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A robust stability indicating HPLC technique for evaluation of Pibrentasvir and Glecaprevir in tablet dosage form

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ABSTRACT

When liver cells gets infected and vandalized, the condition is termed as Hepatitis. HCV therapy is performed with mixture of drugs. For the combined evaluation of Pibrentasvir and Glecaprevir in tablets, a rapid, selective and robust HPLC technique stability indicating was developed herein this work. Analysis was executed by Cosmicsil, with dimensions 250 mm by 4.6 mm column and mobile phase possessing KH_2PO_4 with 0.1M, 65 ml and 35 ml of methanol and 230 nm of PDA analysis. Elution times were found out as were 1.663 min and 2.249 min, for Pibrentasvir and Glecaprevir respectively with linear ranges 20µg/ml, 60 µg/ml and 50 µg/ml, 150 µg/ml, respectively having detection limits as 0.190 µg/ml and 0.207 µg/ml and quantization limits as 0.634 µg/ml and 0.690 µg/ml. This method is explicit having RSD values as 0.097% Pibrentasir & 0.232% Glecaprevir showing an accuracy of between 98.82 and 100.07% for Pibrentasir 99.31, Glecaprevir 100.45% recovery values. During the investigation of degradation, peaks elution times of degradants greatly varied with the elution times of Glecaprevir and Pibrentasvir thus, proving method's power of stability indication and specificity. The validation and degradation stability studies were carried out according to ICH and ICH Q1B Guidelines.

Keywords: Hepatitis; HCV; Pibrentasvir; Glecaprevir; HPLC; Cosmicsil; PDA analysis; ICH.

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INTRODUCTION

When liver cells gets infected and vandalized, the condition is termed as Hepatitis. Although there are varied reasons for its occurrence and types, similar symptoms may be exhibited [1]. The major service of liver is to detoxify blood, store vitamins and manufacture hormones. Disruption of previously stated liver functions may lead to severe health issues in total body [2]. The acute and major kinds are Hepatitis A, B, C caused because of various viruses [3, 4].. Multi-class combination drugs refer to a single pill or pill pack combination of drugs. The combination of used drugs approved is represented in (Table 1). Pibrentasvir acts on NS3A proteases are indispensable to replication of hepatitis C virus RNA and virus assembly. These processes are clogged and hence virus growth is held in by Pibrentasvir [5-7]. Glecaprevir Proteases NS4A and 5A are preconditions for RNA replication and virus assembly of hepatitis C virus. Hence, blocks these two processes and thus virus development is suppressed [8-10].

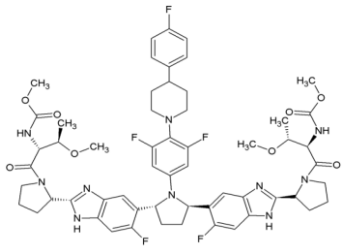


Figure 1: Structure of Pibrentasvir

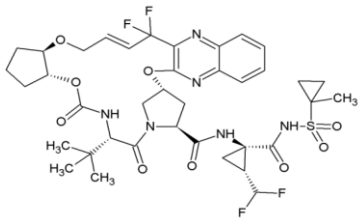


Figure 2: Structure of Glecaprevir

To the finest of our information, handful studies use HPLC and UPLC to assess pibrentasvir together with glecaprevir [11-17].

The main aim of this investigation is an effort to establish the RP-HPLC method which is stability indicating for testing pibrentasvir and glecaprevir which is economically friendly, fast and have a wide accurate range. The established method is validated for parameters such as sensitivity, linearity, specificity, selectivity, accuracy, robustness and precision according to ICH Guidelines [18]. And degradation studies were carried out to represent the method sensitivity according to ICH Q1B Guidelines [19].

MATERIALS AND METHODS

Materials used: The materials employed were Pibrentasvir, Glecaprevir, Methanol, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide, and Potassium dihydrogen phosphate.

The apparatus used were Waters alliance HPLC system, Photodiode array detector and Cosmicsil analytical column, measuring C18, 250 x4. 6 mm, 5 µm

HPLC technique conditions: The Mobile phase flow rate was 1.0ml/Min, Temperature was maintained at 25˚C, Volume subjected was 10µl, Run time was 5min, detected at the wave length of 230nm and maintained pH was 4.5.

Preparation of mobile phase

KH_2PO_4 of 0.1 M is blended in 65:35 parts with methanol: Orthophosphoric acid is used to alter pH to 4.5. This mixture is also applied as a solvent in the development of standard solutions.

Preparation of stock solution

Implicated in the preparation of stock solution of pibrentasvir and glecaprevir, a properly weighed 40 mg pibrentasvir and 100 mg glecaprevir in a 100 ml volumetric flask and exactly diluted with mobile phase. Concentration of stock solutions: pibrentasvir 400 µg/ml and glecaprevir 1000 µg/ml.

Preparation of sample solutions for validation

The standard solution to validate pibrentasvir and glecaprevir was performed in which mobile phase was used to dilute one ml of stock of pibrentasvir and glecaprevir to ten ml in the flask of capacity 10 ml. Standard solution concentration for validation is pibrentasvir 40 µg/ml and glecaprevir 100 µg/ml.

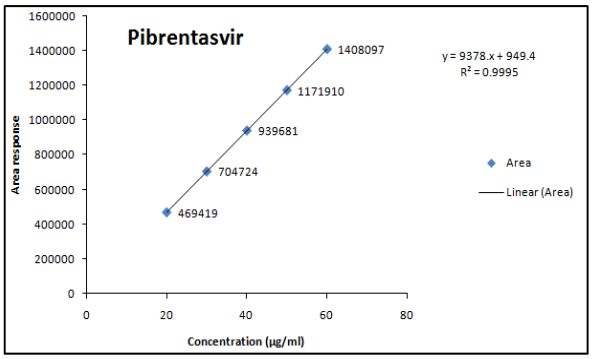
Optimized method

After several trials a method was optimized with following conditions represented in (Table 2) and results are represented in (Figure 3).

Assay of Pibrentasvir and Glecaprevir in Maviret tablet

Involved in the preparation of pibrentasvir and glecaprevir stock tablet solution, a properly weighed finely powdered Marivet tablet equivalent to 40 mg pibrentasvir and 100 mg glecaprevir in a 100 ml volumetric flask with 30 ml mobile phase were mixed. Concentrations of stock tablet solutions are 400µg/ml and 1000 µg/ml of pibrentasvir & glecaprevir respectively. Test tablet solution concentrations are 40µg/ml (pibrentasvir) and 100 µg/ml (glecaprevir).

A sample solution amounting 20 µl was 3 times infused into HPLC. The peak areas are measured at 230 nm and concentrations of pibrentasvir and glecaprevir in tablet specimens were determined with the help of linear regression equation or calibration graphs.



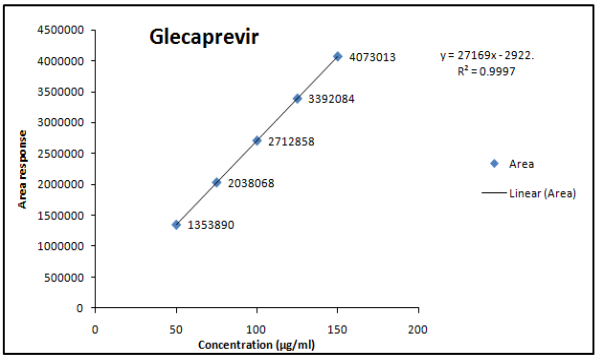


Figure 3: Linearity curves of Pibrentasvir and Glecaprevir

RESULTS AND DISCUSSIONS

A stability indicating method development & validation of Pibrentasvir and Glecaprevir was done by RP-HPLC method. The estimation was done by the analysis in RP-HPLC employing Cosmicsil C18 (250×4. 6mm, 5µm) chromatographic column. The mobile phase was mixture of Phosphate Buffer and Acetonitrile (65:35). The flow rate was 1.0 ml/ min and detection was performed at 230 nm.

Validation

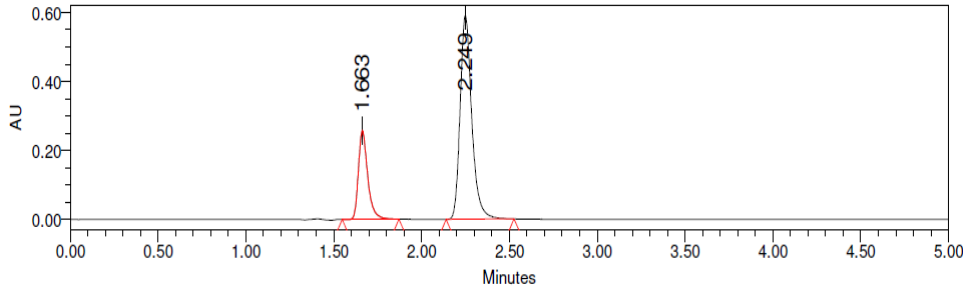
The new technique has been assessed to meet the International Conference on Harmonization (ICH) Q1B criteria, including sensitivity, linearity, selectivity, accuracy, specificity, robustness and precision.

Table 1: Multi-class drug combination to treat Hepatitis C virus

Brand Name	Generic Name	Status	Pharmaceutical Company
Epclusa*	Sofosbuvir + Velpatasvir	Approved	Gilead Sciences
Harvoni*	Ledipasvir + Sofosbuvir	Approved	Gilead Sciences
Mavyret	Glecaprevir + Pibrentasvir	Approved	Abbvie
Vosevi	Sofosbuvir/Velpatasvir/ Voxilaprevir	Approved	Gilead Sciences
Zepatier	Elbasvir + Grazoprevir	Approved	Merck
n/a	Dactlatasvir + Asunaprevir + Beclabuvir	Phase III	Bristol-Myers Squibb

Table 2: Optimized method conditions

Combo and ratio in blend of mobile phase	KH2PO4 (65%) and Acetonitrile (35%)
Trial Column	C18 Cosmicsil, 250 mm × 4.6 mm, 5 µm particle dimension
Flow rate trial	1.0 ml/min
Size of vol. sample injected	10 µl
Column temperature trial	25oC
Time run	5 min
Eluents checked at	230 nm



Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1.663	945840	25.81	257161	-	1.38	5083
2.249	2719026	74.19	589825	5.41	1.28	5868

Figure 4: Chromatogram of optimized method

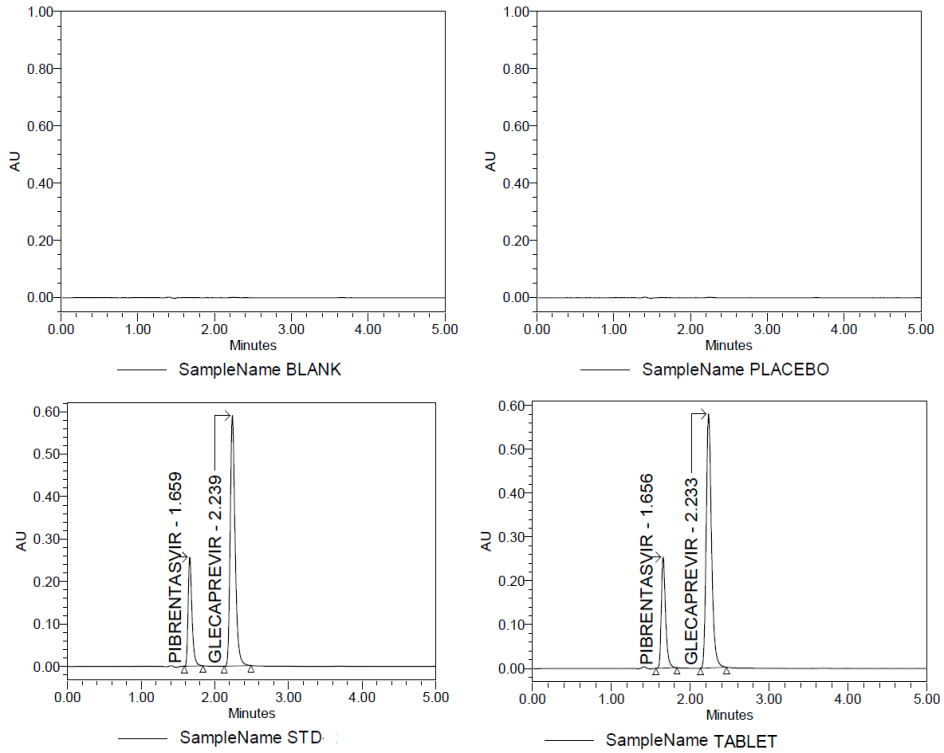


Figure 5: Chromatograms of selectivity

Selectivity

The selectivity evaluation was conducted by incorporating into the chromatographic system a volume of 20 µg solution standard (40 µg/ml pibrentasvir and 100 µg/ml glecaprevir), tablet sample (40 µg/ml pibrentasvir and 100 µg/ml glecaprevir) blank diluent and placebo. No peaks that interfere with peaks when pibrentasvir and glecaprevir are retained. These results are represented in (Figure 4).

Linearity

Linearity solutions were prepared with concentrations pibrentasvir and glecaprevir each solution for concentration was incorporated into the HPLC instrument and assessed according to the similar conditions. The results are represented in (Table 3) and linearity graphs are shown in (Figure 5).

Table 3: Peak area and concentration data

Conc of Glecaprevir µg/ml	Glecaprevir Area response	Conc of Pibrentasvir µg/ml	Pibrentasvir Area response
50	1353890	20	469419
75	2038068	30	704724
100	2712858	40	939681
125	3392084	50	1171910
150	4073013	60	1408097

The linearity range of Pibrentasvir was found to be HPLC 20-60 µg/ml, with R^2 value of 0.9995 and linearity range of Glecaprevir was found to be HPLC 60-150 µg/ml, with R^2 value of 0.9997. The %RSD for intra precision was <2%. The % recovery varies in the range of 95-105. The method also passes the specifications for robustness parameters

Sensitivity

The sensitivity measurement (LOD and LOQ) is performed by the signal to noise (S/N) ratio for both the drugs. Values proved good sensitivity. Results are represented in (Figure 6). LOD and LOQ was calculated by using following formula

$$\text{LOD: } \frac{3.3}{SD} \times 100, \text{ LOQ: } \frac{10}{SD} \times 100$$

LOD: 0.190 µg/ml for Pibrentasvir and 0.20 µg/ml for Glecaprevir. LOQ: 0.634 µg/ml for Pibrentasvir and 0.690 µg/ml for Glecaprevir.

Precision

For precise measurement six area response measurements of standard solution (40 µg/ml pibrentasvir and 100 µg/ml glecaprevir) were used. The RSD was calculated and it was precise.

Table 4: Area response of Glecaprevir and Pibrentasvir for precision

Area response	Area response	
	Glecaprevir	Pibrentasvir
Sample I	2708652	939412
Sample II	2716419	938701
Sample III	2705033	939606
Sample IV	2714205	938661
Sample V	2700375	937041
Sample VI	2714368	938340
Average	2709842	938627
Standard Deviation	6276.050414	914.2279
RSD	0.232	0.097

Accuracy

The precision was evaluated using tablets by pibrentasvir and glecaprevir recovery research. Each level concentration was prepared and analyzed for three times. For replicate specimens, the recovery percentage of added analytes was calculated. The result revealed the accuracy.

Robustness

Robustness was evaluated by inspecting the impacts in assay circumstances generated by minor alternations. The Theoretical plate count, asymmetry factor, resolution of analytes and peak area of glecaprevir and pibrentasvir in every condition shows that there was no major changes observed. Hence, the method is robust.

Degradation Studies for Pibrentasvir and Glecaprevir

Acid Hydrolysis - Degrading With 0.1n Hcl

10 ml solution of tablet with 400 µg/ml concentration of Pibrentasvir and 1000 µg/ml concentration of Glecaprevir was blended to 10 ml of Hcl with normality 0.1N at 27°C up to 30 min by sonication.

Base hydrolysis degrading with 0.1N NaOH

10 ml tablet solution (strength of 400 µg / ml pibrentasvir and 1000 µg / ml glecaprevir) was combined at 27°C to 10 ml 0.1N NaOH for 30 min by sonication.

Oxidative hydrolysis degrading with 30% hydrogen peroxide

10 ml of tablet solution (concentration of 400 µg/ml pibrentasvir and 1000 µg/ml glecaprevir) was combined for 1/2 an hour with 10 ml 30 percent H_2O_2 at 27°C through sonication.

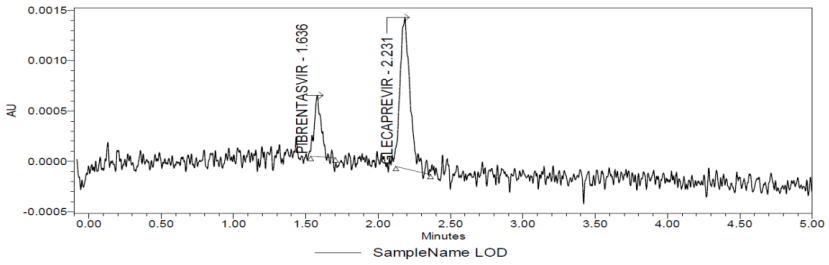
Thermal analysis degrading with dry heat at 105°C

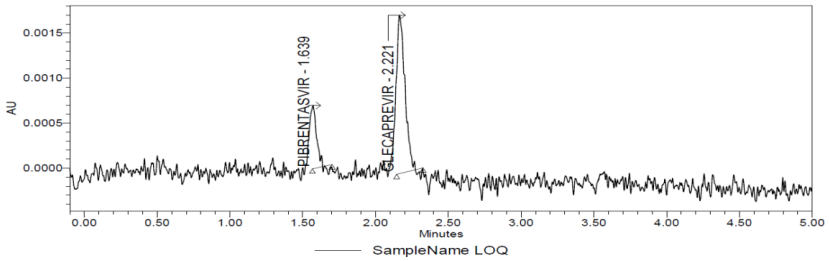
Tablet solution at temperature of 105°C (concentration 400µg/ml pibrentasvir and 1000 µg/ml glecaprevir) is applied for 30 min to 10 ml in hot air oven.

Photolysis Degrading with sunlight

10 ml tablet solution (400 µg/ml pibrentasvir concentration and 1000 µg/ml glecaprevir concentration) is held in sunlight for 6 hours.

Pibrentasvir and glecaprevir was more degraded in dry heat condition and less degraded in peroxide condition. The peak elution times of the degradants are





Peak Name: PIBRENTASVIR

	SampleName	Peak Name	RT	Area	s/n
1	LOD	PIBRENTASVIR	1.636	2035	3.77
2	LOQ	PIBRENTASVIR	1.639	2281	10.38

Peak Name: GLECAPREVIR

	SampleName	Peak Name	RT	Area	s/n
1	LOD	GLECAPREVIR	2.231	7377	3.36
2	LOQ	GLECAPREVIR	2.221	7341	10.10

Figure 6: Sensitivity test chromatograms

Table 5: Recovery data for Pibrentasvir for accuracy

Level	Area of response Pibrentasvir	Conc. of Pibrentasvir added (µg/ml)	Conc. of Pibrentasvir found (µg/ml)	Percentage of Pibrentasvir Recovered
50% spiked	468954	19.8	19.78	99.89
	468666	19.8	19.77	99.83
	469413	19.8	19.80	99.99
100% spiked	938544	39.6	39.58	99.96
	938907	39.6	39.60	99.99
	939645	39.6	39.63	100.07
150% spiked	1397221	59.4	58.93	99.20
	1391781	59.4	58.70	98.82
	1400703	59.4	59.07	99.45

Table 6: Recovery data for Gecaprevir for accuracy

Level	Area of response Glecaprevir	Conc. of Glecaprevir added (µg/ml)	Conc. of Glecaprevir found (µg/ml)	Percentage of Glecaprevir Recovered
50% spiked	1343575	49.5	49.16	99.31
	1350505	49.5	49.41	99.82
	1341895	49.5	49.10	99.18
100% spiked	2703946	99.0	98.93	99.93
	2710263	99.0	99.16	100.16
	2701147	99.0	98.83	99.83
150% spiked	4066263	148.5	148.77	100.18
	4068818	148.5	148.87	100.25
	4076857	148.5	149.16	100.45

Table 7: Robustness data of Pibrentasvir

Sample Name	Peak Name	RT	Area	USP Tailing	USP Plate Count
FLOW-1	Pibrentasvir	1.376	787370	1.38	4713
FLOW-2	Pibrentasvir	1.499	859477	1.39	4946
TEMP-1	Pibrentasvir	1.823	1059372	1.40	5582
TEMP-2	Pibrentasvir	2.054	1191705	1.40	5973
COMP-1	Pibrentasvir	1.376	787370	1.38	4713
COMP-2	Pibrentasvir	1.823	1059372	1.40	5582
pH-1	Pibrentasvir	1.657	942412	1.38	4997
pH-2	Pibrentasvir	1.654	939701	1.37	4973

Table 8: Robustness data of Glecaprevir

Sample Name	Peak Name	RT	Area	USP Tailing	USP Plate Count	USP Resolution
FLOW-1	Glecaprevir	1.847	2291831	1.29	5379	5.06
FLOW-2	Glecaprevir	2.007	2506019	1.29	5551	5.12
TEMP-1	Glecaprevir	2.429	3070004	1.29	6110	5.30
TEMP-2	Glecaprevir	2.751	3463366	1.30	6742	5.63
COMP-1	Glecaprevir	1.847	2291831	1.29	5379	5.06
COMP-2	Glecaprevir	2.429	3070004	1.29	6110	5.30
pH-1	Glecaprevir	2.233	2718652	1.27	5782	5.30
pH-2	Glecaprevir	2.230	2716419	1.27	5703	5.28

Table 9: Data achieved for degradation study

Test	Pibrentasvir			Glecaprevir		
	Area Response	Percentage remained	Percentage degraded	Area Response	Percentage remained	Percentage degraded
Acid	857202	90.38	9.62	2505748	91.68	8.32
Alkali	906340	95.56	4.44	2594536	94.93	5.07
H_2O_2	914387	96.41	3.59	2646545	96.83	3.17
Dry heat	845426	89.14	10.86	2403035	87.92	12.08
Sun light	904050	95.32	4.68	2555828	93.51	6.49

distinct from the time of glecaprevir and pibrentasvir being eluted. So interference will not occur. Results proved stability indicating ability.

CONCLUSION

Stability study on Pibrentasvir and Glecaprevir was carried out was an efficient HPLC method for the quantification of Pibrentasvir and Glecaprevir and identification of its degradation products and validated. The results of stress testing of API, undertaken to the ICH Q1B guidelines, revealed that degradation products were formed under acidic, alkaline, oxidizing and thermal conditions.

The results show the method is accurate, precise, sensitive, and economic friendly and rapid. Hence, the method can be successfully applied to the pharmaceutical dosage form and can be used for routine analysis.

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