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

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ABSTRACT

A simple, rapid, accurate and precise RP-HPLC method is developed for the determination of Glecaprevir and Pibrentasvir in bulk and dosage forms. Separation of the Glecaprevir and Pibrentasvir was achieved on a Cosmicsil C18 Column (250 mm x 4.6 mm, 5µm) using the mobile phase of (0.1M Phosphate buffer :Methanol) in the ratio of (65:35) at pH 4.5. The flow rate was 1.0ml/min using PDA detector at 225nm. The retention times are 1.663 min and 2.249 min, for Pibrentasvir and Glecaprevir 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 (acid and base), oxidation, photolysis and thermal degradation to show the stability-indicating power of the developed RP-HPLC method. The present method can be successfully used for routine analysis of Glecaprevir and Pibrentasvir and stability studies.

KEYWORDS: Glecaprevir, Pibrentasvir, RP-HPLC, Mavyret, validation.

INTRODUCTION

Glecaprevir is an antiviral agent, chemically described as (3aR,7S,10S,12R,21E,24aR)-7-tert-Butyl-N-{(1R,2R)-2-(difluoromethyl)-1-[(1-methylcyclopropanesulfonyl)carbamoyl]cyclopropyl}-20,20-difluoro-5,8-dioxo 2,3,3a,5,6,7,8,11,12,20,23,24 a-dodecahydro-1H, 10H-9, 12-methanocyclopenta (18,19)(1,10,17,3,6) trioxadiazacyclononadecino [11,12-b] quinoxaline-10-carboxamide. Glecaprevir acts nonstructural protease 3/4A protease inhibitor. These two enzymes are essential for hepatitis C viral RNA replication and viron assembly.

Pibrentasvir is also an antiviral agent and chemically known as 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-(methoxycarbonylamino) butanoyl] pyrro lidin-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 (Figure 1). Pibrentasvir serve as nonstructural protease 5A inhibitor. Nonstructural protease 5A enzyme is required for hepatitis C viral RNA replication and viron assembly.

EXPERIMENTAL

Equipments: The chromatographic technique was performed on Alliance waters e2695, with photo diode array detector (2998) employing empower 2 software and Cosmicsil C18(250*4.6mm,5µm) as stationary phase, ultra Sonicator, electronic balance, vacuum micro filtration unit with 0.45 membrane filter, caliberated borosil glassware.

Chemicals and reagents: Pharmaceutically pure samples of Glecaprevir and Pibrentasvir were obtained as gift samples from HETERO Pharmaceuticals jeedimetla, Hyderabad. HPLC grade water from lobachemi, HCl, NaOH, methanol from Merck, KH2PO4 from Finar. Tablet formulation: Mavyret tablet (100mg Glecaprevir and 40mg Pibrentasvir).

Preparation of buffer (0.1M):13.609gm of potassium dihydrogen phosphate is mixed in 1000ml of HPLC grade water and subjected to vacuum filtration and sonication for 15min.

Preparation of mobile phase: KH_2PO_4 of 0.1 M is blended in 65:35 volume/volume parts with Methanol, Orthophosphoric acid is used to alter pH to 4.5. This mixture is also applied as a solvent in the development of standard solutions.

Preparation of standard and sample solutions: Implicated in the preparation of stock solution of Pibrentasvir and glecaprevir, a properly weighed 40 mg Pibrentasvir and 100 mg Glecaprevir in a 100 ml volumetric flask and exactly diluted with mobile phase. Concentration of stock solutions: Pibrentasvir 400 µg/ml and Glecaprevir 1000 µg/ml. Twenty tablets are taken and weighed individually and their average weight is determined. Powder equivalent to 428mg of Glecaprevir and Pibrentasvir is transferred into 100ml volumetric flask and concentrations are made.

RESULTS AND DISCUSSIONS

In developing the new RP-HPLC method a systematic study of the effect of various factors (i.e, the influence of column, aqueous and organic phase for mobile phase, mobile phase proportion, wavelength, diluent, concentration of analyte and other chromatographic parameters) was carried out by varying one parameter at a time and keeping all other conditions constant. From these studies it is revealed that Cosmicsil, C18-Column (4.6 mmx250 mm) having 5 µm particle size was used as stationary phase for separation of Glecaprevir and Pibrentasvir among the other columns because of its advantages of high degree of retention, high resolution capacity, better reproducibility, ability to produce lower back pressure and low degree of tailing. A good symmetrical peaks for Glecaprevir and Pibrentasvir were obtained. Preliminary trials on mobile phase proportion were carried out to provide good resolution for Glecaprevir and Pibrentasvir. Using different compositions of mobile phase. From these trails, the proportion of potassium dihydrogen phosphate buffer (pH-4.5) and methanol in the ratio of 65:35v/v was finalized as it gave good symmetrical peak for Glecaprevir and Pibrentasvir. The appropriate wavelength for determination was scanned by UV-visible spectrophotometer and it was observed that the maximum absorbance (λmax) was obtained at 225nm. At this wavelength both the drugs offered high response with good linearity. The best separation with adequate resolution and symmetric peas for Glecaprevir and Pibrentasvir were obtained with the injection volume of 10 µL at a flow rate of 1.0 ml/min for the mobile phase respectively. On this finalized chromatographic conditions, chromatogram of the drugs exhibited good peak symmetry with higher theoretical plates. The representative chromatogram of Glecaprevir and Pibrentasvir is shown in Fig.2.

METHOD VALIDATION

After fixing the optimization studies the developed method was validated as per ICH guidelines which include system suitability, specificity, linearity, accuracy test, precision, robustness, ruggedness, sensitivity, limit of detection and quantification. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions of Glecaprevir and Pibrentasvir. The values demonstrated the suitability of the system for the analysis of Glecaprevir and Pibrentasvir dosage forms and the results of these studies were summarized in **Table.1**. The specificity of the proposed method for Glecaprevir and Pibrentasvir were studied and calculated basing on the resolution factor of the peak and were found to be free of interference from the excipients used in pharmaceutical formulation and it indicates the specificity of the system. In the present study, the drugs were subjected to

various stress degradation studies as per the ICH recommended guidelines. As Glecaprevir and Pibrentasvir are soluble in methanol all solutions of Glecaprevir and Pibrentasvir for use in forced degradation studies were prepared in methanol. This is done by subjecting Glecaprevir and Pibrentasvir standard reference solution to acidic (0.1N HCl), basic (0.1N NaOH), oxidizing (30% H_2O_2), and photo stability stress conditions. The chromatograms obtained under acidic stress, basic stress and photo stability stress conditions revealed that Glecaprevir and Pibrentasvir are stable, did not show any degradation and is eluted from the column respectively. The oxidative stress studies revealed that Glecaprevir and Pibrentasvir are not fully degraded and its degradation products were eluted separately at different retention times respectively. From the respective chromatograms, it was observed that the degradation products did not interfere in the detection analysis of Glecaprevir and Pibrentasvir establishing the high stability of the developed method, For linearity studies concentration levels corresponding to of test solution[50µg/ml –150 µg/ml] of Glecaprevir and[20-60 µg/ml] Pibrentasvir were prepared separately and was injected into the prescribed HPLC system and the response was read at 225 nm and the corresponding chromatograms were recorded. From these chromatograms, a calibration curve was constructed by plotting the peak areas of the drugs versus concentration of Glecaprevir and Pibrentasvir (Figs.3). The linear regression equation for the calibration curve of Glecaprevir and Pibrentasvir was found to be Glecaprevir Y= 27169x+2922 with a coefficient of regression, R^2=0.9998 respectively, Pibrentesvir Y=9378+949.4, with a regression coefficient R^2=0.998. The calibrated results of Glecaprevir and Pibrentasvir were tabulated in **Table. 2**. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by calculating the signal to noise (S/N) ratio. The LOD values of Glecaprevir and Pibrentasvir were found to be 0.207 µg/ml and 0.190µg/ml respectively. LOQ values of Glecaprevir and Pibrentasvir were found to be 0.690 µg/ml and 0.634 µg/ml respectively.

Precision of the proposed method was determined by repeatability (intra-day precision). It was expressed as % relative standard deviation (%RSD). The percent relative standard deviation (% RSD) was calculated and it was found to be 0.097 for Pibrentasvir and 0.232 for glecaprevir, which are within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in **Table.3**. The accuracy of the proposed method was assessed by determination of recovery for three concentrations in triplicate (corresponding to 50, 100 and 150% level of test solution concentration) of Glecaprevir and Pibrentasvir covering the within the linearity range of the proposed method. The percentage recovery was calculated

and results are compiled in **Table.4.** These results indicate a high degree of accuracy of the proposed method for determination of Glecaprevir and Pibrentasvir. The ruggedness of the present RP-HPLC method was determined by carrying out the experiment by different analysts using different columns of similar types. Robustness of the method was determined by small deliberate changes in flow rate, and temperature. The robustness limit for flow rate variation and temperature variation were well within the limit, revealing that the proposed method is robust under given set of defined experimental conditions **(Table.5)**. The proposed RP-HPLC method has been validated for the assay of Glecaprevir and Pibrentasvir in tablet as per guidelines of ICH. Twenty tablets of MAVYRET [Label claim; 100mg of Glecaprevir and 40g Pibrentasvir] were procured from local pharmacy and were powdered. An accurately weighed portion of powder equivalent to 100 mg of Glecaprevir and 40mg Pibrentasvir dissolved in 30ml of methanol and filtered through 0.45 µm membrane filter. From this filtrate, 1ml was pipetted in to 10 ml graduated test tube and made up to volume with the mobile phase. 20 µL of this sample was injected into the column and the drug content in the tablet was quantified using the regression equation and the chromatogram and the results are shown in **Table 6** and the data of degradation studies was shown in **Table 7.**

Table 1: System suitability parameters.

Parameters	Pibrentasvir	Glecaprevir
Retention time	1.66	2.24
Peak area	945840	2719026
USP tailing	1.38	1.28

Table 2: Calibration of RP-HPLC for Estimation of Glecaprevir and Pibrentasvir.

Concentration (µg.mL) Glecaprevir	Area	Concentration (µg.mL) Pibrentasvir	Area
50	1353890	20	469419
75	2038068	30	704724
100	2712858	40	939681
125	3392084	50	1171910
150	4073013	60	1408097
Intercept(a)	949.4	Intercept(a)	2922.
Slope(b)	9378x	Slope(b)	27169x
LOD	0.20	LOD	0.19
LOQ	0.69	LOQ	0.63

Table 3: Results of Precision.

Area response	Glecaprevir	Pibrentasvir
Injection 1	2708652	939412
Injection 2	2716419	938701
Injection 3	2705033	939606
Injection 4	2714205	938661
Injection 5	2700375	937041
Injection 6	2714368	938340
Average	2709842	938627
Std deviation	6276.050414	914.2279
%RSD	0.232	0.097

Table 4: Recovery studies (Average).

Level spiked	"µg/ml" added pibrentasvir	"µg/ml" found pibrentasvir	"%" recovered pibrentasvir
50%	19.8	19.8	99.99
100%	39.6	39.63	100.07
150%	59.4	59.07	99.4

Level spiked	"µg/ml" added Glecaprevir	"µg/ml" found Glecaprevir	"%" recovered Glecaprevir
50%	49.5	49.10	99.18
100%	99.0	99.16	100.16
150%	148.5	148.87	100.45

Table 5: Evaluation of Robustness data.

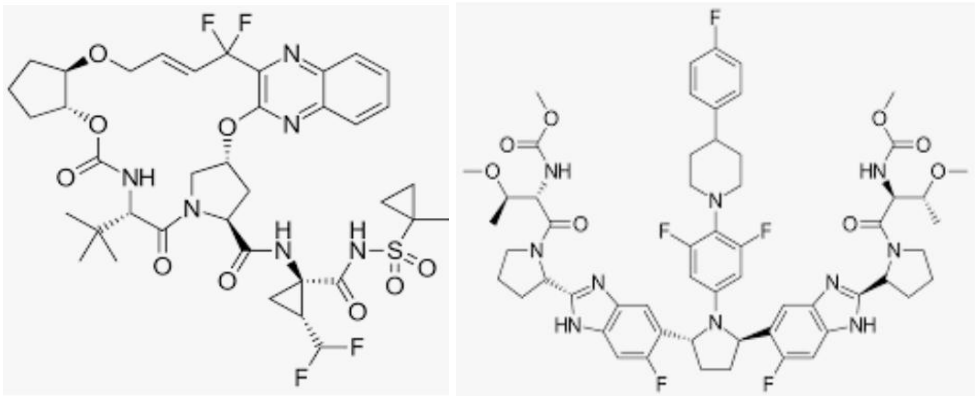
Robust conditions	Pibrentasvir		Glecaprevir	
	RT	Peak area	RT	Peak area
Flow rate 0.9ml/min	1.37	787370	1.84	2291831
Flow rate 1.1ml/min	1.49	859477	2.00	2506019
Temperature $23^{0}C$	1.82	1059372	2.4	3070004
Temperature $27^{0}C$	2.05	1191705	2.7	3463366
Composition (methanol 30%ratio)	1.37	787370	1.8	2291831
Composition (methanol 40%ratio)	1.82	1059372	2.4	3070004
pH4.4	1.65	942412	2.2	2718652
pH4.6	1.65	939701	2.2	2716419

Table 6: Results of Assay.

Sample Name	Pibrentasvir area	Rt	Glecaprevir area	Rt
Standard	945840	1.666	2719026	2.246
Sample	939412	1.656	2708652	2.233

Table 7: Degradation Studies of Pibrentasvir and Glecaprevir.

Test	Pibrentasvir			Glecaprevir		
	Area Response	"%" remained	"%" degraded	Area Response	"%" remained	"%" degraded
Acid	857202	90.38	9.62	2505748	91.68	8.32
Alkali	906340	95.56	4.44	2594536	94.93	5.07
H_2O_2	914387	96.41	3.59	2646545	96.83	3.17
Dry heat	845426	89.14	10.86	2403035	87.92	12.08
Sun light	904050	95.32	4.68	2555828	93.51	6.49



Glecaprevir **Pibrentasvir**

Figure 1: Chemical structures of Glecaprevir and Pibrentasvir.

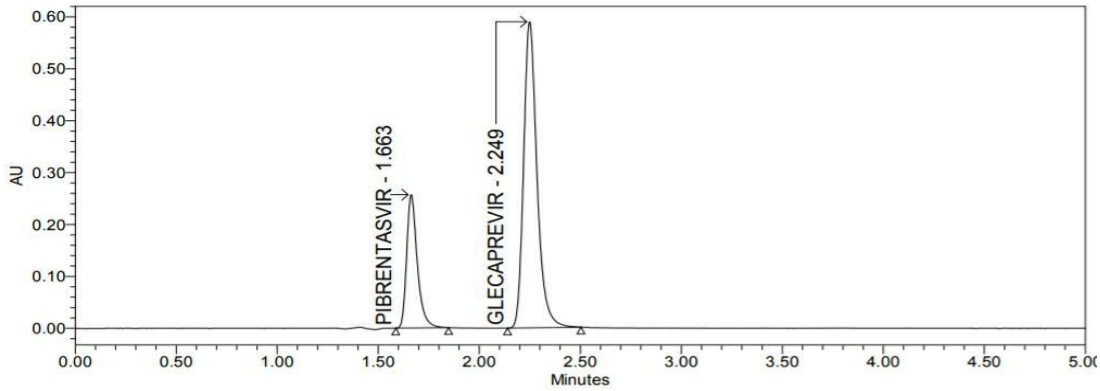


Figure 2: Validative Chromatogram of Glecaprevir and Pibrentasvir.

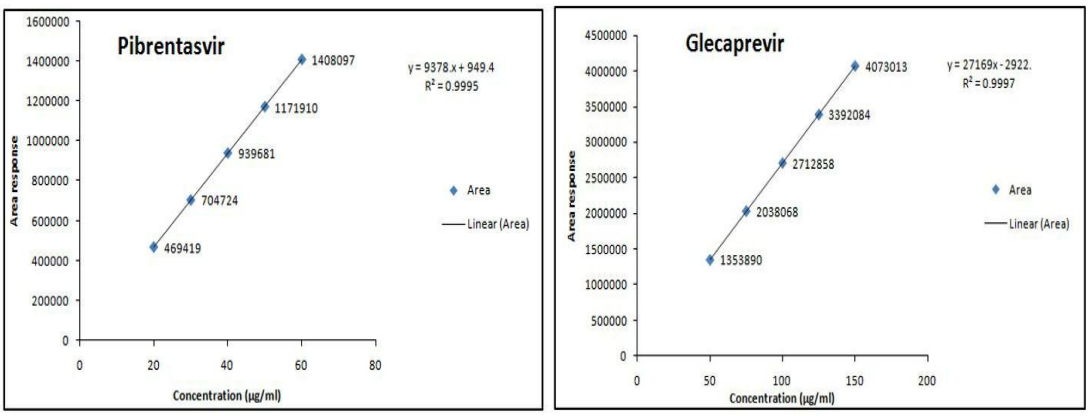


Figure 3: Linearity curves of Pibrentasvir and Glecaprevir.

CONCLUSION

Based on all the results, it can be concluded that a simple, accurate and stability indicating RP-HPLC method has been developed and validated for the analysis of Glecaprevir and Pibrentasvir in bulk and tablet dosage forms. Statistical analysis proved that the method is suitable for the analysis of Glecaprevir and Pibrentasvir in pure and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Glecaprevir and Pibrentasvir can be conveniently used for the routine assay of Glecaprevir and Pibrentasvir by the pharmaceutical manufacturing units.

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