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A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GLECAPREVIR AND PIBRENTASVIR IN PHARMACEUTICAL DOSAGE FORM

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Abstract:
A simple, Accurate, precise technique was produced for the simultaneous estimation of the estimation of the Glecaprevir & Pibrentasvir in Pharmaceutical dosage form. Chromatogram was run through Kromosil C18 250x4.6mm,5µ. MP containing Buffer 0.01N KH2PO4: ACN taken in the ratio 55:45 was pumped through column at a stream rate of 1ml/min. Buffer utilized in this technique was 0.1% Ortho phosphoric acid buffer. Temperature was maintained at 30°C. Improved wavelength chose was 260 nm. Retention time of Glecaprevir and Pibrentasvir were observed to be 2.501 min and 3.113 min. %RSD of the Glecaprevir and Pibrentasvir were and found to be 0.2 and 0.6 correspondingly. %Recovery was obtained as 99.48% and 99.57% for Glecaprevir & Pibrentasvir correspondingly. LOD, LOQ values get from regression equations of Glecaprevir & Pibrentasvir were 0.71, 2.15 and 0.32, 0.96 respectively. Regression equation of Glecaprevir is y = 8171.x + 4225., y = 8748.x + 1373 of Pibrentasvir. Upkeep times were lessened and run time was reduced, so the procedure made was direct and mild that can be grasped in typical Quality control test in Industries.

Key Words: *Glecaprevir, Pibrentasvir, RP-HPLC.*

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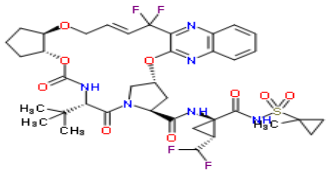
INTRODUCTION:

Glecaprevir is a direct acting antiviral agent and Hepatitis C virus (HCV) NS3/4A protease inhibitor that targets the the viral RNA replication. In combination with Pibrentasvir, glecaprevir is a useful therapy for patients who experienced therapeutic failure from other NS3/4A protease inhibitors.

IUPAC Name:(1R,14E,18R,22R,26S,29S)-26-tert-butyl-N-[(1R,2R)-2-(difluoromethyl)-1-{[(1-methylcyclopropyl)sulfonyl]carbamoyl}cyclopropyl]-13,13-difluoro-24,27-dioxo-2,17,23trioxa4,11,25,28tetraazapentacyclo[26.2.1.0^{3,12}.0^{5,10}.0^{18,22}]hentriaconta-3,5(10),6,8,11,14-hexaene-29-carboxamide.

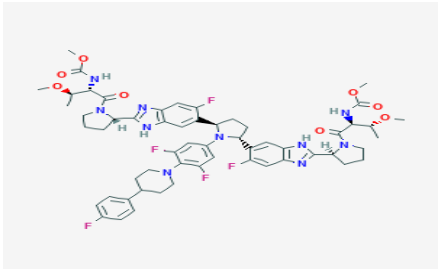
Structure:



Pibrentasvir is an immediate acting antiviral specialist and Hepatitis C infection (HCV) NS5A inhibitor that objectives the viral RNA replication and viron get together. In mix with Glecaprevir, pibrentastiv is a valuable treatment for patients who experienced remedial disappointment from other NS5A inhibitors.

IUPAC Name: (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)methylidene]amino}-3-methoxybutanoyl]pyrrolidin-2-yl]-1H-1,3-benzodiazol-5-yl}pyrrolidin-2-yl]-6-fluoro-1H-1,3-benzodiazol-2-yl}pyrrolidin-1-yl]-2-{[hydroxy(methoxy)methylidene]amino}-3-methoxybutan-1-one

Structure:



As per the literature review, Glecaprevir and Pibrentasvir was estimated individually by few methods like simple HPLC1 , Ultra HPLC2 ,HPLC-MS. The objective of the work is to develop RP-HPLC method for estimation of Glecaprevir and Pibrentasvir in tablet dosage form with simple , rapid, accurate and economical methods and validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution as per ICH guidelines.[1]. The objective of the work is to develop RP-HPLC method for estimation of Glecaprevir and Pibrentasvir in tablet dosage form with simple , rapid, accurate and economical methods and validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution as per ICH guidelines.

MATERIALS AND METHODS:

HPLC Instrumentation & Conditions:

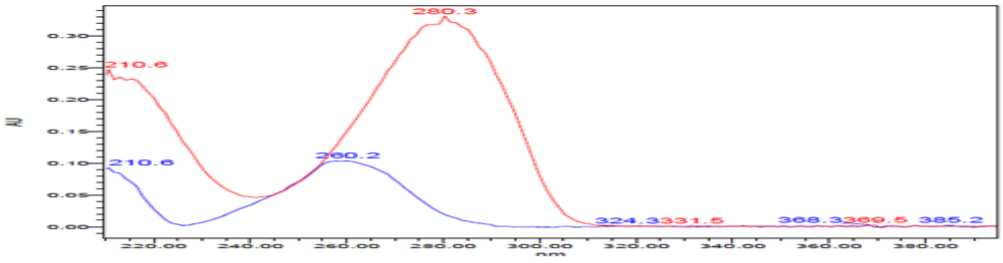
The HPLC system employed was HPLC with Empower2 Software with Isocratic with UV-Visible Detector.

Standard & sample preparation for UV-spectrophotometer analysis:

Standard Preparation:
Exactly weighed & transferred 25mg of Glecaprevir and 10mg of Pibrentasvir working Standards into a 25ml clean dry volumetric cup, include 3/fourth volume of diluent, sonicated for 5 minutes and make up to the last volume with diluents. 1ml from the above stock plan was taken into a 10ml VF and wound up to 10ml

Sample Preparation:
5 Tablets was weighed ,powdered and then was moved into a 100mL volumetric glass, 50mL of diluent included and sonicated for 25 min, further the volume made up with diluent and sifted. From the sifted arrangement 1 ml was pipeted out into a 10 ml volumetric glass and made upto 10ml with diluent.

Overlay UV spectra of Glecaprevir & Pibrentasvir:
λ_{max} of Glecaprevir & Pibrentasvir was 280.3nm and 260.2nm correspondingly.Overlay spectra gave the enhanced wavelength for these 2 drugs.



Enhanced wavelength choose was 260nm.

Optimized Chromatographic Conditions:

Mobile phase : 55% KH2PO4 (0.01N): 45% Acetonitrile
Stream rate : 1 ml/min
Column : Kromosil C18 (4.6 x 150mm, 5µm)
Detector wavelength : 260.0 nm
Column temperature : 30°C
Injection volume : 10µL
Run time : **6** min
Diluent : Water & ACN in the ratio 50:50
Results : Two peaks have good resolution, tailing Factor, theoretical plate count and resolution.

MOBILE PHASE PREPARATION:
Mobile phase was prepared by taking KH2PO4 and Acetonitrile. Mobile phase was filtered through 0.45 m membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

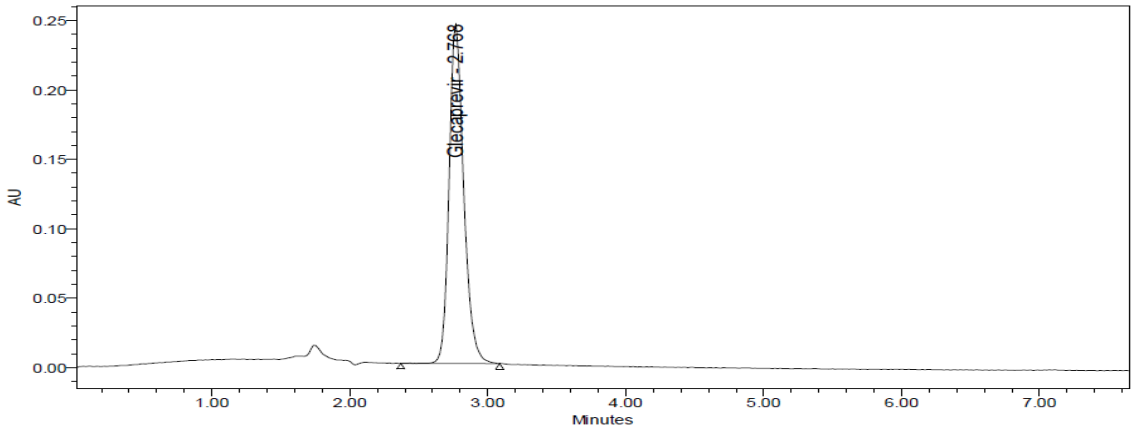
SAMPLE & STANDARD PREPARATION FOR THEANALYSIS:

Exactly weighed & transferred 25mg of Glecaprevir and 10mg of Pibrentasvir working Standards into a 25ml clean dry volumetric cup, include 3/fourth volume of diluent, sonicated for 5 minutes and make up to the last volume with diluents. 1ml from the above stock plan was taken into a 10ml VF and wound up to 10ml.

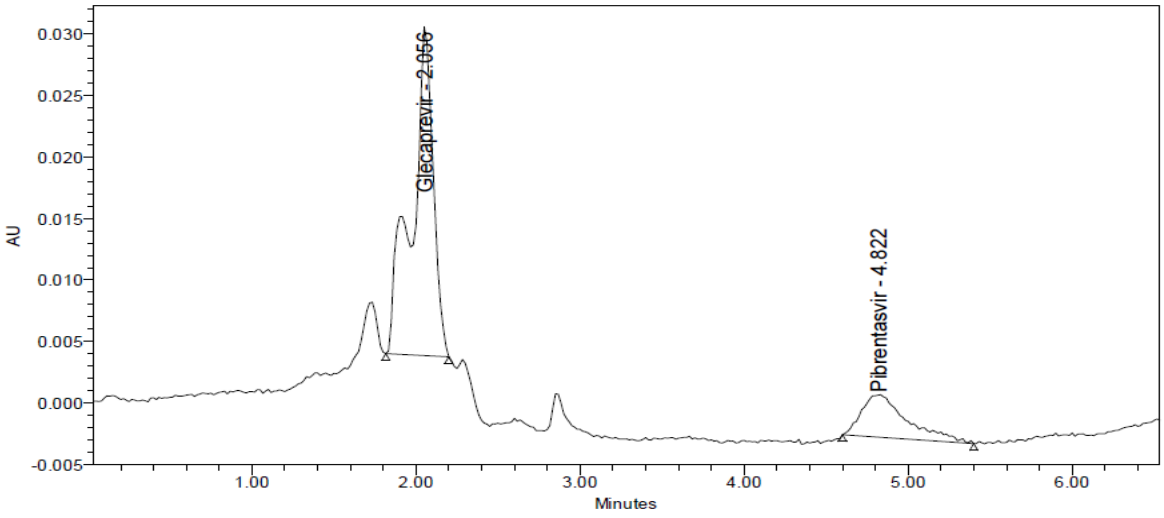
RESULT AND DISCUSSION:

Table-1:Trials for method development

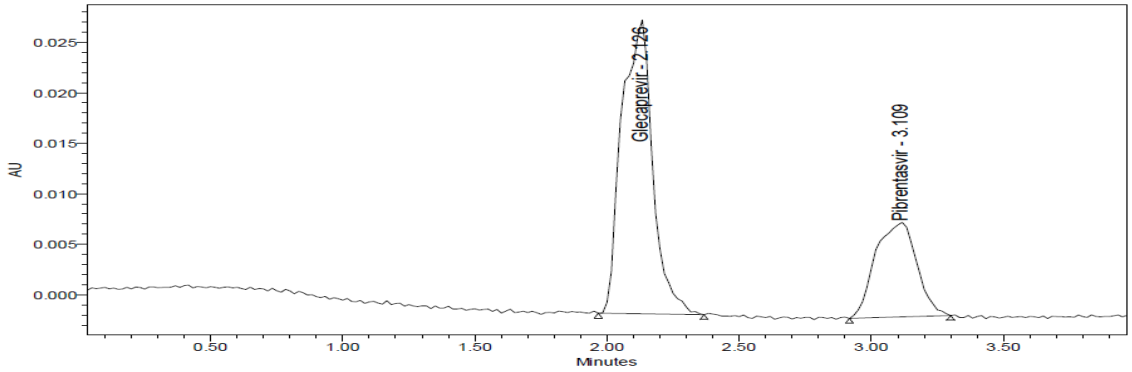
Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Altima C18 (4.6 x 150mm, 5µm)	Water and Methanol taken in the ratio 50:50	1 ml/min	260nm	Peak was not eluted	Method rejected
Altima C18 (4.6 x 150mm, 5µm)	0.1% OPA**:** Methanol (50:50)	1.0 ml/min	260nm	Tailing Peaks	Method rejected
Altima C18 (4.6 x 150mm, 5µm)	50% OPA(0.1%): 50% Acetonitrile	1.0 ml/ min	260nm	Peaks and plate count was not good	Method rejected
Kromosil C18 (4.6 x 150mm, 5µm)	50% 0.1% OPA:50% Acetonitrile	1.0 ml/ min	260nm	Base line is not good	Method rejected
Kromosil C18 (4.6 x 150mm, 5µm)	55% 0.1% OPA: 45% Acetonitrile	1.0 ml/ min	260nm	Base line is not good	Method rejected
Kromosil C18 (4.6 x 150mm, 5µm)	55% KH2PO4 (0.01N): 45% Acetonitrile	1.0 ml/ min	260nm	Good Peak	Method Accepted



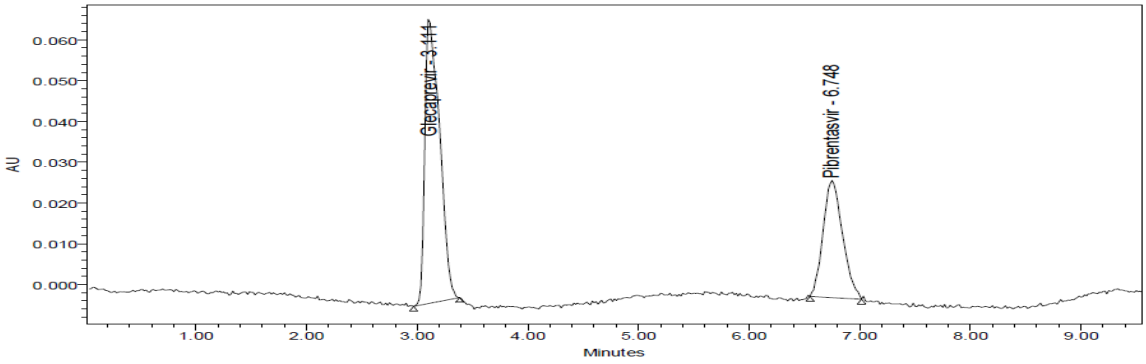
Trial chromatogram 1



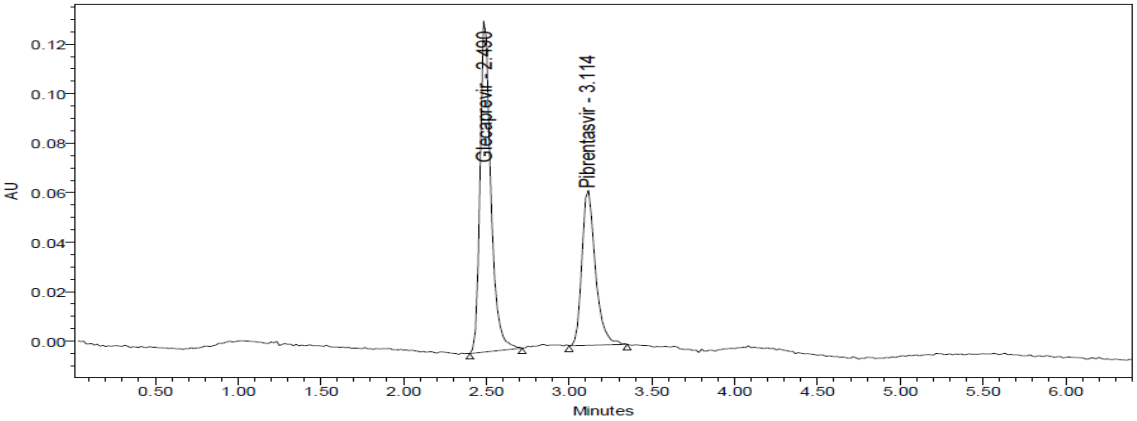
Trial chromatogram 2



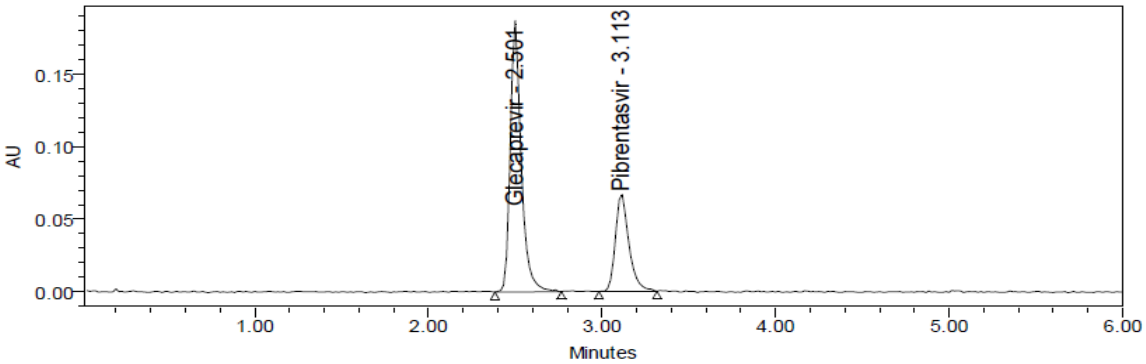
Trial chromatogram 3



Trial chromatogram 4



Trial chromatogram 5



Optimized Chromatom

METHOD VALIDATION:

Accuracy: ***Recovery study:***To determine the accuracy of the projected technique, recovery studies were distributed by adding totally different amounts (50%, 100%, and 150%) of pure drug of Glecaprevir and Pibrentasvi and the values were calculated.

Accuracy Readings

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	50.32248	100.64	99.48%
	50	50.13193	100.26	
	50	49.93183	99.86	
100%	100	98.81312	98.81	
	100	99.05103	99.05	
	100	99.91813	99.92	
150%	150	147.5893	98.39	
	150	148.7543	99.17	
	150	148.8524	99.23	

Precision:

Repeatability-

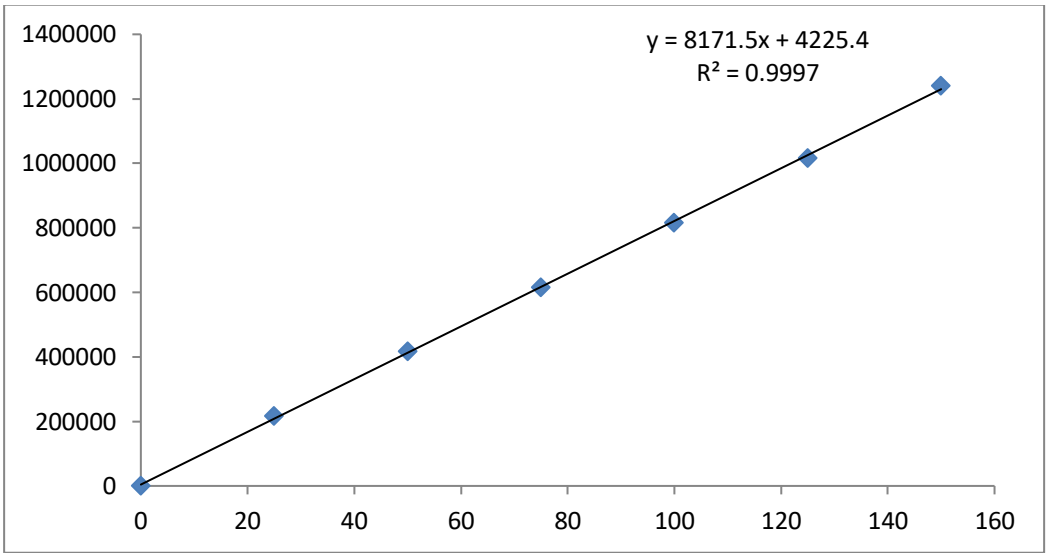
The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug.

Repeatability Results of Precision

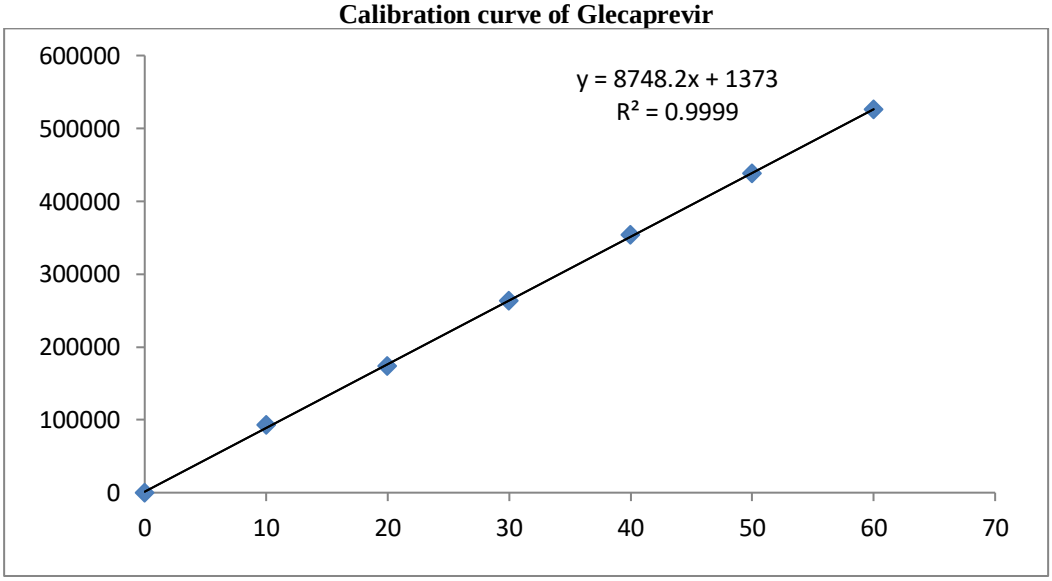
S. No	Area of Glecaprevir	Area of Pibrentasvir
1.	819660	357522
2.	802447	358068
3.	817777	353334
4.	818341	358544
5.	810782	353754
6.	816781	352707
Mean	814298	355655
S.D	6575.3	2658.8
%RSD	0.8	0.7

Linearity and Range

Glecaprevir		Pibrentasvir	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
25	215815	10	92506
50	416445	20	173434
75	615508	30	262916
100	816257	40	353824
125	1014870	50	438046
150	1240701	60	526010



Calibration curve of Glecaprevir



Calibration curve of Pibrentasvir

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) for Glecaprevir were found to be 0.71 and 2.15 and pibrentasvir were found to be 0.32 and0.96 µg/ml respectively.

System Suitability Parameter

System quality testing is Associate in nursing integral a part of several analytical procedures. The tests area unit supported the construct that the instrumentation, physics, Associate in Nursingalytical operations and samples to be analyzed represent an integral system that may be evaluated intrinsically[14]. Following system quality take a look at parameters were established.

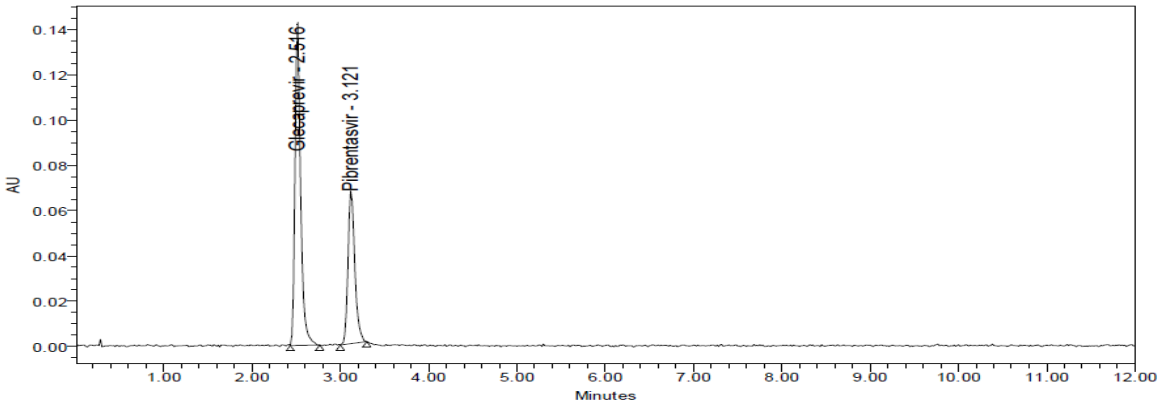
Dataof System Suitability Parameter

S no	Glecaprevir			Pibrentasvir			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	USP Resolution
1	2.497	7701	1.31	3.105	8059	1.34	4.7
2	2.499	7943	1.29	3.109	8087	1.31	4.7
3	2.499	8095	1.30	3.113	8154	1.29	4.7
4	2.501	8207	1.30	3.114	7772	1.29	4.7
5	2.502	8143	1.30	3.115	7856	1.29	4.6
6	2.503	8083	1.35	3.117	7393	1.29	4.6

FORCED DEGRADATION STUDIES:

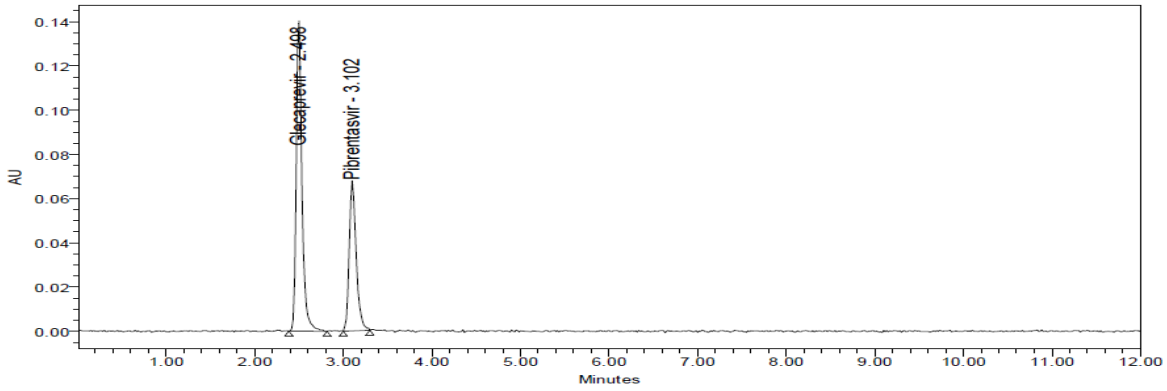
Acid Degradation:

To 1.0ml of stock s arrangement Glecaprevir and Pibrentasvir, 1.0ml of 2N HCL was included and keeping for 30.0 mins at 60^0C. The resultant game plan was debilitated to get 100µg/ml&40µg/ml game plan and 10.0 µl courses of action were injected into the Hplc.



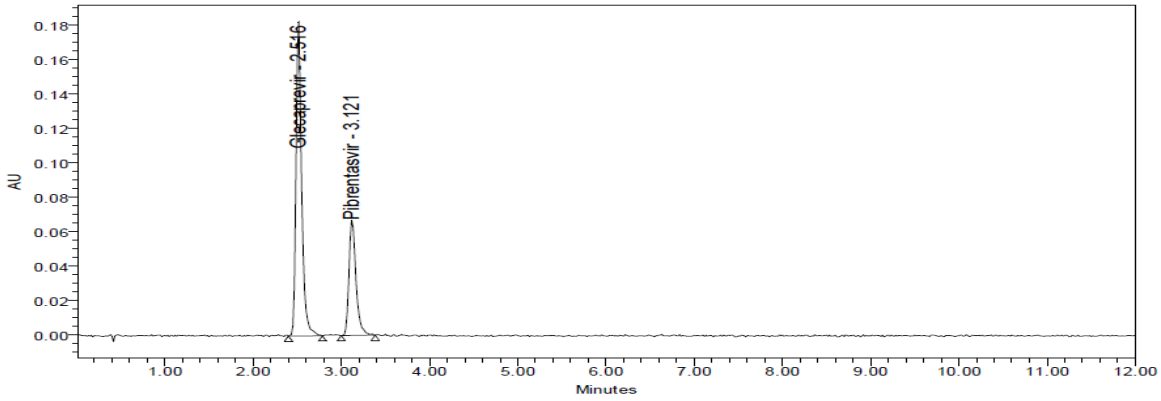
Acid chromatogram of Glecaprevir & Pibrentasvir

2. Basic Degradation: To 1.0ml of stock arrangement Glecaprevir and Pibrentasvir, 1.0ml of 2N NaOH was included and refluxed for 30.0minutes at 600C. The resultant course of action was debilitated to secure 100µg/ml&40µg/ml game plan and 10.0µl were injected into the HPLC.



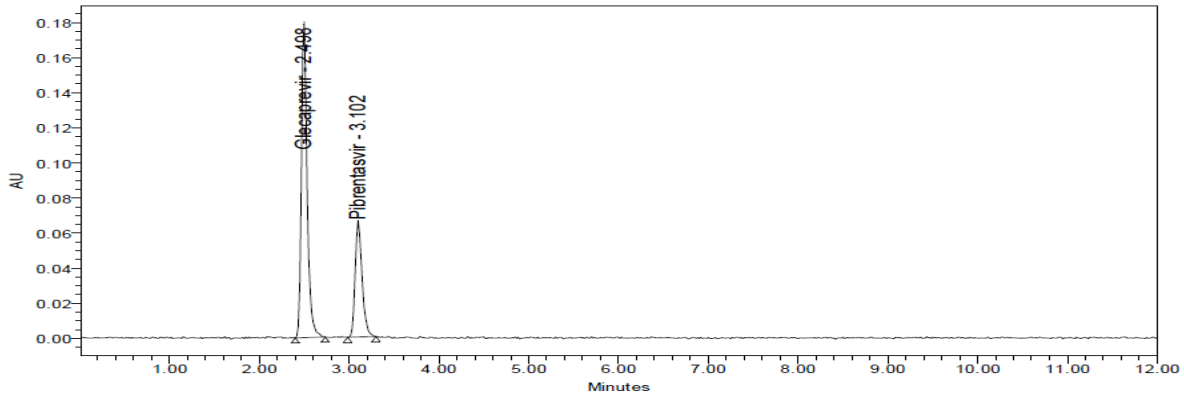
Base chromatogram of Glecaprevir & Pibrentasvir

3. Thermal Degradation: The standard medication arrangement was set in broiler at 105°C for 6.0 hours to consider dry warm corruption. For HPLC think about, the resultant plan was debilitated to 100µg/ml&40µg/ml game plan and 10.0µl were mixed into the system and the chromatograms were recorded to assess the reliability of the example



Thermal chromatogram of Glecaprevir & Pibrentasvir

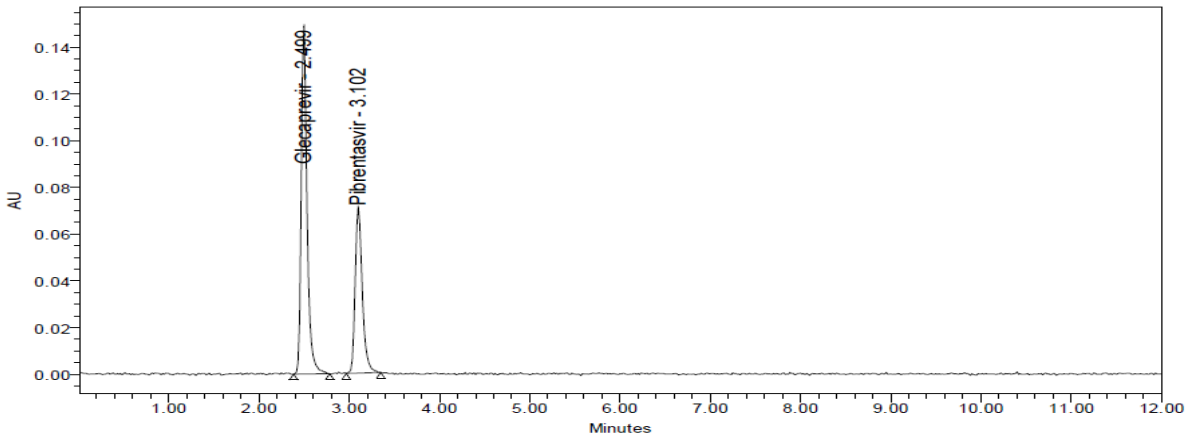
4. Photo Stability studies:
The photochemical dependability of the medication was likewise contemplated by uncovering the 1000µg/ml& &400µg/ml answer for UV Light by keeping the measuring utencil in UV Chamber for 1days or 200 Watt hours/m2 in photograph steadiness chamber. For HPLC mull over, the resultant plan was debilitated to gain 100µg/ml 40µg/ml courses of action and 10.0µl were injected into the system.



Chromatogram showing photolytic degradation.

5. Oxidation

To 1.0ml of stock solution of Glecaprevir & Pibrentasvir, 1 ml of 20% H2O2 was included independently and kept for 30.0min at 60^0c. For HPLC think about, the resultant course of action was debilitated to get 100µg/ml&40µg/ml game plan and 10µl were imbued into the system.



Peroxide chromatogram of Glecaprevir & Pibrentasvir

Degradation Data of Glecaprevir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.38	1.565	2.215
2	Alkali	4.17	1.373	1.627
3	Oxidation	3.74	1.221	1.465
4	Thermal	2.92	1.619	2.361
5	UV	1.01	1.176	1.433
6	Water	1.01	1.143	1.404

Degradation Data of Pibrentasvir

S.NO	**Degradation Condition**	**% Drug Degraded**	**Purity Angle**	**Purity Threshold**
1	Acid	4.38	2.902	4.043
2	Alkali	3.84	2.310	2.587
3	Oxidation	3.51	3.191	2.694
4	Thermal	2.25	3.400	4.561
5	UV	1.11	2.333	2.657
6	Water	0.76	3.139	2.517

CONCLUSION:

A simple, Accurate, precise, sensitive & selective RP-HPLC method has been developed & validated for the simultaneous estimation of the Glecaprevir & Pibrentasvir in Pharmaceutical dosage form. The result shows the developed method is yet another suitable method for assay, purity & stability which can help in the analysis of Glecaprevir & Pibrentasvir in different formulations.

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