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R E S E A R C H A R T I C L E

Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Determination of Glecaprevir and Pibrentasvir in Its Bulk and Their Pharmaceutical Dosage Forms

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A B S T R A C T

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Glecaprevir and Pibrentasvir was done by RP-HPLC. The Phosphate buffer was p^H 4.5 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of P^H 4.5(20:80 v/v). Kromosil C_{18} Column (250mm x 4.6mm) 5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1 ml min^{-1}. The linearity range of Glecaprevir and Pibrentasvir were found to be from 100-500 µg/ml of Glecaprevir and 1-5µg/ml of Pibrentasvir. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. Glecaprevir %RSD 0.2 and Pibrentasvir %RSD 0.6. Intermediate precision for Glecaprevir %RSD 0.2 and Pibrentasvir %RSD 0.1 LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements.

Keywords: Kromosil $C_{18,}$ Glecaprevir and Pibrentasvir, RP-HPLC

A R T I C L E I N F O

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1. Introduction

Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system. Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction.

2. Materials and Methods

Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, KH_2PO_4

Methodology

HPLC Method Development

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 4.5), Methanol in proportion 20: 80 v/v respectively.

Wave length selection:

UV spectrum of 10 µg / ml Glecaprevir and Pibrentasvir in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 254. At this wavelength both the drugs show good absorbance.

Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow.

Table 1: Chromatographic condition

Parameters	Description
Flow rate	1ml min^{-1}
Column	kromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Phosphate buffer: Methanol P^H 4.5(20:80 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 4.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254 nm
Injection volume	20µl
Run time	10min

System Suitability:

Tailing factor for the peaks due to Glecaprevir and Pibrentasvir in Standard solution should not be more than 2.0. Theoretical plates for the Glecaprevir and Pibrentasvir peaks in Standard solution should not be less than 2000.

Precision

Accurately weigh and transfer 25 mg of Glecaprevir and Pibrentasvir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Accuracy

Accurately weigh and transfer 10 mg of Glecaprevir and Pibrentasvir 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Linearity

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Glecaprevir and Pibrentasvir (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Detection

Accurately weigh and transfer 10 mg of Glecaprevir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Quantification

Accurately weigh and transfer 10 mg of Glecaprevir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2ml/min. Standard solution 300ppm of Glecaprevir &3ppm of Pibrentasvir was prepared and analysed using the varied flow rates along with method flow rate.

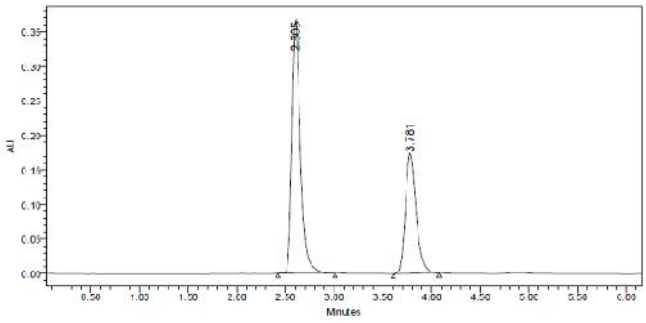

Figure 1: Chromatogram of Trail-6

Observation:

The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

3. Results and Discussion

Table 2: Results of system suitability parameters for Glecaprevir and Pibrentasvir

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Glecaprevir**	2.669	124505	223532	**1.2**	1.2	4523.3
2	**Glecaprevir**	2.5264	123442	134544	1.2	1.2	5020.2
3	**Glecaprevir**	2.5265	123431	124386	1.2	1.2	4061.2
4	**Glecaprevir**	2.5266	125432	134568	1.2	1.2	5032.4
5	**Glecaprevir**	2.5267	122434	146852	1.2	1.2	5076.4
6	**Glecaprevir**	2.5268	124438	145782	1.2	1.2	6024.8
7	**Pibrentasvir**	3.855	1308495	154566	1.3	1.3	6090.3
8	**Pibrentasvir**	3.902	1309496	156428	1.3	1.3	5023.2
9	**Pibrentasvir**	3.903	1306498	152634	1.3	1.3	8060.7
10	**Pibrentasvir**	3.904	1342499	158426	1.3	1.3	7080.1
11	**Pibrentasvir**	3.905	1343451	158484	1.3	1.3	6054.4
12	**Pibrentasvir**	3.906	1346455	158423	1.3	1.3	7080.6

Table 3: Showing %RSD results method precession for Glecaprevir

Injection	Peak Name	Rt	Area	Height
1	**Glecaprevir**	**3.699**	**1302729**	**341432.2**
2	**Glecaprevir**	**3.790**	**1302947**	**523341.4**
3	**Glecaprevir**	**3.663**	**1303236**	**374642.4**
4	**Glecaprevir**	**3.658**	**1303977**	**327514.3**
5	**Glecaprevir**	**3.647**	**1309759**	**374028.1**
6.	**Glecaprevir**	**3.645**	**1309789**	**346280.2**
mean			**1304529.8**	
Std.dev			**2961.1**	
%RSD			**0.2**	

Table 4: Showing % RSD results method precession for Pibrentasvir

Injection	Peak Name	Rt	Area	Height
1	**Pibrentasvir**	**3.616**	**123149**	**248742.3**
2	**Pibrentasvir**	**3.634**	**123766**	**281441.2**
3	**Pibrentasvir**	**3.460**	**124271**	**271721.2**
4	**Pibrentasvir**	**3.446**	**124691**	**284393.8**
5	**Pibrentasvir**	**3.437**	**124956**	**256318.0**
6	**Pibrentasvir**	**3.438**	**125845**	**226813.0**
mean			**124162.7**	
Std.dev			**725.6**	
%RSD			**0.6**	

Table 5: Showing results for intermediate precision of Glecaprevir

INJECTION	Peak name	Rt	Area	Height
1	Glecaprevir	2.554	1300148	438467.1
2	Glecaprevir	2.557	1304520	436873.3
3	Glecaprevir	2.563	1305937	438572.1
4	Glecaprevir	2.562	1306476	435587.5
5	Glecaprevir	2.561	130871	432826.4
6	Glecaprevir	2.561	130872	432838.3
mean			1305070.2	
Std.dev			3061.8	
%RSD			0.2	

Table 6: Showing results for intermediate precision of Pibrentasvir

Injection	Peak name	Rt	Area	Height
1	Pibrentasvir	3.790	122487	241421.6

2	Pibrentasvir	3.657	122626	233417.3
3	Pibrentasvir	3.663	122632	281751.1
4	Pibrentasvir	3.646	122702	241843.6
5	Pibrentasvir	3.662	122962	281564.1
6	Voxilaprevir	3.663	122972	284917.2
mean			122681.8	
Std.dev			174.8	
%RSD			0.1	

Table 7: Details of Accuracy 50 %

Injection	Peak Name	RT	Area	Height
1	Glecaprevir	2.572	132457	86026
2	Glecaprevir	2.573	132458	85549
3	Glecaprevir	2.576	134242	84196
4	Pibrentasvir	3.881	122487	21744
5	Pibrentasvir	3.882	122489	21909
6	Pibrentasvir	3.792	122392	21382
Mean			371513.5	
Std.Dev			253899.3	
% RSD			0.532	

Table 8: Details of Accuracy 100 %

Injection	Peak Name	RT	Area	Height
1	Glecaprevir	2.306	132405	86096
2	Glecaprevir	2.243	132452	86549
3	Glecaprevir	2.223	133232	84176
4	Pibrentasvir	3.546	124465	21784
5	Pibrentasvir	3.542	122428	25909
6	Pibrentasvir	3.546	124345	21372
Mean			372523.5	
Std.Dev			2508918.3	
% RSD			0.535	

Table 9: Details of Accuracy 150 %

Injection	Peak Name	RT	Area	Height
1	Glecaprevir	2.592	142526	76083
2	Glecaprevir	2.573	142527	76348
3	Glecaprevir	2.223	143532	74275
4	Pibrentasvir	3.841	135545	21682
5	Pibrentasvir	3.882	132558	25508
6	Pibrentasvir	3.842	134345	21476
Mean			338742.3	
Std.Dev			840776.2	
% RSD			0.575	

Table 10: Accuracy (recovery) data for Glecaprevir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 11: Accuracy (recovery) data for Pibrentasvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table 12: Area of different concentration of Glecaprevir

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table 13: Area of different concentration of Pibrentasvir

S.No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

Table 14: Analytical performance parameters of Glecaprevir and Pibrentasvir

Parameters	Glecaprevir	Pibrentasvir
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table 15: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Glecaprevir	52	152	2.9
Pibrentasvir	52	156	3

Table 16: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Glecaprevir	52	522	10.03
Pibrentasvir	52	524	10.1

Table 17: Flow Rate (ml/min) data for Glecaprevir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 18: Flow rate (ml/min) data for Pibrentasvir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 19: Change in Organic Composition in the Mobile Phase for Glecaprevir

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 20: Change in Organic Composition in the Mobile Phase for Pibrentasvir

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2

2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

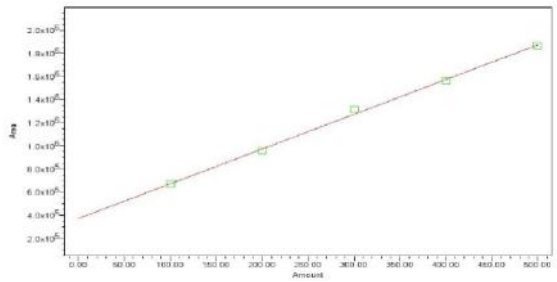

Figure 4: Calibration graph for Glecaprevir at 254 nm

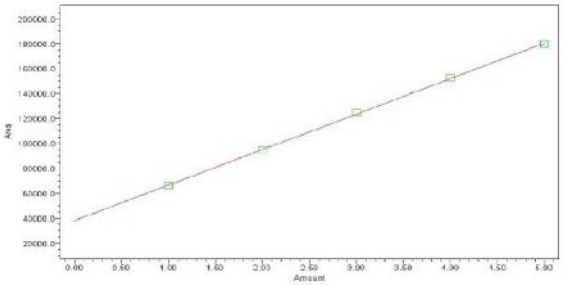

Figure 5: Calibration graph for Pibrentasvir at 254 nm

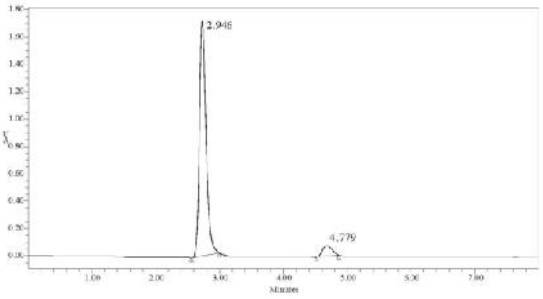

Figure 6: Chromatogram snowing less flow of 0.6ml/min

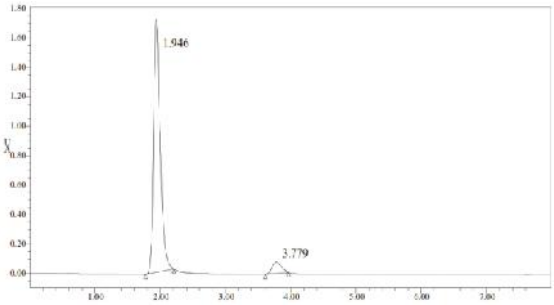

Figure 7 Chromatogram snowing more flow of 1.0ml/min

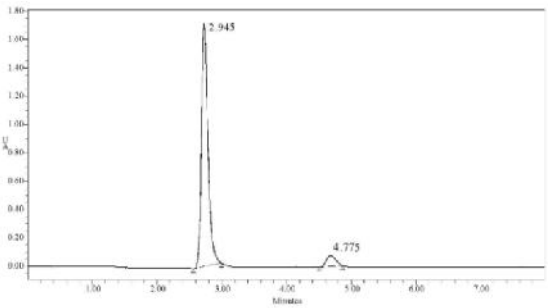

Figure 8: Chromatogram showing less organic composition

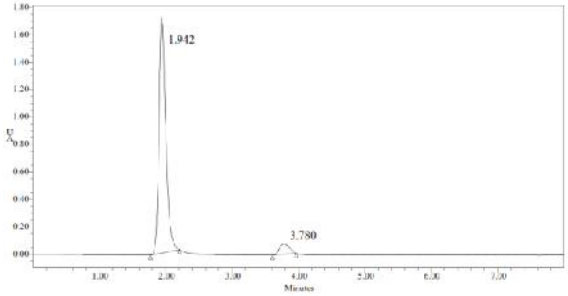

Figure 9: Chromatogram showing more organic composition

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Glecaprevir and Pibrentasvir was done by RP-HPLC. The Phosphate buffer was p^H 4.5 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of P^H 4.5(20:80 v/v). Kromosil C_{18} Column (250mm x 4.6mm)5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1ml min^{-1}. the linearity range of Glecaprevir and Pibrentasvir were found to be from 100-500 µg/ml of Glecaprevir and 1-5µg/ml of Pibrentasvir Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. Glecaprevir %RSD 0.2 and Pibrentasvir %RSD 0.6. Intermediate precision for Glecaprevir %RSD 0.2 and Pibrentasvir %RSD 0.1 the percentage recovery varies from 98-102% of Glecaprevir and Pibrentasvir. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5. References

[1] Sukhadev Pawar et al, Development and Validation of RP-HPLC Method For The Simultaneous Estimation of Glecaprevir and Pibrentasvir In Bulk and Formulations, International Journal of Pharmacy & Pharmaceutical Sciences; 2013 Supplement

[2] Madhukar A. Simple and Sensitive Analytical Method Development and Validation of Glecaprevir Bulk Drug by RP-HPLC. Der Pharma Chemica, 2011, 3 (6): 494-499.

[3] B. Mohan Gandhi, A. Lakshmana Rao, J. Venkateswara Rao. Analytical Method Development and Validation for Simultaneous Estimation of Glecaprevir and Pibrentasvir by RP-HPLC. International Journal of Research and

Development in Pharmacy and Life Sciences, 2015, 4(7): 859-873.

[4] Naresh Kondapalli. Simple and Sensitive Analytical Method Development and Validation of Glecaprevir Bulk Drug by RP-HPLC. Der Pharma Chemica 2011, 3(6): 494-499

[5] Ponnilavarasan. RP-HPLC Method for Simultaneous Estimation of Antiretroviral Drugs Glecaprevir and Pibrentasvir in Tablet Dosage Form. Digest Journal of Nanomaterials & Biostructures (Djnb). 2010, 5(3): 771.

[6] Anusha Tiyyagura. Method Development and Validation for the Simultaneous Estimation of Glecaprevir and Pibrentasvir in Pharmaceutical Dosage Form By RP-HPLC, IJPCBS 2012, 3(1), 44-54.