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A STUDY OF METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION FOR SIMULTANEOUS QUANTIFICATION OF GLECAPREVIR AND PIBRENTASVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT
Objective: The present paper describes a simple, accurate and precise reversed phase HPLC method for rapid and simultaneous quantification of Glecaprevir and Pibrentasvir in bulk and pharmaceutical dosage form. The chromatographic separation was achieved on Luna C18 (150x4.6mm, 3.5µm). Mobile phase contained a mixture of 0.1% Ortho Phosphoric acid (OPA) and Acetonitrile in the ratio of 50:50 v/v, flow rate 1.0ml/min and UV detection at 258nm. The proposed method shows a good linearity in the concentration range of 10-150 µg/ml for Glecaprevir and 4-60 µg/ml for Pibrentasvir under optimised conditions. Precision and recovery study results are in between 98-102%. In the entire robustness conditions %RSD is below 2.0%. Degradation has minimum effect in stress condition and solutions are stable up to 24 hrs.

KEYWORDS: Glecaprevir and Pibrentasvir.

INTRODUCTION

Glecaprevir[1] is hepatitis C virus (HCV)[2-3] nonstructural (NS) 3/4A[4] protease inhibitor that was identified jointly by AbbVie[5] and Enanta pharmaceuticals. It is being developed as a treatment of chronic hepatitis infection in co-formulation with an HCV NS5A[6-7] inhibitor Pibrenstavir.[8] Together they demonstrated potent antiviral activity against major HCV genotypes[9] and high barriers to resistance in vitro.[10]

Pibrentasvir is an antiviral agent. In the United States and Europe, it is approved for use with Glecaprevir as the combination drug Glecaprevir/Pibrentasvir (trade name Mavyret in the US and Maviret in the EU) for the treatment of hepatitis C.[11] It is sold by Abbvie. The chemical structures of Glecaprevir and Pibrentasvir were showed in Fig.1 and 2.

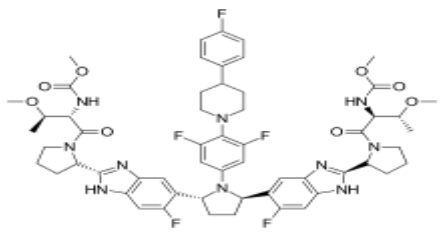

Fig. 1: Chemical structure for Glecaprevir.

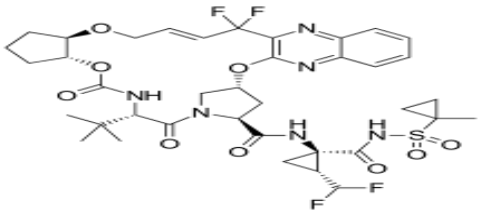

Fig. 2: Chemical structure for Pibrentasvir.

MATERIAL AND METHODS

Instrumentation

The analysis was performed on water alliance e-2695 chromatographic system equipped with a quaternary pump and PDA detector-2996. Chromatographic software empower-2.0 was used for data collection.

Chemicals and reagents

Acetonitrile (HPLC grade), Ortho Phosphoric acid (HPLC grade), Water (HPLC grade) were purchased from Merck (India) Ltd, Worli, Mumbai, India. APIs of Glecaprevir and Pibrentasvir reference standards were produced from Glenmark pharmaceuticals private Ltd., Mumbai, India Mumbai, India.

Chromatographic Conditions

Chromatographic analysis was done using isocratic elution, mobile phase in the ratio of Acetonitrile: Buffer

(0.1% OPA) (50:50 v/v) was filtered through 0.45µ membrane filter paper. The flow rate of the mobile phase was monitored at 1.0ml/min and eluents were detected at 258 nm. Injection volume 10µl with a run time 10min.

Selection of Wavelength

By using PDA detector the absorption spectra of the solution of two drugs were scanned in the UV region 200-400nm spectra shown in Fig.3, the spectra of the Glecaprevir and Pibrentasvir shown at different λ_{max} viz. 258.3 and 264.3 nm respectively. The maximum wavelength of Glecaprevir at 258.3 wave length for HPLC chromatographic method.

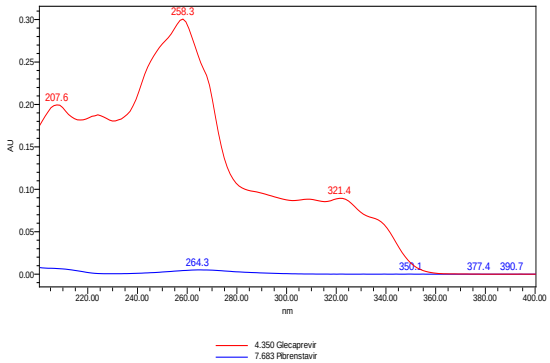

Fig. PDAspectra for Glecaprevirand Pibrentasvir.

Preparation of Standard Solution

100mg of Glecaprevir and 40mg of Pibrentasvir working standards taken into a 100ml volumetric flask. Add 70ml of mobile phase sonicated for 10min to dissolve and makeup to the mark with mobile phase. Further diluted 5ml of above two solutions to 50ml with mobile phase. As shown in Fig.4.

Preparation of Sample Solution

Weigh 10 tablets and take the average weight of one tablet equivalent weight. Crush the 10 tablets into powder form, take 180mg of sample into a 100ml volumetric flask add 70ml mobile phase sonicated for 30min after that make up to the mark with mobile phase. Further dilute 5ml of above solution to 50ml volumetric flask make up to mark with mobile phase. Filter through 0.45µ nylon syringe filter. As shown in Fig.5.

Validation

System suitability

As per the test method the standard solutions were prepared and injected into HPLC system from which the evaluated system suitability parameters are found to be within the limits.

Linearity

The ability of the method to produce results those are directly or indirectly proportional to the concentration of the analyst in samples within the limits.

Precision

The degree of closeness of agreement among individual test results when a method is applied to multiple samplings of a homogeneous sample. It is a measure of either the degree of reproducibility (agreement under different conditions) or repeatability (agreement under the same conditions) of the method.

Accuracy

The closeness of results obtained by a method to the true value. It is a measure of the exactness of the method.

Limit of detection and quantification

The detection limit and quantification limit for each analyse were determined based on a signal-to-noise concept, as the lowest concentration at which signal-to-noise ratio between 3 or 2:1 and 10:1 respectively, with defined precision and accuracy under the given experimental conditions.

Stability

Standard and the sample solutions were subjected to 24hrs stability studies at RT and 2-8°C. The stability of these solutions was studied and observed for changes in the area and retention time of the peaks which were then compared with the pattern of the chromatogram of the freshly prepared solution.

Robustness

Robustness of the method was studied by slightly changes in experimental conditions like flow rate, organic composition. Performed by same analyst with same instrument.

Ruggedness

Ruggedness of the method was studied by using different source of analyst, instruments and columns with same experimental.

RESULTS AND DISCUSSION

Method Validation

In this method system suitability, linearity, precision, accuracy, robustness, LOD, LOQ, forced degradation and stability are validated for the selected Glecaprevir, Pibrentasvir drugs.

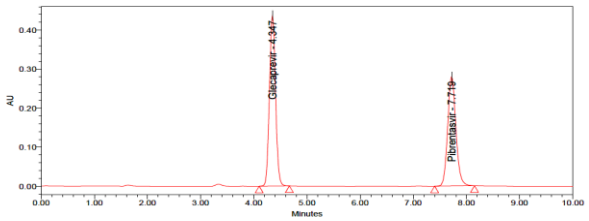

Fig. 4: Standard Chromatogram.

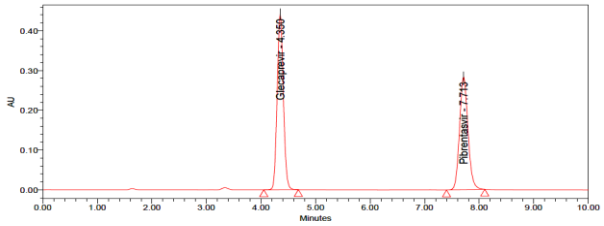

Fig. 5: Sample Chromatogram.

System Suitability

Standard solution is prepared in the concentration of Glecaprevir 100µg/ml and Pibrentasvir 40 µg/ml were prepared and injected into system. The retention times of Glecaprevir and Pibrentasvir were found to be 4.34 and 7.72 min respectively. Resolution of the Pibrentasvir was 8.36 from the Glecaprevir. The number of theoretical plate counts for Glecaprevir and Pibrentasvir were 8560 and 4836 respectively. Tailing factor for Glecaprevir and Pibrentasvir were 1.11 and 0.94 respectively. All the parameters found to be within the limit.

Linearity

Linearity of the method was evaluated by preparing a standard solution containing 100µg/ml of Glecaprevir and 40µg/ml of Pibrentasvir (100% targeted level of the assay concentration).

Sequential dilutions were performed to give solutions at 10, 25, 50, 100, 125 and 150% of the target concentrations. These were injected and peak areas used to plot calibration curves against the concentration. The correlation coefficient values of these three analyte were 0.999. The results are shown in Table 1 and Fig.6 and 7.

Table 1: Linearity Study Results.

Analyte	Linearity Range	Equation of calibration curve	Correlation coefficient
Glecaprevir	10-150 µg/ml	Y=26738x+23225	0.999
Pibrentasvir	4-60 µg/ml	Y=7126x+3365	0.999

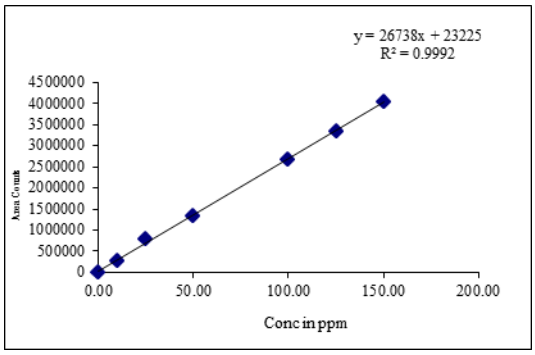

Fig. 6: Linearity plot for Glecaprevir.

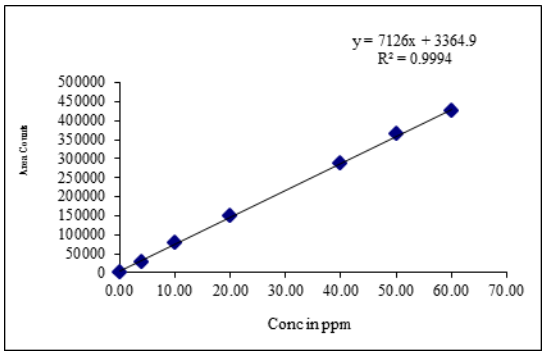

Fig. 7: Linearity plot for Pibrentasvir.

Limit of detection and quantification

Limit of detection and quantification is the minimum concentration level at which the analyte can be reliably detected, quantified by using the standard formulas (3.3times σ/s and 10times σ/s for LOD and LOQ respectively). LOD values for Glecaprevir and Pibrentasvir were 0.2 and 0.05 µg/ml and s/n values are 4 and 7 respectively. LOQ values for Glecaprevir and Pibrentasvir were 0.66 and 0.165 µg/ml and s/n values are 25 and 28 respectively.

Precision

Method precision was investigated by the analysis of six separately prepared samples of the same batch. From this six separate samples solutions was injected and the peak areas obtained used to calculate mean and percentage RSD values. The present method was found to be precise as % RSD of the less than 2%, and also the percentage assay values were closed to be 100%. The results are given in Table 2.

Table 2: Method precision results.

Analyte	Amount present	%Assayas is (mean)	%RSD of assay
Glecaprevir	100 µg/ml	100.23	0.55
Pibrentasvir	40 µg/ml	100.09	0.32

Accuracy

Accuracy was determined by recovery studies which were carried out in three different concentrations levels (50%, 100% and 150%). APIs with concentration 100, 200 and 300µg/ml of Glecaprevir; 25, 50 and 75µg/ml of Pibrentasvir were prepared. As per the test method, the

test solution was injected three preparations each spike level and the assay was performed. The percentage of recovery values were found to be in the range of 100.14-100.26% of Glecaprevir and 100.54-100.72% of Pibrentasvir. RSD values were found to be less than 2%. The results are given in table 3.

Table 3: Accuracy (recovery) study results.

% of Target Conc.	Glecaprevir (%recovery)	Glecaprevir (%RSD)	Pibrentasvir (%recovery)	Pibrentasvir (%RSD)
50	100.23	0.21	100.51	0.43
100	100.45	0.56	100.42	0.55
150	100.33	0.45	100.68	0.89

Ruggedness

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The value of percentage of RSD was below 2% exhibits the ruggedness of the developed method.

Robustness

Robustness of the method found to be %RSD should be less than 2%. Slight variations were done in the optimised method parameters like flow rate (±20%), organic content in mobile phase (±5%). The results are given in Table 4.

Table 4: Robustness Results.

Drug Name	Flow Plus (1.2ml/min) (%RSD)	Flow Minus (0.8ml/min) (%RSD)	Organic Plus (55:45) (%RSD)	Organic Minus (45:55) (%RSD)
Glecaprevir	0.65	0.53	0.47	0.38
Pibrentasvir	0.71	0.69	0.54	0.45

Stability

Stability of standard and sample solutions are studied initial to 24hrs in stored RT and 2-8°C. They are injected at different time intervals different between initial to 24hrs percentage of assay not more than 2%. There no effects in storage conditions for Glecaprevir and Pibrentasvir drugs. The results are shown in below Table 5.

Table 5: Stability Results.

	Glecaprevir % Assay	% Difference	Pibrentasvir % Assay	% Difference
Initial	100.54	0.00	100.56	0.00
12Hr	100.62	0.04	100.49	0.07
18Hr	100.53	0.30	100.36	0.12
24Hr	100.46	0.36	100.22	0.34

Forced Degradation

Forced degradation conditions such as acidic, basic, oxidative, reduction, thermal, hydrolysis and photolytic stresses were attempted as per ICH guidelines Q2B. The effect of assay on their result. The results are shown in Table 6.

Table 6: Forced degradation Results.

Degradation	Glecapevir(% Assay)	% of Degradation	Pibrentasvir (%Assay)	% of Degradation
Control	100.3	0.00	100.35	0.00
Acid	86.45	11.28	84.55	13.22
Alkali	91.56	9.02	91.42	11.04
Peroxide	84.79	15.52	82.36	16.55
Reduction	94.22	4.18	88.75	11.96
Thermal	74.29	23.04	71.93	25.84
Photolytic	87.46	9.86	86.87	10.56
Hydrolysis	82.72	14.92	82.52	14.44

CONCLUSION

Glecaprevir and Pibrentasvir are two drugs reported as novel and method is novel. And is very strong discussion for the developed method in their validation. This method described the quantification of Glecaprevir and Pibrentasvir in bulk and pharmaceutical formulation as per ICH guidelines. The developed method was found to be accurate, precise, linear and reliable. The advantage

lies in the simplicity of sample preparation and economically fewer reagents were used. In addition two compounds are eluted with in 10min. The purposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Statistical analysis of the experimental result indicates that the precision and reproducibility data are satisfactory.

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