ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.pharmascope.org/ijrps

Stability indicating RP-HPLC method development and validation for the simultaneous estimation of Pibrentasvir and Glecaprevir in bulk and pharmaceutical dosage form

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Article History:

Received on: 03.03.2019
Revised on: 12.06.2019
Accepted on: 16.06.2019

Keywords:

Accuracy,
Glecaprevir,
Linearity,
Pibrentasvir,
Precision,
Specificity

ABSTRACT

A plain sailing, unambiguous, speedy and error-free methodology was progressed for the quantifiable concurrent estimation of Pibrentasvir and Glecaprevir in conglomerated pharmaceutical dosage form. The contrivance was based on Chromatographic separation of both the drugs in reverse phase mode using C_{18} (250 X 4.6 mm), 5μ by utilizing phosphate buffer (pH 4.0) and Methyl alcohol in the ratio of 30:70 v/v was allowed to flow through column at a rate of 1.0 ml/min, and the detection wavelength was set at 251 nm. The time of retention was found to be 2.205 min for Glecaprevir and 4.996 min for Pibrentasvir. The dimensionality of Glecaprevir and Pibrentasvir was in linear range with a parametric statistic of 0.999 and 0.999. The acceptance criteria of precision was RSD should be not more than 2.0%, and the method showed precision 0.6 and 0.5 for Glecaprevir and Pibrentasvir, which shows that the method was precise. % Assay was found as 100.83 and 100.23, which show that the method was useful for routine analysis. The total recovery was founded to be100.40% and 100.25% for Glecaprevir and Pibrentasvir. LOD and LOQ for Glecaprevirwas found as 2.98 and 10.00 and LOD and LOQ for Pibrentasvir was found as 3.00 and 9.98. The methodology was assessed by various validation parameters in accordance with ICH Guidelines which indicates the method can be employed for routine quality control analysis.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i3.1381

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INTRODUCTION

Glecaprevir mainly acts as hepatitis C Virus non-structural protein 3/4A protease inhibitor (Babu *et al.*, 2018; GampaVijayKumar and Reddy, 2018; Kumar and Raja, 2018) It is used for the treatment of chronic hepatitis C Infection along Pibrentasvir.

Pibrentasvir acts as NS5A inhibitor anti-viral agent (Sangameshwar *et al.*, 2019; Saradhi *et al.*, 2018; Sreeram and Venkateswarlu, 2018). The combination of Glecaprevir and Pibrentasvir was being approved by US Food and Drug Administration for hepatitis treatment (Issarani *et al.*, 2013). The chemical structures of Glecaprevir and Pibrentasvir are shown in Figure 1 and Figure 2.

MATERIALS AND METHODS

Chemicals and Reagents

Glecaprevir and Pibrentasvir were kindly gifted by

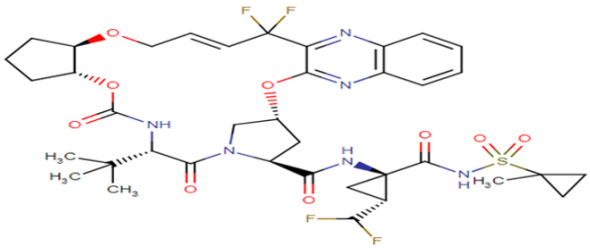

Figure 1: Structure of glecaprevir

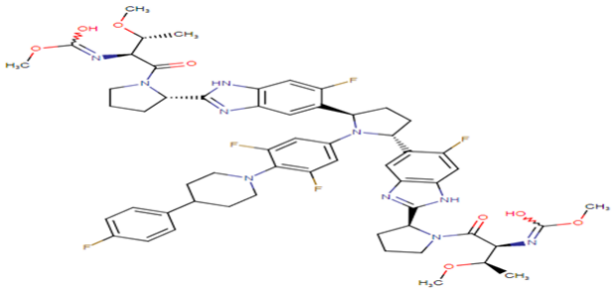

Figure 2: Structure of pibrentasvir

Pharmatrain Labs Pvt Ltd, Hyderabad certified to contain 99.9% and 99.8% purity respectively. Purification was not performed for the gifted samples and are used directly for Analysis. All the solvents utilized in analysis were of HPLC grade. Mavyret Tablets (label claim 100 mg of Glecaprevir and 40 mg of Pibrentasvir) of Abbvie pharma was used in analysis.

Instrument

LC system used consists of Waters HPLC having Empower Software with 2695 separation module having PDA detector with 20 μLinjection capacity. The column used was Xterra C_{18} Column, 5μ (250$\times$ 4.6 mm) at controlled temperature. Various solvents were tested in order to sort out the simplest conditions for drug separation at a particular time.

Optimized Chromatographic conditions

The mobile phase having phosphate buffer (pH 4.0) and methyl alcohol in the proportions of 30:70 v/v was selected because it was found that it ideally resolve the peaks with retention time (RT) 2.205 min and 4.996 min for Glecaprevir and Pibrentasvir respectively. Isobestic point was determined byperforming the spectroscopic analysis in the range of 200 nm to 390nm. Both the components showed reasonably good response at 251 nm with characteristic peak as shown in the Figure 3.

Preparation of Phosphate Buffer

Precisely Weigh 1.732g of monobasic potassium phosphate was taken in a 500ml volumetric flask, dissolved and diluted to 500ml with HPLC water and the volume was adjusted to pH 4.0 with Ortho Phosphoric Acid.

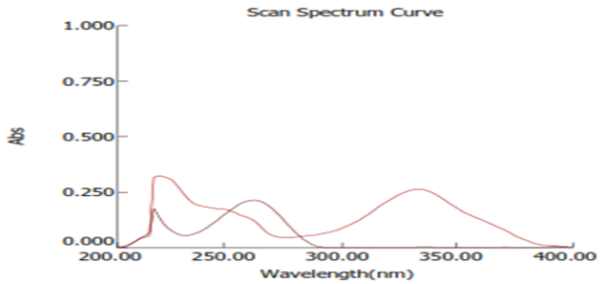

Figure 3: Overlain UV spectrumof glecaprevir and pibrentasvir

Preparation of Mobile Phase

Accurately measured 300 milli litre (30%) of phosphate Buffer and 700 milli litre of Methanol(70%) were mixed and degassed in inaudible water tub for 10 minutes then filtered through 0.45 µfilter under vacuum filtration. Diluent was mobile phase.

Standard Solution Preparation

Weigh precisely a quantity which is equivalent to 10 mg of Pibrentasvir and 25 mg of Glecaprevir and dissolve in 25 ml of mobile phase which comprises of Phosphate buffer and methanol. Sonicate the above solution for few minutes until the drug samples dissolves completely and pipette out 3 ml of PibrentasvirandGlecaprevir from standard stock solution into two separate 10ml volumetric flasks and make up the volume to the mark with diluents. Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for glecaprevir and pibrentasvir peaks and calculate the % Assay by using the formulae. The Chromatogram of standard solution was shown in Figure 4.

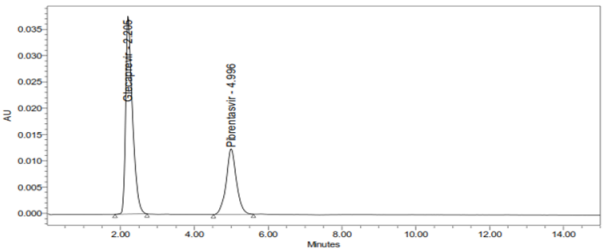

Figure 4: Chromatogram of astandard solution of glecaprevir and pibrentasvir

Sample Solution Preparation

Weigh precisely a quantity which is equivalent to 5 mg of Pibrentasvir and 12.5 mg of Glecaprevir and dissolve in 25 ml of mobile phase which comprises of Phosphate buffer and methanol. Sonicate the above solution for few minutes until the drug samples dissolve completely and pipette out 3 ml of Pibrentasvir and Glecaprevir from standard stock solution into two separate 10ml volumetric flasks and make up the volume to the mark with diluents.

The Chromatogram of sample solution was shown in Figure 5.

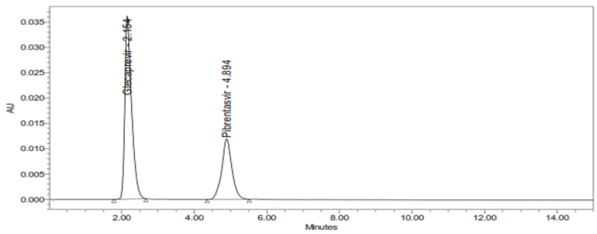

Figure 5: Chromatogram of sample solution of glecapreviranд pibrentasvir

Recovery studies

To analyze the correctness of the sample by the process developed and to study the levels of interference of formulation additives recovery experiments were carried out by standard addition method at various levels. From the overall amount of drug found, recovery was estimated. The results were reported in Table 1.

Table 1: Recovery Data of Formulation

PARAMETER	Glecaprevir	Pibrentasvir
Label claim (mg)	100	40
Drug found	100.42	40.07
% Accuracy	98-102	98-102

RESULTS AND DISCUSSION

Preparation of Calibration Curves by HPLC

Serial dilutions of Glecaprevir (100-500 μg/ml) and Pibrentasvir (40-200 μg/ml) were prepared using a mobile phase as solvent using 10 ml volumetric flasks. Each drug solution was analyzed by HPLC system, and the chromatograms were recorded. The peak spaces of each drug were calculated and also the individual activity curves were planned against magnitude relation of the area underneath curve and concentration of the drug. The chromatogram reports are shown in Table 2.

HPLC Method Validation

Method validation was assessed by different parameters, which include linearity, accuracy, Specificity, precision, the limit of detection, the limit of quantitation in accordance with International Council for Harmonisation guidelines.

Linearity and Range

The one-dimensionality of a technique is its ability to get take a look at results that area unit directly proportional to the sample concentration over a given vary. The peak area and concentration were aforethought to induce atypical standardization plot. The linearity range for Pibrentasvir and Glecaprevir was found to be 40 to 200 µg/ml and 100-500 µg/ml, respectively. The Correlation coefficient value for the calibration plot of Pibrentasvir and Glecaprevir was 0.999; it shows good linearity for both the drugs. The calibration curves were given in Figure 6 and Figure 7.

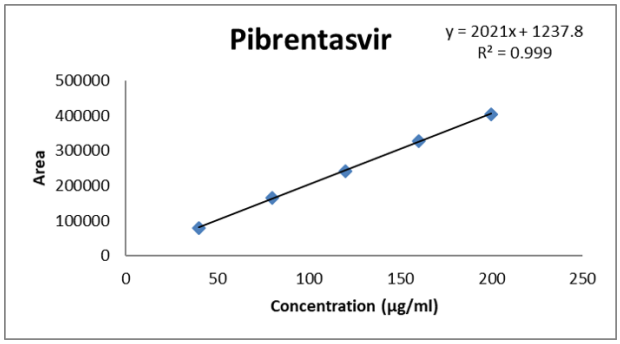

Figure 6: Linearity Curve of Pibrentasvir

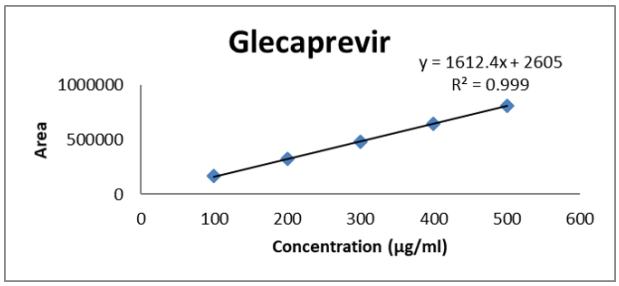

Figure 7: Linearity Curve of Glecaprevir

Accuracy

Accuracy was determined by sneakiness of the measured worth to actuality worth for the sample. The percentage recovery of Pibrentasvir was found to be 100.23%, 100.50% and 100.02% for accuracy 50%, 100% and 150% samples respectively. The mean recovery for Pibrentasvir is 100.25. The %RSD of the samples was found to be less than 2, and Glecaprevir was found to be 100.54%, 100.73% and 99.94% for accuracy 50%, 100% and 150% samples respectively. The mean recovery for Glecaprevir is 100.40. The %RSD of the samples was found to be less than 2. Reports of accuracy were given in Table 3.

Precision

Repeatability studies were studied by a series of measurements are done with pibrentasvir and glecaprevir. Peak area was measured for six replicate injections of both the drugs. % Relative Standard Deviation for the area measured of repeated injections was found to be at intervals the desired range. The reports for precision were given in Table 4.

Limit of Detection and Limit of Quantification

Table 2: Linearity Data of Pibrentasvir and Glecaprevir

S. No.	Linearity Level	Pibrentasvir		Glecaprevir	
		Conc. (mg/ml)	Average Area	Conc. (mg/ml)	Average Area
1	100	40	80059	100	165078
2	200	80	166202	200	323696
3	300	120	241069	300	480199
4	400	160	328202	400	645118
5	500	200	403255	500	807079
	Correlation Coefficient	0.9999		0.9994	
	Slope	2021		1605.4	
	Intercept	1237.8		2605	

Table 3: Accuracy Report of glecaprevir and pibrentasvir

Paramet	Pibrentasvir		Glecaprevir	
	% Estimated	% RSD	% Estimated	% RSD
50 %	100.23	0.40	100.54	0.69
100 %	100.50	0.27	100.73	0.29
150 %	100.02	0.68	99.94	0.35

Table 4: Precision Report of Glecaprevir and Pibrentasvir

Drug	Intraday		Inter day	
	% Obtained	% RSD	% Obtained	% RSD
Pibrentasvir	100.4%	0.14	99.6 %	0. 32
Glecaprevir	99.6%	0.06	100.2%	0.79

The Limit of Detection and Limit of Quantification for Pibrentasvir was 3.00 and 9.98. The Limit of Detection and Limit of Quantification for Glecaprevirwas2.98 and 10.00, respectively. The results obtained are within the limits.

Robustness and Ruggedness

Robustness and Ruggedness studies were studied by five replicate injections of Pibrentasvir and Glecaprevir on different days. The acceptance criteria for robustness and ruggedness can be assessed by measuring the percentage relative standard deviation, which should not be of not more than 2%. The reports were tabulated Table 5 in and Table 6.

Degradation Studies

The International Council for Harmonization (ICH) guideline entitled stability testing of recent pharmaceutical compounds and merchandise needs that stress sampling is dispensed to explain the stability of active pharmaceutical ingredients. The aim of this work was to perform the degradation studies on the Pibrentasvir and Glecaprevir using the developed method.

Hydrolytic degradation under acidic condition

Accurately measure 3 ml of the above solution and add 3 ml of 0.1N HCL. Transfer the above contents into 10 ml volumetric flask. Keep the Volumetric flask at a temperature of 60ºC for one day and neutralization was carried out by 0.1N NaOH. Make up the volume up to 10 ml with diluent and filtration was performed by using whatmann grade filter paper and the filtrate was stored in vials.

Hydrolytic degradation under alkaline condition

Accurately measure 3 ml of the above solution and add 3 ml of 0.1N NaOH. Transfer the above contents into 10 ml volumetric flask. Keep the Volumetric flask at a temperature of 60ºC for one day and neutralization was carried out by 0.1 N HCL. Make up the volume up to 10 ml with diluent and filtration was performed by using whatmann grade filter paper and the filtrate was stored in vials.

Oxidative degradation

Accurately measure 3 ml of the above solution and add 1 ml of 30% w/v of Hydrogen peroxide. Transfer the above contents into 10 ml volumetric flask. Keep the Volumetric flask at a temperature of 60ºC for 15 minutes. Make up the volume up to 10 ml with diluent and filtration was performed by using whatmann grade filter paper and the filtrate was stored in vials.

Thermal-induced degradation

Glecaprevir and Pibrentasvir sample was taken in Petri dish and placed in Hot air oven at 110^0 C for 3 hours. Dilution was performed by adding diluents to the sample and was analyzed by injecting into HPLC.

Table 5: Effect of change in the mobile phase flow rate

S.No.	System Suitability Parameter		Observations		
			As Such	Less flow	More flow
1	Theoretical Plates	Pibrentasvir	4652	4701	4388
		Glecaprevir	3678	3672	3574
2	Tailing factor	Pibrentasvir	1.04	1.08	1.01
		Glecaprevir	1.57	1.59	1.46

Table 6: Effect of change in mobile phase Composition

S.No	System Suitability parameter		Observations		
			Actual	Less Organic Phase	Actual
1	Theoretical Plates	4652	4446	4652	4388
		3678	3668	3678	3574
2	Tailing factor	1.04	0.83	1.04	1.01
		1.57	1.45	1.57	1.46

Table 7: Degradation Data of Glecaprevir and Pibrentasvir

Sample Name	Pibrentasvir		Glecaprevir	
	Area	% Degraded	Area	% Degraded
Acid	230162	4.21	446913	6.99
Base	222493	7.40	453107	5.70
Peroxide	213086	11.32	427337	11.06
Thermal	207448	13.66	421314	12.32

All the results of stability testing were tabulated in Table 7.

CONCLUSIONS

Several trials were carried out to achieve optimized chromatographic conditions. The initial attempt was to use as much low proportion of organic solvents for the chromatographic separation of the compounds. Usage of binary compound solvents results in enhancement of elution time of each the compounds. The proposed method can be used for acceptable results. The proposed technique is simple, speedy and statistically valid for its accuracy. Hence no interfering peaks were found in the chromatograms shows that anyone excipient is not interfered during the chromatographic separation of both the drugs.

ACKNOWLEDGEMENT

The authors are thankful to the Er. A.C.S. Arunkumar, President, Dr.M.G.R. Educational and Research Institute, Deemed to be University, Maduravoyal, Chennai for providing the facilities to carry out the research work.

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