Development and validation of a novel bioanalytical method for the simultaneous determination of glecaprevir and pibrentasvir in human plasma using reversed-phase high-performance liquid chromatography

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Background and objective
For the simultaneous determination of glecaprevir (GPR) and pibrentasvir (PTR) in human plasma, a novel, accurate, and selective reversed-phase high-performance liquid chromatography method was developed and validated.
Materials and methods
Owing to structural resemblance, bictegravir was selected as an internal standard. Anticoagulant used was K_2-EDTA. The GPR-PTR was the first of its kind approved drug by FDA for the treatment of chronic hepatitis C. Precipitation technique with acetonitrile was employed for the extraction of analyte from human plasma. Kromasil C_{18} column (5 µ, 150×4.6 mm) with an isocratic mobile phase of 0.1% orthophosphoric acid buffer pH 4.3, adjusted with dilute hydrochloric acid: acetonitrile in the ratio of 70 : 30 v/v, was used for the resolution. At a flow rate of 1 ml/min, the mobile phase was pumped. Using a photodiode array detector, effluents were monitored at 250 nm.
Results
Over concentration ranges of 5–200 µg/ml and 6.650–266.000 µg/ml, the method was found to be linear for GPR and PTR, respectively, in human plasma, with the precision and accuracy ranging from 0.76 to 9.05% and 90.55 to 98.98% for GPR respectively, whereas for PTR ranged from 0.74 to 9.52% and 91.56 to 105.61%, respectively.
Conclusion
The stability of the analyte was evaluated in plasma under different stress conditions.

Keywords:
acetonitrile, bictegravir, glecaprevir, Kromasil, orthophosphoric acid buffer, pibrentasvir, plasma

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Introduction

Each year, between two and four million new cases of hepatitis C virus (HCV) are reported by the World Health Organization [1]. Infections are primarily associated with intravenous drug use, blood transfusions, and tattoos, which are the few common causes of transmission [2]. The cause of 70–90% of chronic infections is acute infections, which are then followed by cirrhosis, chronic liver failure, hepatocellular carcinoma, and death [3]. Cirrhosis develops in 20–30% of patients with chronic liver disease, end-stage liver disease develops in 5–10%, and 4–8% die owing to liver-related causes after 20 years of infection. There are six genotypes of HCV, with genotypes 1–3 distributed worldwide [4,5]. Globally, genotypes 1a and 1b cause 60% of HCV infections. There is a 54% prevalence of genotypes 1a and 1b and a 37% prevalence of genotypes 3a [6,7]. In the United States and Europe, there is a low incidence of HCV genotype 4 infection (~1 and ~5% on average) [8].

In patients with genotype 1 HCV infection and prior failure of direct-acting antiviral therapy, glecaprevir (GPR) and pibrentasvir (PTR) were highly effective and well tolerated [9]. The sustained virologic response to direct-acting antiviral therapies for chronic HCV infection has been high; however, virologic failure may still occur, potentially resulting in viral resistance, and subsequently decreasing the efficacy of all subsequent treatments [10]. GPR is a potent pangenotypic next-generation HCV NS3/4A. According to enzymatic assays, GPR exhibited high selectivity for HCV

NS3/4A protease over human proteases. PTR inhibits NS5A, whereas GPR inhibits NS3/4A of HCV [9–11]. For the treatment of all six major genotypes of HCV, these drugs have been coformulated as GPR and PTR. After the European Union approved GPR/PTR for chronic HCV infection, it was then approved by the United States for chronic infections [9,11]. Figure 1a and b shows the chemical structures of GPR and PTR, respectively.

As per the literature survey, several methods have been reported for the estimation of GPR and PTR individually or in combination or with the combination of some other drugs in formulations [12–18]. To our knowledge, there is no bioanalytical method for the quantitative measurement of analytes in plasma using reversed-phase high-performance liquid chromatography (RP-HPLC). The present proposed method estimates GPR and PTR in a simple and economical process. It is the objective of this method to develop and validate a method for estimating GPR and PTR simultaneously in plasma using RP-HPLC.

Materials and methods

Chemicals and solvents

Cadila Health Care Ltd (Ahmadabad, India), provided the GPR (99.98%), PTR (100.00%), and internal standard (IS), bictegravir reference samples. During the analysis, Millipore Milli-Q system type II HPLC grade water was employed. Merck (Mumbai, India), provided HPLC grade acetonitrile. All additional chemicals were acquired from SD Fine Chem in Mumbai, India, and were of analytical grade. Brunda blood bank, Kadapa, India, provided human K2-EDTA plasma.

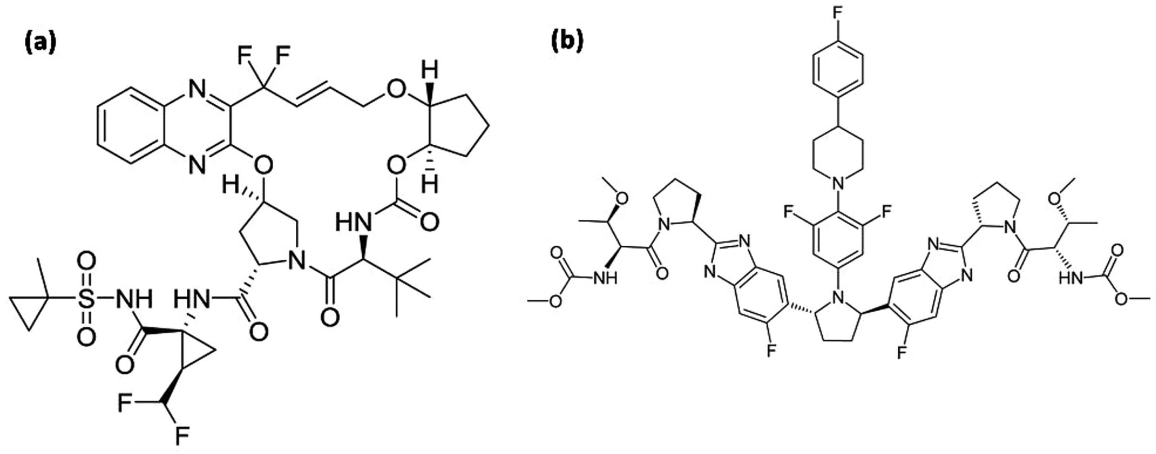
High-performance liquid chromatography–PDA instrumentation and chromatographic conditions

A Waters LC (Waters, Milford, Massachusetts, USA) HPLC system was used, which included a quaternary gradient system (controller 600), inline degasser (Waters, model AF), photodiode array detector (Waters, model 2998), and autosampler (Waters, model 717 plus). Empower Pro was used to process the data (Waters). A Kromasil C18 column (5 µ, 150×4.6 mm) kept at room temperature was used for the chromatographic separation. The mobile phase is 0.1% orthophosphoric acid buffer pH 4.3, adjusted with dilute hydrochloric acid: acetonitrile in the ratio of 70 : 30 v/v. A flow rate of 1.0 ml/min was used to pump the mobile phase. The isosbestic detection wavelength was 250 nm. Mobile phase was used as diluent for the preparation of working standards of GRP and PTR.

Stock and working standards solutions

Overall, 25 mg of GRP and PTR was dissolved in separate 25-ml volumetric flasks to make the stock solutions. Methanol was used to dissolve the drugs. This solution was then diluted in the same solvent to reach GRP and PTR concentrations of 500.00 µg/ml. The calibration standards were made by diluting the working standard solution to get final concentrations of 5.000, 10.000, 20.000, 45.000, 72.500, 100.000, 170.000, and 200.000 µg/ml for GPR and 6.650, 13.300, 26.600, 59.850, 96.425, 133.000, 226.100, and 266.000 µg/ml for PTR. Quality control (QC) samples were prepared for GPR as high-quality control (HQC): 170.00 µg/ml; mid quality control (MQC): 100.00 µg/ml; low-quality control (LQC): 15.00 µg/ml; and lower limit of quantitation quality control (LLOQQC): 2.50 µg/ml. Similarly, for PTR, the HQC, MQC, LQC, and LLOQQC were 226.10,

Figure 1



Chemical structures of (a) glecaprevir and (b) pibrentasvir.

133.00, 19.95, and 3.325 μg/ml, respectively. The IS stock solution was made by dissolving 25.0 mg of bictegravir in 25 ml of methanol. To make a 100.00 μg/ml IS solution, another 10 ml of stock solution was diluted to 100 ml. All of the solutions were kept between 2 and 98°C.

Sample preparation

The protein precipitation procedure was used to prepare the samples. In 2-ml Eppendorf centrifuge tubes, 200-μl aliquots of blank, standard blank (STD), calibration curve standard (STD-1 to STD-8), and QC samples were prepared. All tubes except the STD blank received 50 μl of IS working solution (100 μg/ml was added) and vortexed for about 10 s. The plasma contents were then precipitated by adding 2 ml of acetonitrile to the vials above and vortexed for 10 min. In refrigerated centrifuges at 4°C, all vials were centrifuged for 10 min at 4500 rpm. Approximately 1 ml of supernatant was put into prelabeled glass vials and dried on a nitrogen evaporator at 40±5°C with a moderate stream of nitrogen. The dried samples were then reconstituted with 1 ml of mobile phase and agitated for 1 min before being put onto an autosampler tray and injected into the HPLC system in 10-μl volume.

Method validation

The validation process was validated in accordance with the requirements of US Food and Drug Administration (USFDA) [19,20].

Selectivity

By comparing the signals in six distinct plasma lots (four were K2-EDTA and one was each lipidemic and hemolyzed) with the analyte and IS signals at the LLOQ level, interference owing to endogenous plasma matrix components was investigated. At this concentration level, the reference noise should be around 20% of the analyte response.

Calibration curve and lower limit of quantitation

A series of eight calibration curve standards, 5.000, 10.000, 20.000, 45.000, 72.500, 100.000, 170.000, and 200.000 μg/ml, for GPR and 6.650, 13.300, 26.600, 59.850, 96.425, 133.000, 226.100, and 266.000 μg/ml for PTR were prepared to test the method's linearity. A $1/x^2$ weighted least squares regression analysis of standard plots associated with an eight-point standard curve was used to validate each calibration curve. The standard curve was selected to encompass the patients' clinically relevant concentration range. The curve has been verified; at least six of the eight calibration standards must have a coefficient of variation (CV) of less than 15%. All calibration curves should have a correlation better than 0.99. The lower limit of quantification was set at the lowest concentration on the calibration curve. All calibration standards should have a CV and accuracy error of less than 15%, and LLOQ should have a CV and accuracy error of less than 20%. The response of LLOQ of the analyte should be at least five times higher than the response of in blank.

Precision and accuracy

Precision and accuracy are crucial elements in measuring repeatability. Six repeat samples from each LLOQ, as well as low, medium, and HQC samples, were used to establish the method's precision and accuracy. Plasma samples were examined on the same day to establish intraday precision and accuracy. By assessing six batches for precision and accuracy on various days, we were able to determine between-day precision and accuracy. Accuracy was calculated as the difference in percentage between the theoretical value and the one obtained using the following equation:

$$\text{Accuracy (\%)} = \frac{(Measured\ Concentration - Theoretical\ Concentation)}{(Theoretical\ Concentation\ )} X100\%$$

With the exception of LLOQ, which should not exceed 20%, the percent variation of each concentration level from the nominal concentration in accuracy and precision should be less than 15%.

Recovery from plasma

The ratio of the mean analyte concentrations in plasma following extraction of the HQC, MQC, and LQC samples to the corresponding quantities dissolved directly in the elution solution was used to determine the method's extraction (recovery) efficiency. Similarly, the IS recovery was determined by comparing the mean concentration of six plasma samples to a reference IS solution of identical concentration. According to FDA requirements, analyte recovery does not have to be 100%, but it must be consistent, accurate, and repeatable.

Matrix effect

By comparing the mean peak area of a given concentration analyte with IS spiked with white plasma samples, the matrix effect was determined. By adding the same quantity of analyte and its IS to 1000 μl of final elution solution, this reaction may be compared. Six distinct chromatographically screened human plasma plots were used to assess the matrix effect for the HPLC procedure. Sample concentrations

corresponding to LQC and HQC at each level were made and injected in triplicate with each batch of plasma. If the percent CV did not surpass 15% of all CCs, the matrix effect was validated.

Stability

Stock solutions for the analytes were tested for stability at 8°C for seven days and at room temperature for 7 h. The freshly created solutions' mean peak area was compared with that of the stability solutions and represented as a mean % change. The study of each analyte was evaluated at the concentration level of LQC and HQC using six replicates in each concentration at the benchtop (at room temperature for 6 h), freeze-thaw (at −20°C for 24 h), short-term stability (6 h at 8°C and 7 h at room temperature), and long-term stability (at −20°C). According to USFDA regulations, the analyte is deemed stable if the percent change is less than 15%. Under varied temperature and time settings, the analytes' laboratory stability, short-term stability, and freeze-thaw stability were assessed. The stability samples' and newly created samples' concentrations were computed, and the stability was given as a percentage of the mean difference from the estimated concentration. Long-term stability was tested for 30 days at −20°C and then treated and compared with newly made solutions.

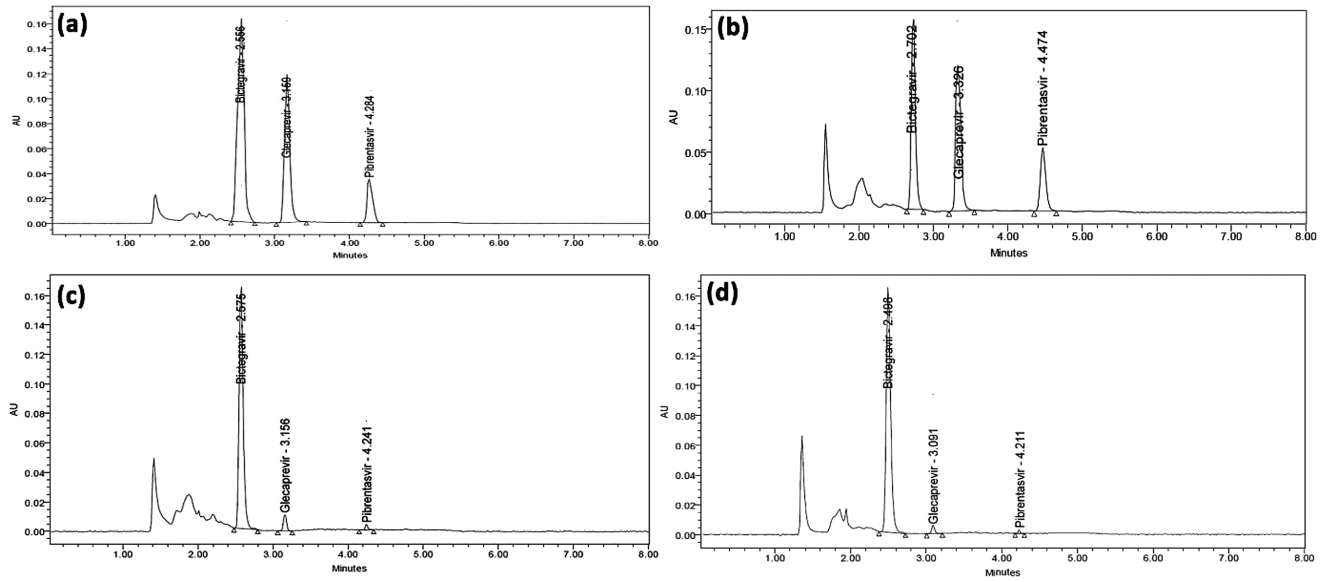
Results and discussion

Optimization of sample preparation and chromatographic conditions

One of the most important phases in the development of a bioanalytical approach is sample preparation. Sample preparation must be rapid, simple, and straightforward, with the use of the fewest possible chemicals and solvents to obtain maximal analyte recovery. Solid-phase extraction was the first step in the extraction process (SPE). In comparison with the LLE approach, SPE is a costly and time-consuming procedure. As a result, we apply the LLE approach to prepare samples to save money, save processing time, and achieve the appropriate analyte recoveries. However, the LLE technique was also proved to be very laborious and the % recovery of the analytes was also too low. Hence, we tried with protein precipitation technique, which proved to be a very simple and cost-effective procedure with excellent recoveries and reproducibility. Higher plasma volumes for sample preparation and injection volume for chromatographic development have been observed in some procedures. Despite the fact that the present approach was designed with a lower plasma and injection volume, it has a higher acceptance. To extract the analyte from human plasma, a range of solvents and precipitation buffers were employed, including diethyl ether, ethyl acetate, ethanol : n-hexane (80 : 20), acetonitrile, and others. Only acetonitrile yielded a response. There was no interference from the plasma matrix, both exogenous and endogenous, and IS had no effect on analyte recovery or sensitivity.

Sofosbuvir, velpatasvir, voxilaprevir, bictegravir, and daclatasvir were tried as IS but bictegravir was selected as IS owing to its physiochemical property like pKa 9.4 and log P:3.58 and detection with Photo

Figure 2



Representative chromatograms of (a) HQC; (b) MQC; (c) LQC; and (d) LLOQ. HQC, high-quality control; LLOQ, lower limit of quantitation; LQC, low-quality control; MQC, mid quality control.

Table 1 Calibration curve results

STD ID	STD-1	STD-2	STD-3	STD-4	STD-5	STD-6	STD-7	STD-8			
Glecaprevir											
Nominal concentration (μg/ml)	5.000	10.000	20.000	45.000	72.500	100.000	170.000	200.000	Slope	Intercept	R2
Mean back calculated concentration (μg/ml)											
Mean*	4.5580	9.2085	19.5740	44.4625	72.0775	99.1370	169.7050	200.0510			
SD	0.23900	0.00071	0.42285	0.45891	0.13081	0.04525	0.65902	0.62225	1.0029	–0.5360	0.9991
% CV	5.24	0.01	2.16	1.03	0.18	0.05	0.39	0.31			
% Mean accuracy	91.16	92.09	97.87	98.81	99.42	99.14	99.83	100.03			
Pibrentasvir											
Nominal concentration (μg/ml)	6.650	13.300	26.600	59.850	96.425	133.000	226.100	266.000	Slope	Intercept	R2
Mean*	6.3580	12.7085	26.0740	59.4625	96.5775	133.0370	225.7050	266.5510			
SD	0.52184	0.70640	0.28426	0.45891	0.57629	0.09617	0.65902	0.08485	1.0027	–0.4275	0.9991
% CV	8.21	5.56	1.09	0.77	0.60	0.07	0.29	0.03			
% Mean accuracy	95.61	95.55	98.02	99.35	100.16	100.03	99.83	100.21			

CV, coefficient of variation; STD, standard blank. *Average of three replicates.

Table 2 Accuracy and precision data

Analytes	Glecaprevir				Pibrentasvir			
QC ID	HQC	MQC	LQC	LLOQQC	HQC	MQC	LQC	LLOQQC
Nominal concentration (μg/ml)	170.000	100.000	15.000	2.500	226.100	133.000	19.950	3.325
P and A ID	Calculated concentration (μg/ml)							
Within batch precision and accuracy								
Mean*	169.7107	98.4608	14.8725	2.4125	225.3370	133.9608	17.3725	3.7368
SD	1.97494	0.97941	0.81691	0.22862	1.05701	1.68019	0.99496	0.34749
% CV	1.16	0.99	5.49	9.48	0.47	1.25	5.73	9.30
% Mean accuracy	99.83	98.46	99.15	96.50	99.66	100.72	87.08	112.39
Between batch precision and accuracy								
Mean	168.2660	97.8884	13.5829	2.3537	223.5881	132.6604	18.2667	3.5114
SD	1.68863	0.74574	1.22862	0.13796	1.65587	1.48495	0.96690	0.33428
% CV	1.00	0.76	9.05	5.86	0.74	1.12	5.29	9.52
% Mean accuracy	98.98	97.89	90.55	94.15	98.89	99.74	91.56	105.61

HQC, high-quality control; LLOQQC, lower limit of quantitation quality control; LQC, low-quality control; MQC, mid quality control.
*Average of six replicates.

Diode Array (PDA) detector. Moreover, a small volume of bictegravir was sufficient for a strong signal in PDA. To achieve good sensitivity, peak shape, and symmetry as well as short chromatographic run time for both analytes and IS, all chromatographic conditions were adjusted and optimized. The thermo, Gemini, symmetry, water, and moon columns were exhausted in this experiment with various mobile phases such as acetonitrile, methanol, formic acid, ammonium formate, ammonium acetate, phosphate buffers, and aqueous ammonia, among others. Finally, the Kromasil C18 column (5 μ, 150×4.6 mm) was chosen for better separation and detection, with the mobile phase consisting of 0.1% orthophosphoric acid buffer pH 4.3, adjusted with diluted hydrochloric acid: acetonitrile in the ratio of 70 : 30 v/v to a flow rate of 1.0 ml/min maintained at room temperature. Column overload with analytes has been decreased because of the modest injection volume of 10 μl, resulting in more runs on the same column. Finally, at 2.56, 3.15, and 4.26 min, IS, GPR, and PTR were eluted, respectively. Figure 2a–d represents the chromatograms of HQC, MQC, LQC, and LLOQ, respectively.

Selectivity

In a retention period of the GPR, PTR, and IS samples isolated from human plasma, no interference peaks attributable to endogenous or exogenous components were identified, as shown in Fig. 1. Drugs had a response rate of less than 2% in white plasma. IS, GPR, and PTR retention times were 2.56, 3.15, and 4.26 min, respectively.

Calibration and lower limit of quantitation

The calibration curves were linear in the range of 5–200 μg/ml for GRP and 6.650–266.000 for PTR,

with lower quantification limits of 2.500 µg/ml for GRP and 3.325 µg/ml for PTR. For the eight calibrated curves studied, the coefficient of correlation is better than 0.999. The current bioanalytical method has a lower quantification limit and a good linearity interval. The mean concentrations obtained for the calibration curve are shown in Table 1.

Precision and accuracy

The within the batch accuracy of the method was between 98.46 and 99.86% for GPR and 87.08 and 112.39% for PTR, with a % CV of 1.16–9.48% for GPR and 0.47–9.30% for PTR. Between the batch, the accuracy was from 90.55 to 98.89% and 91.56 to 105.61% for GRP and PTR, respectively, with a % CV of 0.76–5.86% for GRP and 0.74–9.52% PTR. The findings show that the procedure was repeatable and reproducible enough. The precision and accuracy values are shown in Table 2.

Recovery

By comparing the mean peak area in the samples collected with new unextracted samples generated at three concentrations, the percentage of recovery was calculated. The concentrations of plasma QC samples taken in HQC, MQC, and LQC were compared with QC samples collected in HQC, MQC, and LQC to estimate the mean percent recoveries. The average recovery rate for GPR HQC, MQC, and LQC was 99.52, 99.19, and 103.97%, respectively. Similarly, for PTR HQC, MQC and LQC, the rates were 99.26, 99.63, and 105.49%, respectively. The results of recovery are given in Table 3. The IS was recovered 99.40% the time. In analytical procedures, it has been reported that the percent recovery should be at least 80%. Although the creation of a bioanalytical technique for the aim of recovery is not regarded as an issue if the approach provides sensitivity, precision, and precision, it is considered a problem if the method produces sensitivity, precision, and precision.

Table 3 Results of recovery

Parameters	Glecaprevir						Pibrentasvir						ISTD	
	HQC		MQC		LQC		HQC		MQC		LQC		HQC	
	Aqueous response	Extracted response	Aqueous response	Extracted response	Aqueous response	Extracted response	Aqueous response	Extracted response	Aqueous response	Extracted response	Aqueous response	Extracted response	Aqueous response	Extracted response
Mean*	165.40	164.60	98.30	97.50	15.10	15.70	229.20	227.50	134.40	133.90	18.20	19.20	100.50	99.90
SD	0.50	0.50	0.70	0.20	0.20	0.30	1.10	0.40	0.40	0.30	0.10	0.40	0.90	0.30
% CV	0.30	0.30	0.71	0.21	1.32	1.91	0.48	0.18	0.30	0.22	0.55	2.08	0.90	0.30
% Mean recovery	99.52		99.19		103.97		99.26		99.63		105.49		99.40	
Overall % mean recovery			100.89						101.46				99.40	
Overall SD			2.67						3.50				0.60	
Overall % CV			2.65						3.44				0.60	

HQC, high-quality control; LQC, low-quality control; MQC, mid quality control. *Average of six replicates.

Matrix effect

LQC and HQC concentrations of GPR and PTR were prepared with six different batches of human plasma and screened chromatographically to assess the matrix effect. Table 4 shows the matrix effect data. %Mean accuracy proves that the plasma lots selected do not have any matrix effect.

Stability

Benchtop stability

A six-hour benchtop stability test of plasma samples of HQC and LQC concentrations was conducted. Samples prepared fresh were compared to analyze

stability. Based on the % mean stability for HQC and LQC, GPR was 101.67 and 107.12%, and PTR was 100.15 and 98.33%.

Freeze-thaw stability

The stability of frozen samples was assessed by performing three freeze-thaw cycles, and the resulting samples were stored below −20°C. Freeze-thaw stability % mean values for GPR and PTR were 100.20 and 101.03%, respectively, and 100.84 and 98.60% for HQC.

Autosampler stability

In an autosampler maintained at 10°C, HQC and LQC samples were stored. A comparison of the samples injected at 0 h and those injected at stability time was undertaken for the assessment of autosampler stability. In terms of percent mean stability, the % mean for HQC and LQC for GPR was 98.79 and 104.56% and for PTR was 101.50 and 92.89%.

Short-term stability of drug in plasma

During six and a half hours, postextracted HQC and LQC samples were stored at ambient temperature for determining drug concentrations. The samples were compared against freshly prepared samples to determine stability. During the last six and a half hours of testing, the % mean stability of HQC and LQC was 99.95 and 94.69% for GPR and PTR, respectively. The HQC grade was 100.58% and the LQC grade was 98.62%. The IS %mean stability was 99.97%.

Long-term stability of drug in plasma

A 30-day long-term stability test of the spiked quality control samples (HQC and LQC) was performed below −20°C and below −50°C. The stability of the freshly spiked quality control samples was evaluated by comparing them to samples that were frozen for about 15 and 30 min and then thawed. HQC and LQC had stable % mean values of 99.67 and 102.29%, respectively. In contrast, the overall HQC and LQC for PTR were 99.98 and 97.94%. The % mean stability of IS was found to be 97.67%. GPR and PTR stability results are shown in Table 5 and Table 6, respectively.

$$\% \text{ Change} = \frac{\text{Mean Stability Sample} - \text{Mean Comparision Sample}}{Mean\ Comparision\ Sample} X100$$

Table 4 Results of matrix effect

Parameters	HQC 170.000 µg/ml	LQC 15.000 µg/ml	HQC 226.100 µg/ml	LQC 19.950 µg/ml
Mean calculated concentration (µg/ml)*	166.6348	14.4050	225.7243	18.0662
% Mean accuracy	98.02	96.03	99.83	90.56
SD	1.5651	0.6001	2.9468	0.6801
% CV	0.94	4.17	1.31	3.76

HQC, high-quality control; LQC, low-quality control. *Average of six plasma batches.

Conclusion

The suggested RP-HPLC methodology for determination of GPR and PRT in human plasma is rapid, sensitive, and repeatable, with linear dynamic ranges of 5.00–200.00 and 6.650–266.00 µg/ml, respectively. It has been verified and has met all requirements with a high degree of accuracy and precision in compliance with the guidelines of USFDA standards. The lack of matrix effects has been proved enough. Furthermore, the stability investigation revealed that both analytes were stable in plasma throughout sample preparation and storage. Our innovative approach is particularly suited and

Table 5 Stability data of glecaprevir at low and high-quality control levels

		Mean measured concentrations (µg/ml) (n=6)				
Stability	QC level	Comparison sample	Stability sample	% Change	% CV	% Mean stability*
Benchtop	HQC	171.142	173.994	−1.64	0.55	101.67
	LQC	13.649	14.6211	−6.65	5.83	107.12
Freeze-thaw	HQC	170.947	171.293	−0.20	0.29	100.56
	LQC	14.823	14.947	−0.83	3.99	100.76
Auto sampler	HQC	171.111	169.045	1.22	0.56	100.65
	LQC	14.850	15.527	−4.36	0.50	99.44
Short term	HQC	169.880	169.801	0.05	0.10	99.95
	LQC	15.640	14.810	5.60	0.78	94.69
Long term	HQC	168.410	167.850	0.33	0.91	99.67
	LQC	14.100	14.420	−2.22	0.78	102.29

HQC, high-quality control; LQC, low-quality control. *% Stability=%mean change in the concentration of the stability samples when compared to the freshly spiked samples.

Table 6 Stability data of pibrentasvir at low and high-quality control levels

		Mean measured concentrations (μg/ml) (*n*=6)				
Stability	QC Level	Comparison sample	Stability sample	% Change	% CV	% Mean stability*
Benchtop	HQC	225.9605	226.2892	–0.15	0.52	100.15
	LQC	20.1021	19.7672	1.69	6.53	98.33
Freeze-thaw	HQC	223.8298	226.1254	–1.02	0.68	101.03
	LQC	20.3188	20.0340	1.42	1.11	98.60
Auto sampler	HQC	224.3999	226.6536	–0.99	0.40	101.00
	LQC	21.7765	20.2274	7.66	2.21	92.89
Short term	HQC	225.18	226.47	–0.57	0.76	100.58
	LQC	20.66	20.38	1.37	1.30	98.62
Long term	HQC	226.16	226.12	0.02	0.92	99.98
	LQC	20.85	20.42	2.11	1.29	97.94

HQC high-quality control; LQC, low-quality control. * % Stability=%mean change in the concentration of the stability samples when compared to the freshly spiked samples.

useful for describing clinical pharmacokinetics and bioequivalence analysis investigations of GPR and PRT in humans due to its lower LLOQ, smaller plasma volume, and shorter run time (just 8 min).

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Conflicts of interest

There are no conflicts of interest.

References

1 World Health Organization. Global hepatit is report 2017: web Annex B: WHO estimates of the prevalence and incidence of hepatitis C virus infection by WHO region, 2015. Geneva, Switzerland: World Health Organization; 2018.

2 Pawlotsky JM, Tsakiris L, Roudot-Thoraval F, Pellet C, Stuyver L, Duval J, Dhumeaux D. Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. J Infect Dis 1995; 171:1607–1610.

3 El-Serag HB. Hepatocellular carcinoma: an epidemiologic view. J Clin Gastroenterol 2002; 35:S72–S78.

4 Omata M, Kanda T, Wei L, Yu ML, Chuang WL, Ibrahim A, *et al.* APASL consensus statements and recommendations for hepatitis C prevention, epidemiology, and laboratory testing. Hepatol Int 2016; 10:681–701.

5 Varma V, Webb K, Mirza DF. Liver transplantation for alcoholic liver disease. World J Gastroenterol 2010; 16:4377–4378.

6 Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 2015; 61:77–87.

7 Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: an up-date of the distribution and circulation of hepatitis C virus genotypes. World J Gastroenterol 2016; 22:7824–7825.

8 Kamal SM, Nasser IA. Hepatitis C genotype 4: what we know and what we don't yet know. Hepatology 2008; 47:1371–1383.

9 Yousif MM, Ahmed H, Elsadek HM, Shendi AM, Gouda TM, Elsayed IA, *et al.* Real-world safety and effectiveness of retreatment of Egyptian chronic hepatitis C patients not responding to NS5A inhibitor-based therapies. J Viral Hepat 2020; 27:1190–1201.

10 Shrivastava S, Steele R, Ray R, Ray RB. MicroRNAs: role in hepatitis C virus pathogenesis. Genes Dis 2015; 2:35–45.

11 Said EM, Abdulaziz BA, El Kassas M, El Attar IH, Emadeldeen M, Abd-Elsalam SM. High success rates for the use of sofosbuvir/ombitasvir/paritaprevir/ritonavir+ ribavirin and sofosbuvir/simeprevir/daclatasvir+ ribavirin in retreatment of chronic hepatitis C infection after unsuccessful sofosbuvir/daclatasvir therapy: a real-life experience. Arch Virol 2020; 165:1633–1639.

12 Reddy TK, Kistayya C, Nizamuddhin ND, Aparna T. Simultaneous estimation of new analytical method development and validation of ranitidine and magaldrate by high performance liquid chromatography. Innovat Int J Med Pharma Sci 2018; 3:112–115.

13 Saradhi ND, Jat RK, Reddy MV. A new technical method for Glecaprevir and Pibrentasvir in combined dosage forms using non polar HPLC. World J Pharm Med Res 2018; 4:173–178.

14 Babu DC, Chetty CM, Mastanamma SK. 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; 9:79–86.

15 Sreeram V, Venkateswarlu C. Stability indicating RP-HPLC method for the simultaneous estimation of glecaprevir and pibrentasvir in drug product. J Pharma Sci Res 2018; 10:2757–2761.

16 Sakuntala Mamidi SV, Atmakuri LR, Mamidipalli WC. Stability-indicating high performance liquid chromatographic method for simultaneous assay of pibrentasvir and glecaprevir: method development, validation and application to tablet dosage forms. J Res Pharma 2019; 23:1–3.

17 Sunitha N, Bhargav S, Marihal SC, Rao BA. Method development and validation of glecaprevir and pibrentasvir in pure and pharmaceutical dosage forms by RP-HPLC method. Indian J Res Pharma Biotechnol 2019; 7:3–5.

18 Reddy YN, Sreeramulu J, Balaswami B. A new stability-indicating RP-HPLC-PDA method for simultaneous estimation of glecaprevir and pibrentasvir in tablet dosage form. Res J Pharma Technol 2019; 12:625–631.

19 Ishaq BM, Reddy LS, Basha GM, Sreenivasulu M, Chetty CM, Ahad HA. Rapid and sensitive bioanalytical method development and validation for quantification of metoprolol using LC-MS/MS in human plasma. J Chem Soc Pak 2020; 42:171–175.

20 Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration Centre for Drug Evaluation and Research (CDER), Centre for Veternary Medicine (CVM), (2001) BP. Available at: http://www.fda.gov/cder/guidance/index.htm [Accessed May 2, 2022].