INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.12.10.2021.11(44)

DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LECAPREVIR AND PIBRENTASVIR IN COMBINED TABLET DOSAGE FORMS

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ABSTRACT

The study describes method development and subsequent validation of RP-HPLC method for simultaneous estimation of glecaprevir and pibrentasvir in combined tablet dosage forms. Chromatographic separation was achieved on a thermo C18 column (150 mm x 4.6 mm, 5 µm) using a mobile phase consisting of (65:35v/v) phosphate buffer: methanol at a flow rate of 1 mL/min. The detection wavelength is 226 nm. The retention times of glecaprevir and pibrentasvir were found to be 3.14 min and 3.73 min respectively. The developed method was validated as per ICH guidelines. The developed and validated method was successfully used for the quantitative analysis of glecaprevir and pibrentasvirin tablet dosage forms.

Keywords: Glecaprevir, Pibrentasvir, RP-HPLC method and Validation.

INTRODUCTION

Glecaprevir[1] chemically, (1R,14E,18R,22R,26S,29S)-N-[(1R,2R)-2-(Difluoromethyl)-1-{[(1-methylcyclopropyl) sulfonyl]carbamoyl}cyclopropyl]-13,13-difluoro-26-(2-methyl-2-propanyl)-24,27-dioxo-2,17,23-trioxa 4,11,25,28 tetraaza penta cyclo [26.2.1.$0^{3,12}$.$0^{5,10}$.$0^{18,22}$]hentriaconta-3,5(10), 6,8,11,14-hexaene-29-carboxamide**(Figure 1)** is adirect acting antiviral agent that targets the viral RNA replication.Pibrentasvir[2,3]chemically,(2S,3R)-1-[(2S)-2-{5-[(2R,5R)-1-{3,5-difluoro-4-[4-(4-fluorophenyl) piperidin-1-yl]phenyl}-5-{6-fluoro-2-[(2S)-1-[(2S,3R)-2-{[hydroxy(methoxy) methyl idene]amino}-3-methoxybutanoyl]pyrrolidin-2-yl]-1H-1,3-benzodiazol-5-yl} pyrrolidin-2-yl]-6-fluoro-1H-1,3 benzo diazol-2-yl}pyrrolidin-1-yl]-2-{[hydroxy (methoxy) methyl idene]amino}-3-methoxybutan-1-one **(Figure 2)** is an oral antiviral agent that acts on the viral RNA replication and viron assembly. It is useful for patients who experienced therapeutic failure from other NS5A protease inhibitors. Literature survey reveals few analytical methods[4-7] have been reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms. The present work is aimed to develop and validate simple, sensitive and more precise RP- HPLC method for simultaneous estimation of glecaprevir and pibrentasvirin pharmaceutical tablet dosage form.

MATERIALS AND METHODS

Equipment

Separation was carried out by using Shimadzu LC20A system equipped with LC20AT pump. SPD 20A Prominence UV-Visible detector and the peak areas were integrated by using spinchrome software CFR. Analysis was carried out on thermo C18(150 mm x 4.6 mm, 5 µm) column.

Chemicals and reagents

HPLC grade methanol and analytical grade potassium dihydrogen phosphate obtained from M/s. Rankem Chemicals Ltd, Mumbai, India. Milli-Q water dispensed through a 0.22 µ filter of the Milli-Q water purification system

(Millipore, Merck KGaA, Darmstadt, Germany) was used throughout the experiment.

Preparation of solutions

Preparation of Mobile phase

Mobile phase was prepared by mixing phosphate buffer and methanol in the ratio 65:35 v/v. Mobile phase was filtered through 0.45µ membrane filter and sonicated for 10 min to remove dissolved gases before transferring in to the reservoir.

Preparation of Diluent

A 50:50 v/v mixture of water and methanol was prepared and used as the diluent in the preparation of drug dilutions.

Preparation of mixed standard solution of glecaprevir and pibrentasvir

About 100 mg of glecaprevir and 40 mg of pibrentasvirwere accurately weighed and transferred into a 100 mL clean dry volumetric flask containing 70 mL of the diluent. The solution was sonicated for 20 min and then volume was made up to the mark with a further quantity of the diluent to get a concentration of 1000 µg/mL of glecaprevir and 400 µg/mL of pibrentasvir (Stock solution).

Preparation of the tablet solution

Twenty tablets of the commercial sample of Mavyret were weighed and finely powdered. An accurately weighed portion of powdered sample equivalent to 100 mg of glecaprevir and 40 mg of pibrentasvir was transferred into a 100 mL volumetric flask containing 70 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drugs and the volume made up with a further quantity of the diluent. Then, this mixture was filtered through a 0.45 µ membrane filter. This filtrate was used for further analysis.

Chromatographic conditions

A reverse phase column thermo C18 column (150 mm x 4.6 mm, 5 µm particle size), equilibrated with mobile phase (phosphate buffer: methanol in the ratio of 65:35 v/v) was used. Mobile phase flow rate was maintained at 1 mL/min and effluents were monitored at 226 nm. The sample was injected using 10 micro litre manual sample injector and run time was 6 min.

Procedure

Under optimized chromatographic conditions 20 µl of each standard of linearity range was injected and chromatograms were recorded. Typical chromatogram of glecaprevir and pibrentasvir is given in **Figure3**.

METHOD VALIDATION

System suitability

The system suitability studies were done for parameters like theoretical plates, tailing factor, retention time, resolution by injecting the standard solution in to the optimized chromatographic system for six times and the results are given in the **Table 1**.

Linearity

Linear calibrations plots of the proposed method were obtained over concentration ranges of 50-150 µg/mL for glecaprevir (50, 75, 100, 125, 150µg/mL) and20-60 µg/mL for pibrentasvir (20, 30, 40, 50, 60µg/mL). Each solution was prepared in triplicate. Regression coefficient was found to be 0.999 and 0.998 for both the drugs**.** Standard curve had a reliable reproducible over the standard concentrations across the calibration range. All back calculated concentrations did not differ from the theoretical value as no single calibration standard point was dropped during the validation.

Accuracy

The standard addition method was used to demonstrate the accuracy of the proposed method. For this purpose, known quantities of glecaprevir and pibrentasvir were supplemented to the previously analysed sample solution and then experimental and true values compared. Three levels of solutions were made corresponding to 50, 100 and 150 % of nominal analytical concentration (100µg/ml glecaprevir and 40 µg/ml for pibrentasvir). Standard preparation & sample preparation was injected into the HPLC and % RSD for glecaprevir and pibrentasvir peaks in standard preparation was calculated and tabulated in **Table2.**The mean recovery values of glecaprevir and pibrentasvir were found to be 99.46, 99.45 % respectively.

Precision

For precision same concentration solution i.e. 100 µg/ml of glecaprevir and 40µg/ml of pibrentasvir solution was injected 6 times and observed for any peculiar change in the areas and % RSD was calculated for each drug.

System Precision

For system precision study the standard solution replicates was injected repeatedly for six times and was observed. The standard deviation values were found to be 2131.66 and 4448.36 for glecaprevir and pibrentasvir and

the % RSD values were 0.11 and 0.10 for glecaprevir and pibrentasvir and the results are tabulated in the **Table 3.**

Intermediate precision

Six replicate injections of the same dilution were analyzed on two different days by different analyst for verifying the variation in the precision. The % RSD of the results for glecaprevir and pibrentasvir were found to be 0.1 and 0.08 respectively, which are within acceptable limit of ≤2. Hence, the method is reproducible on different days with different analyst. This indicates that the method is precise. The results are shown in the **Table 4a** and **4b.**

Robustness

Robustness is generally done by changing the parameters like flow rate, organic phase of the mobile phase and column temperature.The results are shown in the following data is given in the **Table 5**and **6.**

Limit of detection (LOD)

The LOD for this method was found to be 0.381µg/mL and 0.069µg/mL for glecaprevir and pibrentasvir respectively.

Limit of quantitation (LOQ)

The LOQ for this method was found to be 1.27µg/mL and 0.232µg/mL for glecaprevir and pibrentasvir respectively.

Assay

Twenty micro litres of standard and sample solutions were injected separately in to the chromatographic system and the peak areas for the analyte peaks were measured. The % content of each drug was calculated.

RESULTS AND DISCUSSION

To develop a new RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation with good peak symmetry was obtained. In the present study, a new simple, precise and accurate HPLC method was developed and validated for the simultaneous estimation of glecaprevir and pibrentasvir in tablet dosage forms. In this method, a thermo C18 (250 x 4.6 mm; 5 µm) column using mobile phase containing phosphate buffer and methanol (65:35 v/v) at a flow rate of 1 mL/min. Quantification was achieved with UV detection at 226 nm based on peak area. The retention time for glecaprevir and pibrentasvir were found to be 3.140 and 3.733 min, respectively. The optimized method was validated as per ICH guidelines. The System suitability parameters observed by using this optimized conditions were reported. A linearity range of 50-150 µg/mL with correlation coefficient 0.999 was established for glecaprevir and 20-60 µg/mL with correlation coefficient 0.998 was established for pibrentasvir. The precision of the proposed method was carried in terms of the repeatability and the %RSD values of glecaprevir was found to be 0.11 % and of pibrentasvir was found to be 0.10 % and reveal that the proposed method is precise. The LOD and LOQ values for glecaprevir were0.381 and 1.27 µg/mL respectively and for pibrentasvir were found to be 0.069 and 0.232 µg/mL. The study of robustness in the present method shows no significant changes either in the peak area or Room temperature. The results of analysis of commercial formulation indicated that there is no interference due to common formulation excipients with the developed method. Therefore, the proposed method can be used for routine analysis of these two drugs in their combined pharmaceutical dosage form.

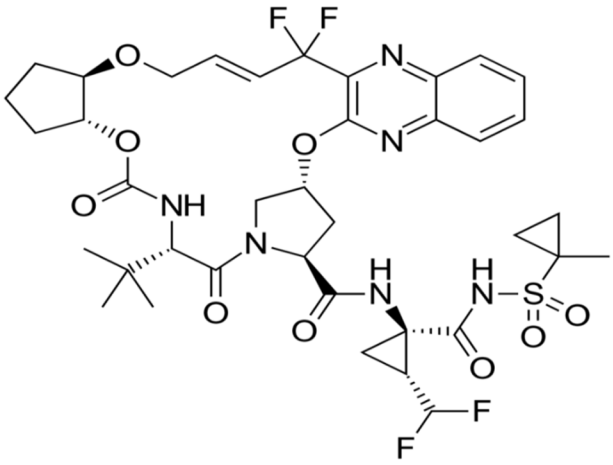

Fig. 1: Chemical structure of Glecaprevir

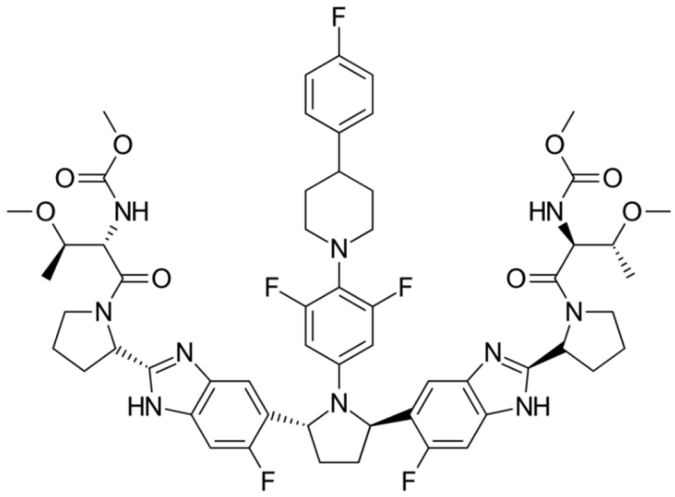

Fig. 2: Chemical structure of Pibrentasvir

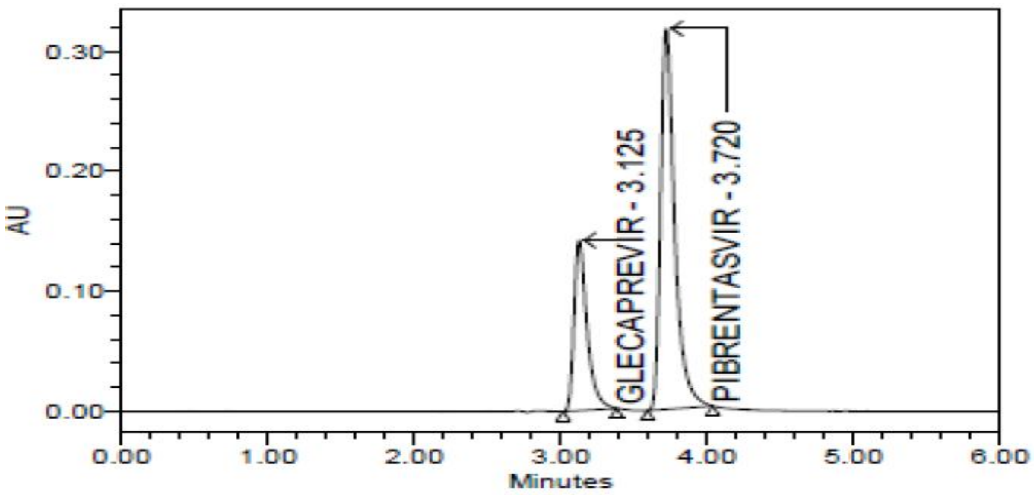

Fig. 3: Chromatogram showing separation of glecaprevir and pibrentasvir

Table 1: System suitability of glecaprevir and pibrentasvir

S. No.	Glecaprevir			Pibrentasvir		
	Area	USP Plate Count	USP tailing	Area	USP Plate Count	USP tailing
1	1894469	6543	1.61	4582633	7688	1.56
2	1895483	6612	1.59	4588371	7721	1.43
3	1891924	6537	1.55	4584942	7671	1.52
4	1895721	6551	1.63	4582499	7665	1.40
5	1890259	6609	1.52	4589132	7679	1.37
6	1893589	6556	1.65	4582121	7658	1.51
Mean	1893571.2			4584949.66		
Std. Dev.	2385.71			3116.77		
% RSD	**0.13**			**0.07**		

Table 2: Results of recovery experiments of glecaprevir and pibrentasvir

Preanalysed amount (µg/mL)		Spiked Amount (µg/mL)		% Recovered	
Glecaprevir	Pibrentasvir	Glecaprevir	Pibrentasvir	Glecaprevir	Pibrentasvir
100	40	50	20	98.99	99.69
100	40	50	20	99.37	99.35
100	40	50	20	99.87	99.88
100	40	100	40	99.23	99.38
100	40	100	40	99.50	99.29
100	40	100	40	99.77	99.27
100	40	150	60	98.95	99.36
100	40	150	60	99.76	99.55
100	40	150	60	99.67	99.26
			MEAN	99.46	99.45
			SD	0.323	0.202
			% RSD	**0.324**	**0.203**

Table 3: Results of repeatability of glecaprevir andpibrentasvir

S. No.	Glecaprevir			Pibrentasvir		
	Area	USP Plate Count	USP Tailing	Area	USP Plate Count	USP Tailing
1	1894472	6525	1.58	4582637	7685	1.54
2	1895485	6622	1.54	4590369	7723	1.42
3	1891935	6532	1.57	4584930	7669	1.55
4	1895708	6555	1.62	4582581	7664	1.41
5	1890256	6621	1.51	4589144	7672	1.36
6	1893693	6545	1.67	4578515	7655	1.50
MEAN	1893591.5			4584696		
SD	2131.66			4448.36		
% RSD	**0.11**			**0.10**		

Table 4a: Results of intermediate precision of glecaprevir

S. No.	Average area (n=6)	USP Plate Count	USP Tailing
Day 1	1892814	6552	1.68
Day 2	1892809	6545	1.54
Overall average	1892811.5		
SD	1850.33		
% RSD	**0.10**		

Table 4b: Results of intermediate precision of pibrentasvir

S. No.	Average area (n=6)	USP Plate Count	USP Tailing
Day 1	4583037	7656	1.48
Day 2	4583035	7442	1.42
Overall average	4583036		
SD	3810.6		
% RSD	**0.08**		

Table 5: Robustness study for glecaprevir

Condition	Mean area	% assay	% difference
Optimized	1892254	99.65	-----
Flow rate at 0.9 mL/min Flow rate at 1.1 mL/min	1891774 1892988	99.04 99.79	0.61 0.14
Mobile phase • Buffer-acetonitrile (70:30) • Buffer-acetonitrile (60:40)	1893287 1893335	100.11 100.15	0.46 0.50
Column Temperature • at 25°C • at 35°C	1891563 1892221	99.03 99.59	0.62 0.06

Table 6: Robustness study for pibrentasvir

Condition	Mean area	% Assay	% difference
Optimized	4581345	99.81	----
Flow rate at 0.9 mL/min Flow rate at 1.1 mL/min	4582166 4581387	100.03 99.97	0.22 0.16
Mobile phase • Buffer-acetonitrile (70:30) • Buffer-acetonitrile (60:40)	4581234 4581195	99.65 99.23	0.16 0.58
Column Temperature • at 25°C • at 35°C	4580728 4582212	99.02 100.05	0.79 0.24

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of glecaprevir and pibrentasvir from its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of glecaprevir and pibrentasvirin pure form and its dosage form and also can be used for dissolution or similar studies.

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