RESEARCH ARTICLE

Simultaneous RP-HPLC Estimation, Validation and Stability Indicating Assay of Two-Component Tablet Formulation Containing Grazoprevir and Pibrentasvir

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ABSTRACT

The determination of a two-component tablet formulation containing grazoprevir and pibrentasvir for method development and validation has been done using the reverse-phase high-performance liquid chromatography (RP-HPLC) method as per International Council of Harmonization (ICH) guidelines. Analytical grade acetonitrile and 0.01 N of potassium dihydrogen phosphate buffer were used as mobile phase while Kromasil C_{18} column was opted for separation. In order to elute the analyte a flow rate of 1-mL/min having 260 nm λ has been maintained. An RT of 2.909 and 2.358 minutes while linearity concentration range from 25 to 150 µg/mL and 10 to 60 µg/mL was obtained for grazoprevir and pibrentasvir, respectively, with 4.4 resolution. Both had a high correlation coefficient of 0.999. The limit of quantification was 0.24 and 0.09 µg/mL, while the detection limit at 0.72 and 0.27 µg/mL was noticed for grazoprevir and pibrentasvir, respectively. For grazoprevir and pibrentasvir, the regression equation was determined to be y = 16332x + 5313.1 and y = 17261x + 601.95, correspondingly.

Keywords: Grazoprevir, Pibrentasvir, ICH guidelines, Retention time.

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INTRODUCTION

A combinational form of grazoprevir and pibrentasvir is being considered to medicate hepatitis C virus (HCV) infection. A 2nd-generation HCV protease inhibitor, grazoprevir acts at non-structural protein whereas, as an antiviral agent pibrentasvir inhibits non-structural protein 5A (NS5A).[1-8]

When formulating a single dosage form with a drug combination, a simultaneous analytical method for routine and stability testing of the product must be developed. It is clear from reading the literature that there aren't enough published methods of measuring pibrentasvir and grazoprevir on alone or in combination with other drugs.[9-18] Grazoprevir and pibrentasvir were methods developed and validated in tablet formulation using reverse-phase high-performance liquid chromatography (RP-HPLC) for determination, in line with International Council of Harmonization (ICH) Q2(R1) guidelines as the primary objective of this research paper. Additionally, stress experiments were carried out in compliance with ICH guidelines Q1A (R2).[8,19-22]

An extensive search of the literature was seen on glecaprevir and pibrentasvir estimation, grazpprevir and elbasvir combinations, but grazoprevir and pibrentasvir combination method by using RP-HPLC has not been reported in the literature regarding analysis, hence the present work deals in developing and validating simple, economic, rapid, reproducible, selective, precise, accurate, cost-effective method using RP-HPLC method along with stability indicating studies for the two combinational drug of glecaprevir and pibrentasvir in tablet form,[23-28] the respective structure is depicted in Figure 1.

MATERIALS AND METHODS

A column of Kromasil C_{18} having 4.6 x 150 mm size and diameter of 5 µm was opted. Frontline FS 4 ultrasonic bath sonicator from Mumbai, India, a Denver electronic balance, and Whatman filter paper No. 41 were used for the studies.

Instruments Required

A Waters Alliance 2695 HPLC system with a model of "2998 PDA (Photo-Diode array detector)", autosampler, along with

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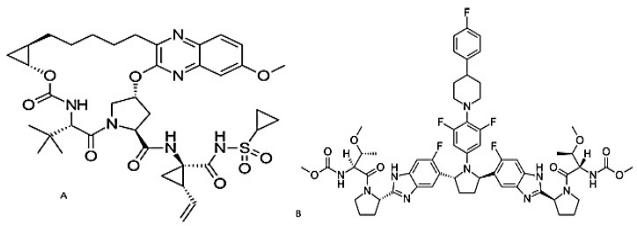

Figure 1: Structure of A) Grazoprevir B) Pibrentasvir

an 'Empower 2 software' for data processing, was used for an RP-HPLC method. Kromasil C_{18} (4.6 x 150 mm and 5 µm) was. Frontline FS 4 ultrasonic bath sonicator from Mumbai, India, a Denver electronic balance, and Whatman filter paper No. 41 were used for the studies.

Required Reagents

Grazoprevir and pibrentasvir were obtained from Hetero Drugs Limited in Hyderabad, India. Analytical grade acetonitrile was purchased from SD Fine Chemicals, India. Water was sourced from Milli-Q. 'Analytical grade OPA and KH_2PO_4 were purchased from Rankem Ltd., India'.

Mobile Phase Preparation

To 1000 mL milli Q water, about 1.36 gm potassium dihydrogen phosphate was added in a reagent bottle. The solution was agitated and water diluted with a pH adjustment of 3.5 with the help of 0.1% OPA. The so-prepared buffer is filtered *via* 0.45 µ filter paper.

After filtration, the buffer was mixed with HPLC-grade Acetonitrile in a 65:35 v/v ratio before being ready for use.

Diluent Preparation

Depending on drug solubility, the selection of diluents was done. Hence, for these drugs are diluted with acetonitrile and water with a 50:50 ratio.

Standard Stock Solution Preparation

Weighed 10 mg of grazoprevir and 4 mg of pibrentasvir was taken in a 10 mL volumetric flask. Later, a diluent of about 10 mL was poured into the flask, followed by 10 minutess of sonication. A flask was prepared by mixing a diluent to obtain a 1000 µg/mL concentration of grazoprevir and 400 µg/mL pibrentasvir.

Standard Working solution preparation

An mL of the above-prepared stock solution was taken in a volumetric flask (10 mL) and a makeup with diluent was done to a 100 µg/ml concentration of grazoprevir and 40 µg/mL of pibrentasvir.

Preparation of Sample Stock Solutions

The weight of 5 tablets was measured to calculate each tablet's average weight. One tablet was weighed again and was finely powdered using a mortar and pestle and then added to a volumetric flask (10 mL). About 10 mL of the diluent was added to make a mixture and passed for sonication for about 25 minutes. Further volume was adjusted by diluent followed by filtration and later centrifugation was carried out for 5 minutes at around 3000 rpm. Following appropriate dilution, the sample solution undergoes filtration using a 0.45-µm nylon filter to attain concentrations of 1000 µg/mL of grazoprevir and 400 µg/mL of pibrentasvir.

Sample working solution preparation

About an mL was pipetted from filtered solution to a volumetric flask (10 mL) and made up using diluent to result in a 100 µg/mL concentration of grazoprevir and 40 µg/mL of pibrentasvir.

Solution preparation for assay studies

For about six times, the aliquots of grazoprevir and pibrentasvir standards were injected into the instrument. For both, the peak areas have been calculated, followed by the percentage of assay was calculated *via* peak comparison.

RESULTS AND DISCUSSION

Method Optimization

Several trials were made through varied compositions of mobile phase and various parameters in order to get apt conditions for RP-HPLC analysis. Using a Symmetry column of Kromasil C_{18} having 4.6 x 150 mm size and diameter of 5µm and 0.1% OPA and acetonitrile (65:35 v/v) composition was taken as mobile phase with a flow rate of 1-mL/min resulting in better repeatability and reproducibility at 260 nm wavelength. The column's temperature was at 25°C with 5 minutes of runtime. The RT was at 2.909 and 2.358 for both drugs, respectively. Figure 2 displays chromatograms of standard solutions.

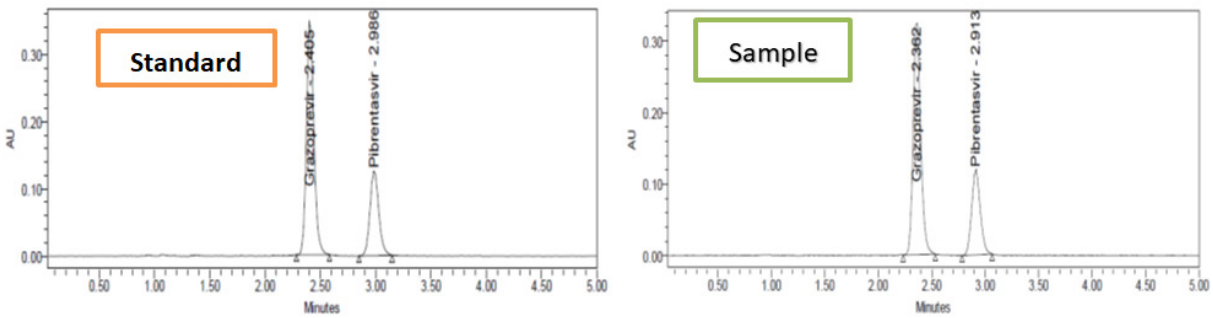

Figure 2: Chromatogram of working standard solution and sample solution

Table 1: %Assay for grazoprevir and pibrentasvir

Drug	Label claim (mg/tablet)	Amount found* (mg/tablet)	Label claim %	RSD %
Grazoprevir	100	99.95	99.95	0.1
Pibrentasvir	40	39.82	99.56	0.2

* mean of six determinations

Assay

The above solutions were taken for assay studies. For grazoprevir and pibrentasvir, the 'average percentage assay' was observed to be at '99.95 and 99.56%' correspondingly. The values are mentioned in Table 1.

Validation

Parameters of system suitability

The parameters were analyzed for the standard solutions of grazoprevir and pibrentasvir at specific concentrations. Six injections of these solutions were made in order to estimate tailing, plate count and resolution from the peak. For all six chromatograms, the percentage RSD (Relative standard deviation) were calculated and it should not be beyond 2%. The data were presented in the Table 2.

Specificity

The study examined the impact of some of the additives and excipients usually noticed in combinational dosages of grazoprevir and pibrentasvir in optimal conditions and verified the absence of any interference. Figure 3 displayed the specificity.

Precision

The closeness among several measurements made from various homogenous samples under particular circumstances is known as precision. It is in 2 forms as standard deviation (SD) and RSD. The analysis method's repeatability and reproducibility can be referred to as precision.

Method precision

Under ideal circumstances, sample solutions were injected six times for 6 days in order to measure the peak areas. For all the six injections, the %RSD has to be below 2% and not beyond. The values are displayed in Table 3.

Ruggedness (Intermediate precision)

About six injections of sample solution were made under the optimized condition for the same day and the peak areas were noted down. A %RSD of 25 is allowed for peak areas of all six replicas. The values are displayed in Table 4.

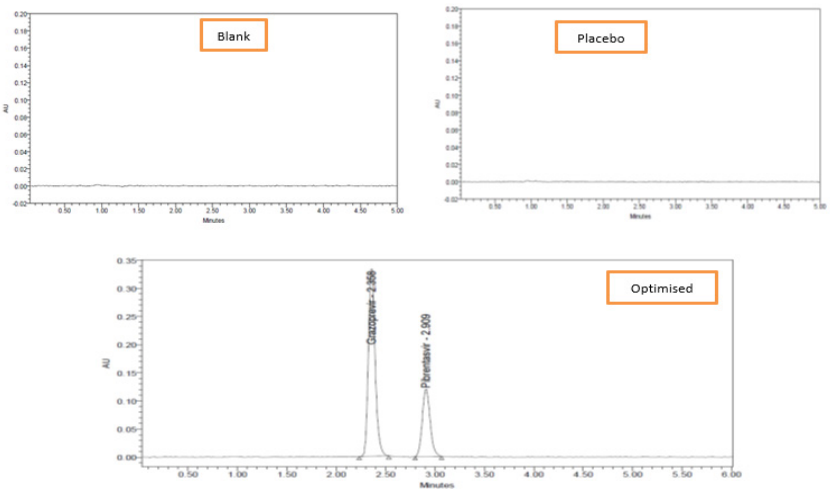

Figure 3: Specificity chromatogram

Table 2: System suitability parameters for grazoprevir and pibrentasvir

S No	*Grazoprevir*			*Pibrentasvir*			
Inj	*RT (minutes)*	*TP*	*Tailing*	*RT (min)*	*TP*	*Tailing*	*Resolution*
1	2.404	6203	1.19	2.986	7058	1.13	4.4
2	2.405	6182	1.17	2.986	6667	1.13	4.4
3	2.405	6077	1.20	2.987	6604	1.13	4.3
4	2.413	5940	1.16	2.998	6282	1.08	4.2
5	2.421	5912	1.17	3.013	6816	1.14	4.4
6	2.433	6244	1.17	3.036	6746	1.11	4.5

Table 3: Method precision

S. No	*Conc. (µg/mL)*	*Area of grazoprevir*	*Conc. (Mg/mL)*	*Area of pibrentasvir*
1.	100	1626575	40	692382
2.	100	1626196	40	691415
3.	100	1630202	40	693944
4.	100	1630709	40	694041
5.	100	1627849	40	691597
6.	100	1627275	40	691213
Mean		1628134	Mean	692432
S.D		1892.8	S.D	1272.4
%RSD		0.1	%RSD	0.2

Table 4: Intermediate precision

S. No	*Conc (µg/mL)*	*Area of grazoprevir*	*Conc. (Mg/mL)*	*Area of pibrentasvir*
1.	100	1593683	40	688592
2.	100	1579704	40	681594
3.	100	1564562	40	676163
4.	100	1588360	40	681835
5.	100	1583022	40	678080
6.	100	1579557	40	677689
Mean		1581481	Mean	680659
S.D		9916.6	S.D	4491.7
%RSD		0.6	%RSD	0.7

Linearity

About six concentration ranges linearly were taken for both grazoprevir (25–150 µg/mL) and pibrentasvir (10–60 µg/mL) and injected twice. The linearity equations obtained were y = 16332x + 5313.1 for grazoprevir and y = 17261x + 601.95 for pibrentasvir. The correlation coefficient for both drugs was 0.999. calibration curve graphs were shown in in Figure 4, and values were in Table 5.

Accuracy

The recovery results are taken for the method' s accuracy. The sample solution was mixed with a predetermined amount of pure standard drug and recovered by peak area. Standard was added to the sample at 50, 100, and 150% test concentrations. The spiked sample was triple-analyzed. As the values were

Table 5: Linearity table for grazoprevir and pibrentasvir

Grazoprevir		*Pibrentasvir*	
Conc (µg/mL)	*Peak area*	*Conc (µg/mL)*	*Peak area*
25	413889	10	175626
50	834883	20	349256
75	1237960	30	511834
100	1632913	40	691136
125	2013340	50	861123
150	2478476	60	1040083

Table 6: Accuracy table of grazoprevir

%Level	*Amount Spiked (µg/mL)*	*Amount recovered (µg/mL)*	*%Recovery*	*Mean %Recovery*
	50	49.82	99.64	
50	50	49.80	99.61	
	50	48.99	97.98	
	100	100.32	100.32	
100	100	99.08	99.08	99.32
	100	98.00	98.00	
	150	149.41	99.61	
150	150	149.84	99.90	
	150	149.58	99.72	

Table 7: Accuracy table of pibrentasvir

%Level	*Amount Spiked (µg/mL)*	*Amount recovered (µg/mL)*	*%Recovery*	*Mean %Recovery*
	20	19.95	99.77	
50	20	19.81	99.04	
	20	19.85	99.27	
	40	39.95	99.87	
100	40	39.71	99.27	99.43
	40	39.57	98.93	
	60	59.89	99.82	
150	60	59.75	99.59	
	60	59.56	99.27	

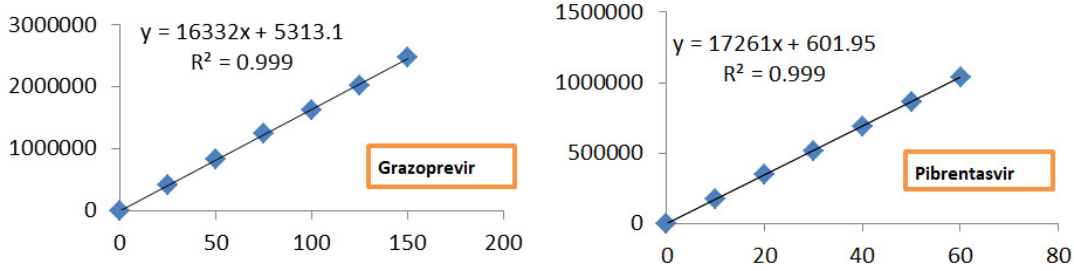

Figure 4: Calibration curve of grazoprevir and pibrentasvir

Table 8: Robustness data for grazoprevir and pibrentasvir

S.no	Condition	%RSD of Grazoprevir	%RSD of Pibrentasvir
1	Flow rate (-) 0.9ml/min	0.5	0.4
2	Flow rate (+) 1.1ml/min	0.4	0.3
3	Mobile phase (-) 60B:40A	0.9	0.7
4	Mobile phase (+) 70B:30A	0.5	0.6
5	Temperature (-) 25°C	0.9	0.1
6	Temperature (+) 35°C	1.4	0.4

Table 9: Sensitivity table of grazoprevir and pibrentasvir

Drugs	*LoD (µg/mL)*	*LoQ (µg/mL)*
Grazoprevir	0.24	0.72
Pibrentasvir	0.09	0.27

Table 10: Degradation data of grazoprevir and pibrentasvir

S. No.	*Degradation condition*	*Grazoprevir %drug degraded*	*Pibrentasvir %drug degraded*
1	Acid	7.85	9.94
2	Alkali	4.23	4.96
3	Oxidation	2.87	3.52
4	Thermal	2.39	2.41
5	UV	1.40	1.21
6	Water	1.40	0.67

shown in Tables 6 and 7, grazoprevir and pibrentasvir had 99.32 and 99.43% mean rates of recovery, respectively.[21-23]

Robustness

The determination of the method's robustness was made by varying a few factors, like a change in the composition of the mobile phase and flow ratio, as shown in Table 8. The system suitability parameters remained largely unaffected, and all of them met the required criteria. The %RSD was within the acceptable range.[24-27]

Detection limit and Limit of quantification

As portrayed in Table 9 the detection limit of grazoprevir and pibrentasvir, was at 0.24 and 0.09 µg/mL, correspondingly. Similarly, LoQ values were calculated and were observed to be 0.72 and 0.27 µg/mL, correspondingly. This indicates that the sensitivity of the method is adequate

Degradation studies

Both grazoprevir and pibrentasvir were taken for different conditions to be noted as alkaline, acidic, neutral, photolytic, thermal and oxidative. For the study, the samples with 100 µg/mL concentration of grazoprevir and 40 µg/mL of pibrentasvir were taken for analysis. The %degradation and recovery were calculated as in Table 10.

SUMMARY AND CONCLUSION

The simple, accurate, and precise method of simultaneously estimating grazoprevir and pibrentasvir in combination tablet form was created. As for pibrentasvir and grazoprevir, The RT was at 2.909 and 2.358 minutes, respectively. Grazoprevir and pibrentasvir had %RSD of intermediate precision values of 0.6 and 0.7, correspondingly. The method's precision for grazoprevir was at 0.1 %RSD, while for pibrentasvir, it was 0.2 %RSD. Grazoprevir had a recovery rate of 99.32% and pibrentasvir of 99.43%. Grazoprevir and pibrentasvir has detection limits of 0.24 and 0.09 µg/mL. Correspondingly, LoQ at 0.72 and 0.27 µg/mL. Grazoprevir and pibrentasvir both have regression equations that look like y = 16332x + 5313.1 and y = 17261x + 601.95, respectively. Creating a straightforward and inexpensive technique that works for regular quality control tests in industries allowed us to decrease retention times.

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