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UPLC STABILITY INDICATING METHOD FOR SIMULTANEOUS ESTIMATION OF GLECAPREVIR AND PIBRENTASVIR

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Glecaprevir, Pibrentasvir, UPLC, Validation

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ABSTRACT: A forced stability-indicating method for simultaneous estimation of glecaprevir and pibrentasvir by Ultra Performance Liquid Chromatography (UPLC) was developed and validated. The separation was done on column BEH C18 50 × 2.1mm 1.7µ which was maintained at 30 °C, eluted with isocratic mobile phase and eluents were detected at 260nm. Mobile phase containing Buffer 0.01NKH_2PO_4: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 0.3 ml/min. The retention time of glecaprevir and pibrentasvir was found to be 0.392 min and 0.607 min respectively. Validation with respect to specificity, system suitability, precision, accuracy, linearity, and robustness was performed as per ICH guidelines. % RSD of the glecaprevir and pibrentasvir were and found to be 0.5 and 0.7 respectively. % Recovery was obtained as 100.40 and 99.90% for glecaprevir and pibrentasvir respectively. LOD, LOQ values obtained from regression equations of glecaprevir and pibrentasvir were 0.79, 2.38 and 0.51, 1.55 respectively. Regression equation of glecaprevir is y = 2140x + 994.9 and of pibrentasvir was y = 2138.x + 314.3. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control tests in Industries.

INTRODUCTION: Protease inhibitors [1] act by inhibiting the virus-specific protease enzyme which converts polyproteins into various structural and functional proteins by cleavage at the appropriate positions. Both glecaprevir and pibrentasvir comes under the class of protease inhibitors, where glecaprevir [2, 3] is directly acting antiviral drug, which is chemically (3aR, 7S, 10S, 12R, 21E, 24aR)-7-tert-butyl-N-{(1R, 2R)-2(difluoromethyl)-1-[(1-methylcyclopropane-1- sulfonyl)carbamoyl] cyclopropyl}-20,20-difluoro5, 8-dioxo-2, 3, 3a, 5, 6, 7, 8, 11, 12, 20, 23, 24a-dodecahydro-1H, 10H-9, 12 methanocyclopenta[18, 19] [1, 10, 17, 3, 6] trioxodiazacyclononadecino [11, 12-b]quinoxaline-10carboxamide hydrate is a HCV NS3/4A protease inhibitor acts by inhibiting protease enzyme which is required for cleavage of HCV polyprotein into mature viral proteins required for viral RNA replication and pibrentasvir [4] which is chemically Methyl {(2S, 3R)-1-[(2S)-2-{5-[(2R, 5R)-1-{3, 5-difluoro- 4[4- (4- fluorophenyl) piperidin- 1- yl] phenyl}- 5- 96- fluoro- 2- {(2S)- 1- [N-(methoxy-carbonyl)- Omethyl- L- threonyl]pyrrolidine-2-yl}-1H-benzimidazol-5-yl)pyrrolidine-2-yl]- 6- fluoro-1Hbenzimidazol- 2- yl} pyrrolidine- 1-yl]- 3-methoxy-1-oxobutan-2-yl}carbamate is a HCV NS5A protease inhibitor, reduces viral replication and virion assembly by inhibiting the viral NS5A

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enzyme. A fixed dose combination of glecaprevir and pibrentasvir was used to treat chronic Hepatitis C virus (HCV) genotype 1-6 infection in adults. Chemical structure of glecaprevir and pibrentasvir was given as **Fig. 1** and **Fig. 2**.

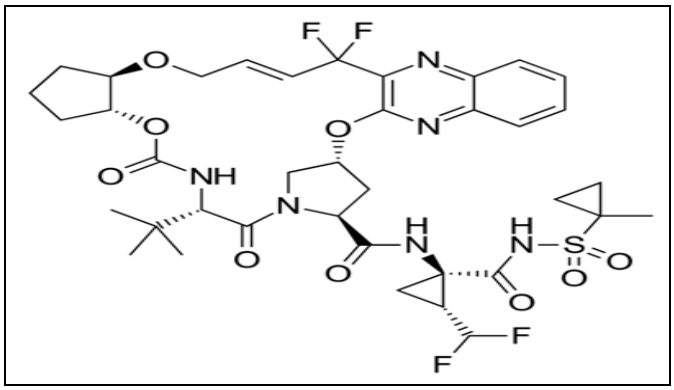

FIG. 1: CHEMICAL STRUCTURE OF GLECAPREVIR

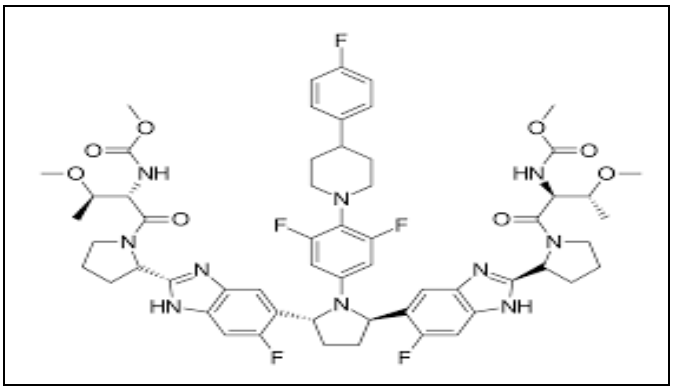

FIG. 2: CHEMICAL STRUCTURE OF PIBRENTASVIR

There are several methods [5-9] for estimation of glecaprevir and pibrentasvir by RP-HPLC were reported. In the present study stability indicating UPLC method for simultaneous estimation of glecaprevir and pibrentasvir was developed and validated as per ICH guidelines [10].

MATERIALS AND METHODS:

Instrumentation and Reagents: Acquity Waters Ultra performance chromatographic system with UV detector and Empower software for data integration was used. The other instruments used in this study include weighing balance (Labindia), pH meter (Labindia), sonicator (Labman) and vacuum pump (Crompton). The drugs glecaprevir and pibrentasvir samples are procured from Merck. All the reagents and chemicals used in the method are of analytical grade which includes Potassium dihydrogen orthophosphate, triethylamine, and Orthophosphoric acid. Acetonitrile (Merck) is of HPLC grade.

Preparation of Solutions:

Preparation of Mobile Phase: 0.01 N potassium dihydrogen orthophosphate buffer having pH 4.8 and acetonitrile in the ratio of 60:40 was used as a mobile phase.

Preparation of Diluent: 50: 50 v/v of water and acetonitrile was used as diluent. It was prepared by mixing 500 ml of water and 500 ml of acetonitrile. The solution was sonicated and filtered through a 0.5 µ filter before use.

Standard Stock Solution: Weighed and transferred 25 mg of glecaprevir and 10 mg of pibrentasvir into 25 ml volumetric flask, diluent was added to dissolve the contents and the resulting solution was sonicated for 15 min to obtain a standard stock solution (1000 µg/ml of glecaprevir and 400 µg/ml of pibrentasvir). From this the working standard solution of 100 µg/ml of glecaprevir and 40 µg/ml of pibrentasvir was prepared.

Assay Sample Preparation: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50 ml of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered (1000 ppm of glecaprevir and 400 ppm of pibrentasvir).

From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent. (100 ppm of glecaprevir and 40 ppm of pibrentasvir). Included chromatogram of assay sample solution as **Fig. 3**.

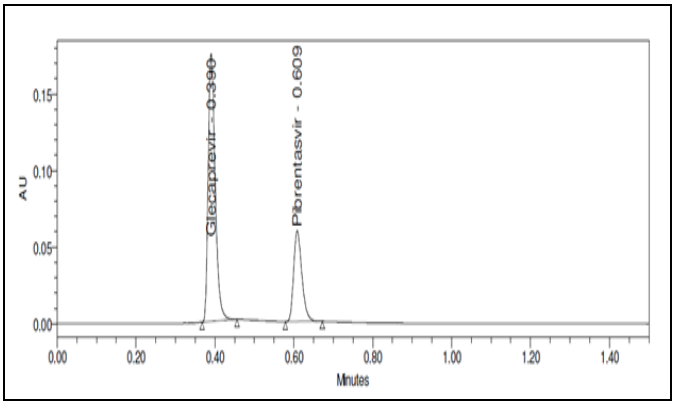

FIG. 3: CHROMATOGRAM OF ASSAY SAMPLE SOLUTION

Optimized Chromatographic Conditions: The separation was performed on the UPLC system equipped with column BEH C18 50 × 2.1mm 1.7µ which was maintained at 30 °C, eluted with isocratic mobile phase at flow rate of 0.3 ml/min and eluents were detected at 260 nm. The total run time was 1.5 min.

RESULTS AND DISCUSSION:

Method Validation: Before the application of the method for regular analysis, the developed and optimized method should satisfy the validation parameters drafted by International Conference on Harmonization (ICH). The validation parameters include system suitability, specificity, precision, linearity, accuracy, Limit of Detection (LOD), Limit of Quantification (LOQ), and robustness.

System Suitability: This is to test the performance of the system. It was analyzed by injecting a working standard solution (n=6) and measuring parameters such as tailing factor, theoretical plates and % Relative standard deviation (RSD). As per ICH guidelines % RSD should be not more than 2.0%. Refer **Table 1.**

TABLE 1: SYSTEM SUITABILITY DATA FOR GLECAPREVIR AND PIBRENTASVIR

Parameter	% RSD	
	Glecaprevir	Pibrentasvir
Retention time	0.392	0.607
Theoretical plates	2624	4587
Tailing factor	1.24	1.29

Specificity: This was checked by analyzing the chromatograms of blank, working standard, placebo, and forced degradation solutions. The method was specific when the analyte of interest was determined without any interference. By comparing **Fig. 4, 5** and **6,** the method was said to be specific.

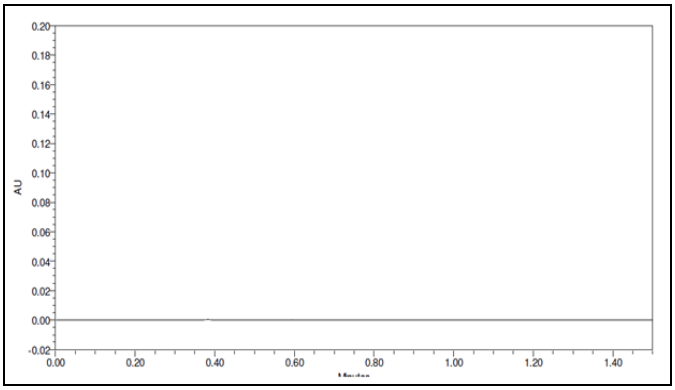

FIG. 4: CHROMATOGRAM OF BLANK SOLUTION

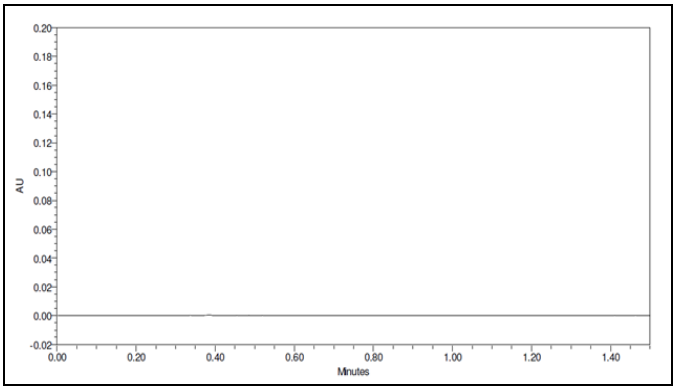

FIG. 5: CHROMATOGRAM OF PLACEBO SOLUTION

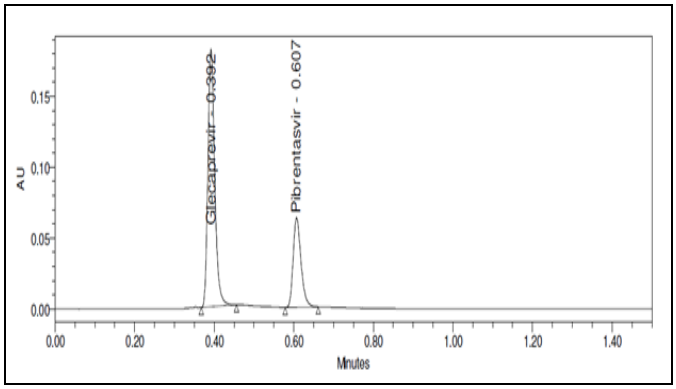

FIG. 6: CHROMATOGRAM OF WORKING STANDARD SOLUTION

Linearity: 25%, 50%, 75%, 100%, 125% and 150% of working standard solution (n=3) were prepared and analyzed. The measured data were subjected to the least square regression analysis. The method was linear when there was proportionate increase in peak area with respect to concentration and correlation coefficient was more than 0.999. **Table 2** represents linearity data and **Fig. 7** and **8** represent calibration graphs of glecaprevir and pibrentasvir. From the calibration graphs the correlation coefficient of glecaprevir and pibrentasvir was found to be 0.9994 and 0.9993 respectively.

TABLE 2: LINEARITY DATA

Linearity level	Glecaprevir		Pibrentasvir	
	Concentration µg/ml	Area	Concentration µg/ml	Area
25%	25	58568	10	23645
50%	50	104311	20	41706
75%	75	161439	30	63485
100%	100	217001	40	86606
125%	125	265174	50	106332
150%	150	324081	60	129495
	Correlation coefficient	0.9994	Correlation coefficient	0.9993

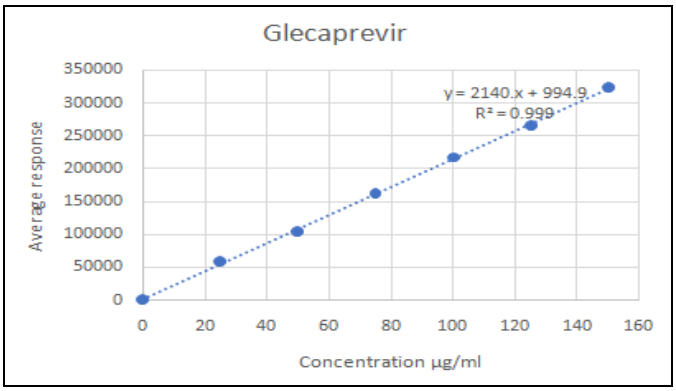

FIG. 7: CALIBRATION CURVE OF GLECAPREVIR

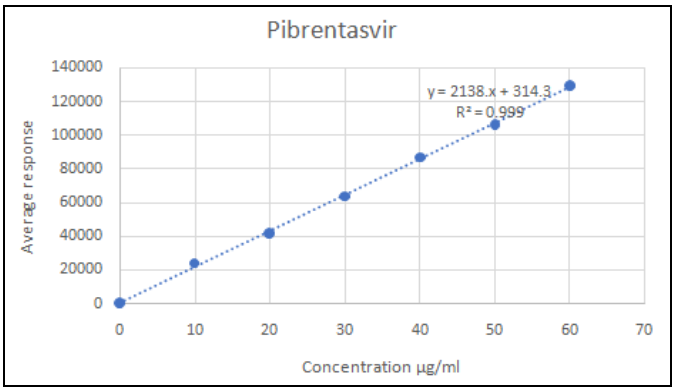

FIG. 8: CALIBRATION CURVE OF PIBRENTASVIR

TABLE 3: REPRODUCIBILTY DATA OF GLECAPREVIR AND PIBRENTASVIR

S. no.	Glecaprevir		Pibrentasvir	
	Day 1	Day 2	Day 1	Day 2
1	218553	202288	88338	81283
2	216264	201805	87562	81404
3	216754	200864	87872	81910
4	218311	201361	86949	81838
5	217324	203726	86814	81601
6	218729	204688	87512	81479
AVG	217656	202455	87508	81586
ST. DEV	1024.3	1468.3	568.9	247.0
% RSD	0.5	0.7	0.7	0.3

Precision: The reproducibility of the method on different systems by another analyst was checked by analyzing the working standard solution (n=6) and measuring % RSD. The method was said to be reproducible based on the results summarized in **Table 3**. For method precision, the assay sample solution was analyzed per the developed method and the amount of the drugs glecaprevir and pibrentasvir in the marketed formulation (MAVYRET) was determined. The % assay was found to be 99.93 ± 0.47 for glecaprevir and 99.81 ± 0.65 for pibrentasvir.

LOD and LOQ: LOD and LOQ amounts were determined from the formula 3.3*(σ/s) and 10*(σ/s) where σ is the standard deviation of y-intercept and s is average slope of the linear line. The LOD and LOQ of glecaprevir were 0.79 and 2.38 µg/ml respectively. The LOD and LOQ of pibrentasvir were 0.51 and 1.55 µg/ml respectively.

TABLE 4: ACCURACY DATA

Accuracy level		Glecaprevir				Pibrentasvir			
		Concentration of standard µg/ml	Amount spiked µg/ml	Amount found µg/ml	% recovery	Concentration of standard µg/ml	Amount spiked µg/ml	Amount found µg/ml	% recovery
50%	1	100	50	49.94	99.88	40	20	20.0794	100.40
	2	100	50	50.486	100.97	40	20	19.9928	99.96
	3	100	50	50.425	100.85	40	20	19.8357	99.18
100%	1	100	100	100.34	100.34	40	40	39.6486	99.12
	2	100	100	100.34	100.34	40	40	39.9746	99.94
	3	100	100	99.864	99.86	40	40	40.3371	100.84
150%	1	100	150	150.17	100.12	40	60	60.2913	100.49
	2	100	150	151.14	100.76	40	60	59.6163	99.36
	3	100	150	150.68	100.46	40	60	59.9068	99.84

Accuracy: The percentage recovery was calculated by adding a known amount of working standard solution to the sample solution. Accuracy was carried at three different levels 50%, 100% and 150% where n=3 and the mean % recovery was determined. Refer **Table 4** for results.

Robustness: The effect of minor changes in flow rate, percentage of organic content in the mobile phase and column temperature on separation of components were studied. Based on the observations % RSD was calculated, it should be not more than 2%. The results were summarized in **Table 5**.

TABLE 5: ROBUSTNESS DATA

S. no.	Condition	% RSD (Peak Areas)	
		Glecaprevir	Pibrentasvir
1	Flow rate (-10%)	0.9	1.0
2	Flow rate (+10%)	0.9	0.8
3	Mobile phase (-10% organic phase)	1.2	1.5
4	Mobile phase (+10% organic phase)	1.1	1.4
5	Temperature (-10%)	1.5	1.4
6	Temperature (+10%)	1.3	0.4

Forced Degradation Studies: The drug samples (n=3) were accelerated to degrade by treating with acid, base, water, heat, oxidizing agent and light. For acid degradation, 1 ml of 1N hydrochloric acid was added to 1 ml of stock solution and refluxed for 30 min at 60 ºC. For alkali degradation, 1 ml of 1N sodium hydroxide was added to 1 ml of stock solution and refluxed for 30 min at 60 ºC. For oxidative degradation, 1 ml of oxidizing agent 20% hydrogen peroxide was added to 1ml of stock solution and heated to 60 ºC for 30 min. for dry heat study, the required stock solution was placed in oven for 6 h at 105 ºC. for photolytic study; stock solution was placed in UV chamber for seven days.

For neutral degradation study, the stock solution was treated with water for 6 h at 60 ºC. The resultant solutions if required neutralized and diluted to obtain 100 ppm of glecaprevir & 40 ppm of pibrentasvir solution and 10 µl of this solution was injected into the system and the chromatograms were recorded to assess the stability of sample. Based on the results it was found to be that more degradation of both the drugs was observed when stressed with acid and alkali. Refer to **Table 6** for results.

TABLE 6: DATA OF FORCED DEGRADATION STUDIES

Stress condition	% of drug degraded	
	Glecaprevir	Pibrentasvir
Acid	6.52	5.51
Alkali	4.14	4.76
Peroxide	3.71	3.68
Thermal	2.85	2.09
UV	1.91	1.44
Water	0.80	0.82

CONCLUSION: The estimation of glecaprevir and pibrentasvir in the presence of degradants by UPLC was developed and validated. The retention time of both the drugs was found to be less than 1 min with a good resolution of 6.0. When the drugs were stressed under accelerated conditions more percentage of degradation was found when treated with acid and alkali. The degradants were well resolved from the eluents. Shorter run time made this method applicable for regular analysis of these drugs in quality control laboratories.

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CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Rang HP, Dale MM, Ritter JM and Moore PK: Pharmacology. Published by Elseiver, Fifth Edition 2003.
2. Teresa I. Ng, Rakesh Tripathi, Thomas Reisch, Liangjun Lu, Timothy Middleton, Todd A. Hopkins, Ron Pithawalla, Michelle Irvin, Tatyana Dekhtyar, Preethi Krishnan, Gretja Schnell, Jill Beyer, Keith F. McDaniel, Jun Ma, Guoqiang Wang, Li- Juan Jiang, Yat Sun Or, Dale Kempf, Tami Pilot- Matias and Christine Collins: In Vitro antiviral activity and resistance profile of the next-generation hepatitis c virus NS3/4A protease inhibitor glecaprevir. Antimicrobial agents and Chemotherapy 2018; 62 (1).
3. https://pubchem.ncbi.nlm.nih.gov/compound/Glecaprevir
4. https://pubchem.ncbi.nlm.nih.gov/compound/Pibrentasvir
5. Hemalatha K, Kistayya C, Nizammudhin ND and Dastiagiriamma D: Simultaneous estimation of new analytical method development and validation of glecaprevir and pibrentasvir by high performance liquid chromatography. Innovat International Journal of Medical and Pharmaceutical Sciences 2018; 3 (s1).
6. Saradhi NDVR, Jat RK and Reddy MV: A new technical method for glecaprevir and pibrentasvir in combined dosage forms using non polar HPLC. World Journal of

Pharmaceutical and Medical Research 2018; 4(12): 173-78.
7. Babu DC, Chetty CM and Mastanamma S: A new force indicating RP-HPLC method development and validation for the simultaneous estimation of pibrentasvir and glecaprevir in bulk and its tablet dosage form. Pharm Methods 2018; 9(2): 79-86.
8. Sreeram V and Venkateswarlu C: Stability indicating RP-HPLC method for the simultaneous estimation of glecaprevir and pibrentasvir in drug product. Journal of Pharmaceutical Sciences and Research 2018; 10(11): 2757- 61.
9. Kumar KR and Raja S: Simultaneous assay of two antiviral agents, pibrentasvir and glecaprevir using stability-indicating RP-HPLC method in bulk and tablets. Der Pharmacia Lettre 2018; 10(8): 33-47.
10. ICH Harmonized Tripartite guideline, Validation of Analytical Procedures: Text and Methodology Q2: 2005, 1-13.

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