129851		0.98	5643
2	Glecaprevir	3.536	2255924	2531247	2.06	1.23	5845

Method Validation & Approval

1. Specificity: a study should be conducted for the interference of the excipients or mobile phase with the given sample of injection and observe the response of the placebo and sample injections to the selected system. The result would be discussed in table No: 2&3.

2. System suitability/ selectivity: multiple injections of same concentrations were introduced into the HPLC system and observe the responses of each individual injection and integrate the peak to measure the peak area, resolution, theoretical plates and asymmetric factor and calculate the mean standard deviations of each injection. The result would be discussed in table No: 2&3.

3. Linearity: the chosen concentrations were prepared with the above said mobile phase and inject the each concentration into the instrument and find their responses of peak area. Draw a linear graph by fitting concentration and peak area on x & y axis respectively. Calculate the slope and correlation of the all concentrations. It will reveal that the selected method is obeying the beer lambart law. The result would be discussed in table No: 04.

4. Precision: it provides the method closeness of the multiple injections of same concentration on multiple days by multiple analysts on multiple systems. The precision carries by its repeatability, intermediate precision between the days and between the analysts. The result would be discussed in table No: 2&3.

5. Accuracy: this validation parameter is evaluated by injecting the three different levels of sample into the HPLC system with respect to the standard assay solution and in each case finds the percentage of recovery from the injected sample. This accuracy is carried by two methods one is spike method and the other is direct assay method. The results were described in the table No: 5, 6 &7.

6. Assay: the assay of the method is done by injecting the same concentration of sample and standard with the selected instrumental conditions and measure the peak

area and calculate the percentage of purity and drug quantity in the given or selected dosage form which were be shown in the result table No: 2&3

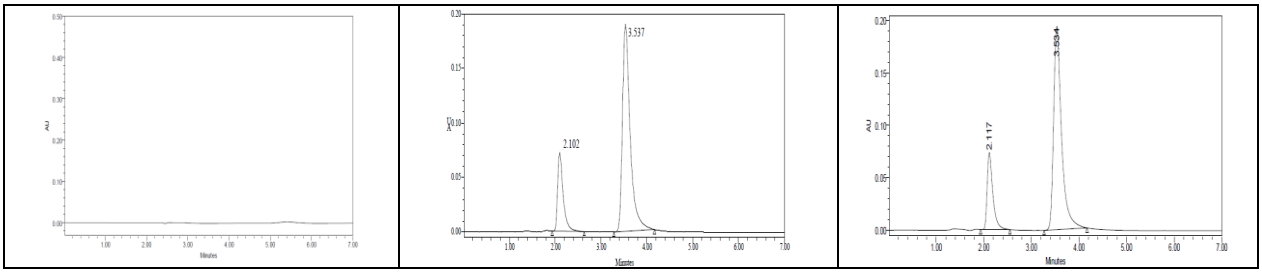
7. Limit of detection and Limit of Quantification: the LOD and LOQ were calculated to know the least amount of drug quantity to be identified and measured in the selected method by the system. This will be calculated by theoretically with help of slope formula and inject the same quantity of drug into the HPLC to find the voltage signal and compare with the placebo result and it should not be less than 3.3 and 10 respectively this is represented in the table No: 08.

8. Robustness and ruggedness: the ability of the method will be read by conducting the robustness of the method it can be carried by injecting the same concentration of the solution with change in the flow rate, injection volume, mobile phase pH, temperature and other conditions of the HPLC system. This would be represented in the table No: 9 & 10.

All the represented values should be presented as per the Q3 guideline of ICH and if the analytical value is less than 1.0 it would be represented two numeric after decimal point and if the value is greater than 1.0 it would be represented one numeric after the decimal point.

RESULTS AND DISCUSSION

Optimized Method: The method is developed by using column filled with non polar stationary phase it is a C18 column makes Altima having 4.6*150*5 micron dimension. The HPLC instrument made setting with flow rate of 1 ml/ min. and 40^0c column temperature maintained and eluent is detected at 225 nm. Each protruded injection having 10 µl capacity and run for 7 min. The placebo, standard and sample graphs are represented as:



Validation of method

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Pibrentasvir	2.120	607770	130275		0.98	1253
2	Glecaprevir	3.536	2225592	93740	2.0	1.23	1836

Table No. 2: Pibrentasvir.

	System suitability	Precision	Ruggedness/IP	Assay %
Inj – 1	608452	602223	596608	98.5
Inj – 2	606820	607748	598959	99.4
Inj – 3	608452	607302	595728	99.3
Inj – 4	595267	608674	594485	99.5
Inj – 5	596608	607376	595267	99.3
Inj – 6	603119.8	606665	596608	99.2
Mean	603119.8	606664.6	596209	99.2
SD	5909.75	2273.83	1718.7	0.37
RSD	0.97	0.37	0.29	0.37

Table No. 3: Glecaprevir.

	System suitability	Precision	Ruggedness/IP	Assay %
Inj – 1	2234724	2220333	2231134	100.3
Inj – 2	2240080	2221573	2334210	100.5
Inj – 3	2234724	2215483	2347461	100.3
Inj – 4	2204466	2217379	2354301	98.9
Inj – 5	2209574	2211255	2334710	99.1
Inj – 6	2224714	2217205	2331247	99.8
Mean	2224713.6	2217204.6	2322177.1	99.8
SD	14667.7	3667.7	45484.4	0.65
RSD	0.65	0.16	1.9	0.65

Table No. 4: Linearity.

Conc.	Pibrentasvir	Glecaprevir
10 & 4	205035	757881
20 & 8	381239	757881
30 & 12	561128	1458941
40 & 16	740162	2132457
50 & 20	909922	2901811
Slope	36199	56304
Correlation	0.999	0.999

Accuracy: The three different level concentrations are protruded into the HPLC and studied for its recovery in formulations at each level. The results will be shown in the following tables. The analytical data is calculated for its percentage recovery in each time. 50%, 100% and 150% with respect to original concentration.

Table No. 5.

Inj No	Name	Rt	50%	100%	150%
1	Pibrentasvir	2.101	287560	551452	826948
	Glecaprevir	3.525	1105806	2134724	3169317
2	Pibrentasvir	2.111	287610	551820	818888
	Glecaprevir	3.541	1102788	2140080	3174580
3	Pibrentasvir	2.112	288152	551213	829689
	Glecaprevir	3.539	1105752	2041160	3290022

Table No. 6: The accuracy results for Pibrentasvir.

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	287774	7.5	7.56	100.8	99.6%
100%	551495	15	14.8	98.6	
150%	825175	22.5	22.4	99.5	

Table No. 7: The accuracy results for Glecaprevir.

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1104782	18.75	18.73	100%	100%
100%	2105321	37.5	37.4	99.9%	
150%	3211306	56.25	56.21	100%	

Table No. 8: Limit of Detection & Limit of Quantitation.

	Pibrentasvir in ppm	Glecaprevir in ppm
LOD	0.2	2.3
LOQ	0.8	7.04

Robustness: This is checking the developed method for its robustness when change in environmental factors like change in flow rate, mobile phase proportions, wave length and study of their effect in the identification of drug. The results are summarized as.

Table No. 9: Pibrentasvir.

Parameter used for sample analysis	Peak Area	Retention time	USP plates	Tailing
Actual Flow rate of 1.0 mL./min	551495	2.102	5586	1.7
Less Flow rate of 0.9 mL./min	564735	2.330	5231	1.7
More Flow rate of 1.1 mL./min	508920	1.950	5234	1.7
Less organic phase	566093	2.290	5643	1.4
More organic phase	543559	1.998	5298	1.5

Table No. 10: Glecaprevir.

Parameter used for sample analysis	Peak Area	Retention Time	USP Plates	Tailing
Actual Flow rate of 1.0 mL./min	2105321	3.537	5371	1.6
Less Flow rate of 0.9 mL./min	2005636	3.885	5324	1.7
More Flow rate of 1.1 mL./min	2108920	3.263	5098	1.7
Less organic phase	2239255	4.435	5239	1.2
More organic phase	2200346	3.009	5647	1.0

SUMMARY AND CONCLUSION

This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Pibrentasvir and Glecaprevir was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) was chosen as the mobile phase. The solvent system used in this method was economical. The retention times were achieved 2.102 & 3.537 Min respectively. The % RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

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