A ROBUST AND ECONIMICAL REVERSE PHASE-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF GLECAPREVIR AND PIBRENTASVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A new analytical simple, rapid, economical and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Glecaprevir and Pibrentasvir in its pure form as well as in combined marketed formulation. Chromatography was carried out on a Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size column using a mixture of Methanol: Phosphate Buffer (pH-4.2) (37:63% v/v)as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 275nm. The retention time of the Glecaprevir and Pibrentasvir was found to be was 2.133, 3.692±0.02min respectively. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The method produce linear responses in the concentration range of 20-60mg/ml of Glecaprevir and 10-30mg/ml of Pibrentasvir. The inter-day and intra-day precisions were found to be within limits. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Glecaprevir and Pibrentasvir, RP-HPLC, Validation, Accuracy.*

Globally, an estimated 58 million people have chronic hepatitis C virus infection, which has currently no effective vaccine, with roughly 15 lakh people getting infected every year. There are an estimated 32 lakh children with chronic hepatitis C infection. WHO estimated that in 2019, approximately 290 000 people died from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma i.e. liver cancer[1]. Glecaprevir is in a class of medications called HCV NS3/4A protease inhibitors. It works by decreasing the amount of hepatitis C virus (HCV) in the body. Pibrentasvir is in a class of medications called HCV NS5A inhibitors. It works by stopping the virus that causes hepatitis C to spread inside the body. The combination of Glecaprevir and Pibrentasvir which comes in tablet dosage form is used to treat certain types of chronic (long-term) hepatitis C infection in adults and children which was highly efficacious and well tolerated in patients who were non-responsive to directly acting antiviral therapy [2].

Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Quality is an important element in every product or service, but it is vital in medicines as it involves lives of people [3]. There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This is due to the uncertainties in the usage of these drugs, report of new toxicities and development of resistance and introduction of better drugs by the competitors. Under these conditions, standardized and analytical procedures for the new drugs may not be available [4].

The combined dosage form of Glecaprevir and Pibrentasvir is not included in any pharmacopoeia till date although the Food and Drug administration (FDA) of USA approved it in 2017 [5]. Till date HPLC and UPLC and some RP-HPLC methods were reported for simultaneous quantification of these drugs in bulk and tablet dosage forms, no procedure has been standardized [6]. In the current study, a simple, economical, precise, novel, rapid RP-HPLC method was described for estimation of Glecaprevir and Pibrentasvir in bulk and pharmaceutical dosage forms with a runtime of only 6 minutes.

MATERIALS AND METHODS

HPLC grade Methanol, Acetonitrile (ACN), Potassium Dihydrogen Phosphate purchased from local chemist of make Merk. Watr was double distilled and filtered with a membrane filter. Pharmaceutical grade Glecaprevir and Pibrentasvir gifted by Sura labs ltd, Hyderabad The combined tablet formulation contains 40mg of Pibrentasvir and 100mg of Glecaprevir sold by brand name Mavyret purchased from chemist at Hyderabad, India.

Preparation of standard solution:

10 mg of Glecaprevir and Pibrentasvir earlier, were weighed and transferred to separate volumetric flasks and a small quantity (about 7ml) of Methanol was added to each flask and sonicated to dissolve and remove air. The volume was made up to give a concentration of 1000µg/mL. Further, 0.4ml of Glecaprevir and 0.2ml of Pibrentasvir were taken into separate 10ml flasks and further diluted with Methanol to get 200 µg/mL and 100 µg/mL of

Glecaprevir and Pibrentasvir respectively. These were serial diluted to get working standard solutions of 20, 30, 40, 50 and 60 µg/mL for Glecaprevir and 10,15,20,25,30 µg/mL for Pibrentasvir.

Procedure

Samples were injected by changing the chromatographic conditions and the chromatograms recorded, the conditions of proper peak elution for performing validation parameters as per International conference of Harmonization (ICH) guidelines [7] were obtained.

Mobile Phase Optimization:

The mobile phase tried initially was a) methanol: Water, b) Methanol: Phosphate buffer and c) ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer with pH-4.2 in the ratio 37:63 v/v respectively.

Optimization of Column:

The method was performed with various C18 columns like Symmetry, X-terra, Avantor ODS-3 and Phenomenex Luna columns. Out of them Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow. It was noted that the UV detector response of the drugs gave good sensitivity at wavelength of 275nm.

Validation Parameters

The standard solution was injected for five times and the areas for all five injections in HPLC were measured. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Sample Solution:

Four Tablets are taken and crushed into powder in a mortar by using pestle and weighted 10 mg equivalent of Glecaprevir and Pibrentasvir sample into a 100mL clean dry volumetric flask and added about 70mL of mobile phase and sonicated to dissolve it completely and volume made up to the mark for a concentration of 100µg/mL and 40 µg/mL of Glecaprevir and Pibrentasvir respectively. The sample solution is filtered by using 0.45µ pore size injection filter.

Procedure:

Three replicate injections of standard and sample solutions were injected and calculated the assay by using formula [8]:

$$\%Assay = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: Phosphate Buffer (pH-4.2) (37:63v/v)

Column : Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size

Column temperature : 35ºC

Wavelength : 275nm

Flow rate : 1ml/min

Injection volume : 10µl

Run time : 6minutes

From the chromatograms it can be observed that the Glecaprevir and Pibrentasvir peaks are well separated and they shows proper retention time, resolution, peak tail and plate count.

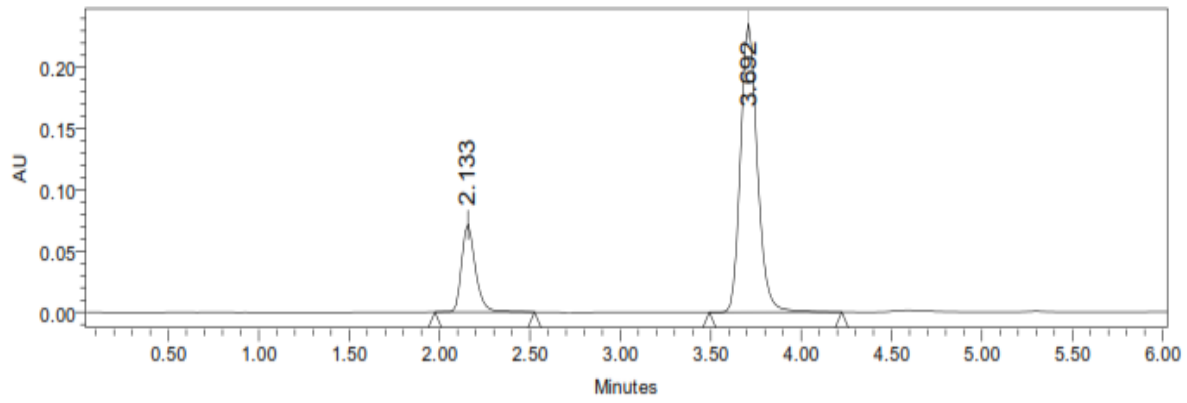

Fig-1: HPLC Chromatogram of Standard solution

Table-3: Parameters of Optimized Chromatogram of Standard

S.No.	Name	Retention time	Area	Height	USPTailing	USP Plate Count	Resolution
1	Glecaprevir	2.133	526389	86756	1.56	5679	
2	Pibrentasvir	3.692	1687285	367532	1.79	8685	9.8

Optimized Chromatogram (Sample)

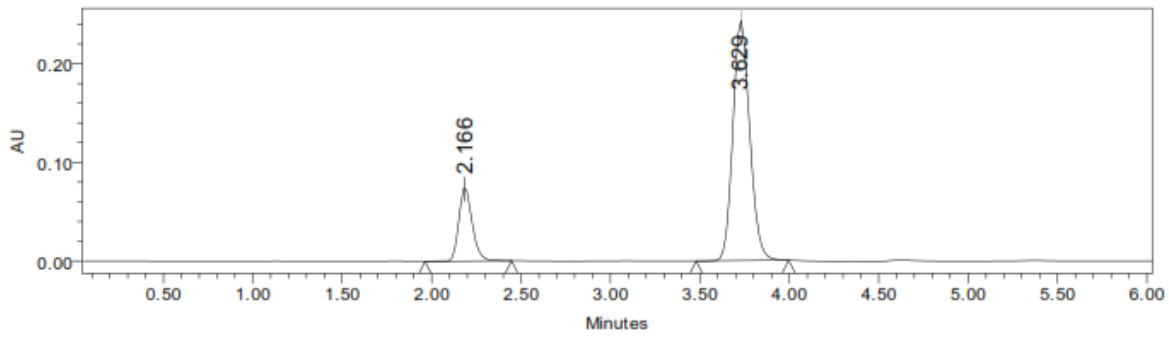

Figure-2: HPLC Chromatogram of of Sample solution

Table-4: Parameters of Optimized Chromatogram (Sample)

S.No.	Name	Retention time	Area	Height	USPTailing	USPPlate Count	Resolution
1	Glecaprevir	2.166	536587	77464	1.57	5789	
2	Pibrentasvir	3.629	1695846	378564	1.80	8795	10.01

Validation

Blank:

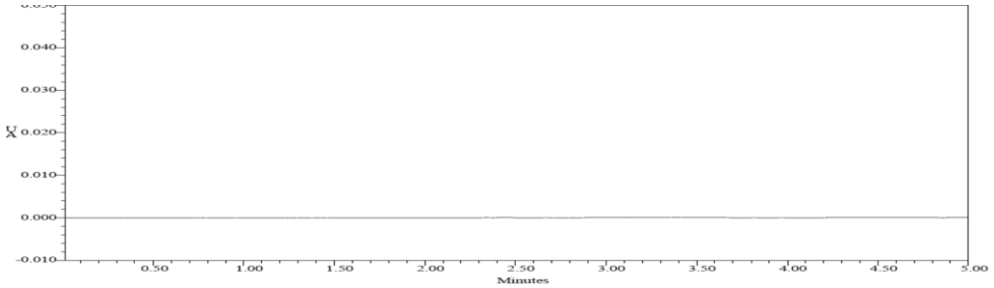

Fig-3: Chromatogram showing blank Solution (Mobile Phase Preparation)

System Suitability:

Table-5: Results of system suitability for Glecaprevir

S.No.	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	2.152	526358	86598	5695	1.56
2	2.157	526548	86254	5652	1.57
3	2.141	526854	86598	5627	1.56
4	2.133	526598	86245	5692	1.57

5	2.166	524874	86521	5641	1.56
Mean		526246.4			
Std.Dev.		787.353			
%RSD		0.149617			

Table-6: Results of system suitability for Pibrentasvir

S.No.	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	3.674	1682821	1686958	8659	1.56	9.8
2	3.631	1682726	1685745	8675	1.57	9.9
3	3.625	1687361	1685421	8692	1.56	9.8
4	3.692	1682811	1685242	8642	1.57	9.8
5	3.629	1683816	1685364	8635	1.58	9.8
Mean		1683907				
Std.Dev.		1982.03				
%RSD		0.117704				

Assay (Standard):

Table-7: Peak results for assay standard of Glecaprevir

S.No	Retention time	Area	Height	USP Tailing	USPPlateCount	Injection
1	2.152	526358	86598	1.56	5698	1
2	2.198	526584	86784	1.57	5687	2
3	2.179	529658	86253	1.56	5639	3

Table-8: Peak results for assay standard of Pibrentasvir

S.No.	Retention time	Area	Height	USPTailing	USP Plate Count	Injection
1	3.646	1687589	365879	1.80	8659	1
2	3.604	1685987	365854	1.79	8697	2
3	3.610	1685974	369854	1.80	8675	3

Assay (Sample):

Table-9: Peak results for Assay sample of Glecaprevir

S.No	Retention time	Area	Height	USP Tailing	USP Plate Count	Injection
1	2.152	536859	87584	1.58	5789	1
2	2.150	532654	87965	1.59	5784	2
3	2.187	532685	87465	1.58	5769	3

Table-10: Peak results for Assay sample of Pibrentasvir

Injection	Retention time	Area	Height	USP Tailing	USP Plate Count
1	3.646	1698568	378562	1.81	8759
2	3.651	1698574	375847	1.80	8795
3	3.601	1698547	376584	1.81	8745

Table-11: Chromatographic Data for Linearity Study of Glecaprevir

Concentration µg/ml	Average Peak Area
20	272897
30	402986
40	526389
50	649785
60	769287

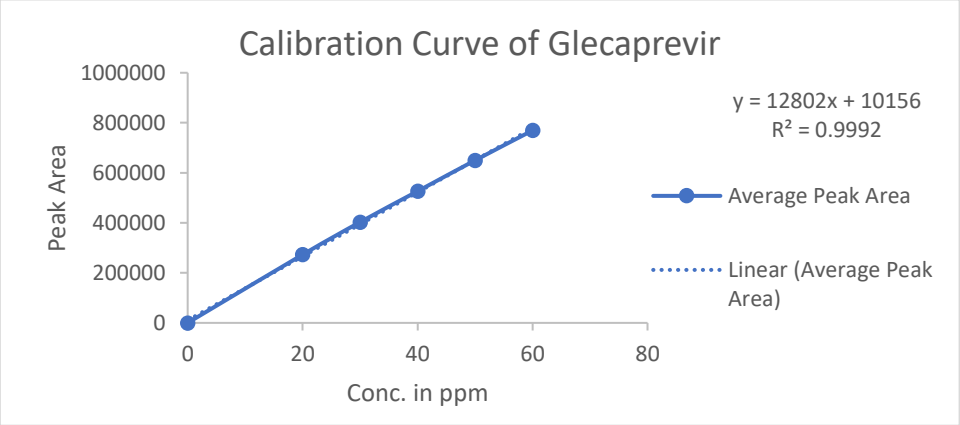

Fig-4: Calibration Curve of Glecaprevir

Table-12: Chromatographic Data for Linearity Study of Pibrentasvir

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845

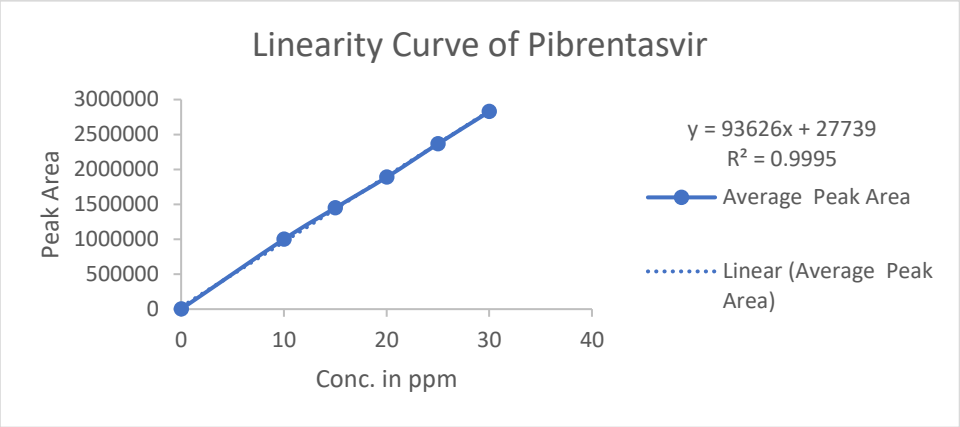

Fig-5: Calibration Curve of Pibrentasvir

Table-13: The accuracy results for Glecaprevir

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

Table-14: The accuracy results for Pibrentasvir

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value [9]

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where, σ = Standard deviation of the response and

S = Slope of the calibration curve

Therefore, result for Glecaprevir is 1.04µg/ml and Pibrentasvir is 3.12µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined [10]

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where , σ = Standard deviation of the response and

S = Slope of the calibration curve

Therefore, result for Glecaprevir is 2.1µg/ml and Pibrentasvir is 6.3µg/ml

Summary of Validation data for Glecaprevir:

S.No.	Parameter	Observation	Acceptance Criteria
1	**System suitability** Theoretical plates Tailing %RSD	 5679 1.56 0.14	 Not less than 2000 Not more than 2 Not more than 2.0%
2	**Specificity** %Assay	 99.89%	 98-102%
3	**Method Precision** (%RSD)	0.29	Not more than 2.0%
4	**Linearity** Slope Correlation coefficient(r^2)	20-60 µg/ml 12802 0.999	 ≤0.99
5	**Accuracy**		

	Mean % recovery	100.28%	98 - 102%
6	**Robustness** a) Flow rate variation b) Organic phase variation	All the system suitability parameters are within the limits.	

Summary of validation data for Pibrentasvir:

S.No	Parameter	Observation	Acceptance criteria
1	**System suitability** Theoretical plates Tailing %RSD	 8685 1.79 0.11	 Not less than 2000 Not more than 2 Not more than 2.0%
2	**Specificity** %Assay	 99.89%	 98-102%
3	**Method Precision** (%RSD)	0.044	Not more than 2.0%
4	**Linearity** Slope Correlation coefficient(r^2)	10-30 µg/ml 93626 0.999	 ≤0.99
5	**Accuracy** Mean % recovery	 100.48%	 98 - 102%
6	**Robustness** a) Flow rate variation b) Organic phase variation	All the system suitability parameters are within the limits.	

Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Glecaprevir and Pibrentasvirin bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

Glecaprevir was found to be Soluble in DMSO, freely soluble in ethanol, Methanol.Pibrentasvir was found to bepractically insoluble in water, but is freely soluble in ethanol, Methanol, dimethyl formamide and Acetonitrile. Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v)was chosen as the mobile phase. The solvent system used in this method was economical. 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. This RP-HPLC method is more sensitive, accurate, economical and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Glecaprevir and Pibrentasvirin bulk drug and in Pharmaceutical dosage forms.

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