INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.10 | Issue 2 | Apr - Jun -2021
Journal Home page: www.ijpar.com

Research Study Open Access

A novel UPLC method to estimate glecaprevir and pibrentasvir simultaneously in tablets

B.V.V.S. Jagadeesh*, A.K.M.Pawar, J.V.L.N. Seshagiri Rao

Pharmaceutical Analysis and Quality Assurance Division, A.U. College of Pharmaceutical Sciences Andhra University, Visakhapatnam-530003, Andhra Pradesh, India

***Corresponding Author: B.V.V.S. Jagadeesh**
Email: suryapharma2006@gmail.com

ABSTRACT

An accurate, sensitive and precise UPLC method has been established for the simultaneous quantitative analysis of glecaprevir and pibrentasvir and validated as per ICH guidelines. The resolutionof both the drugs was accomplished utilizingAcquity UPLC CSH C18 column (100 x 2.1 mm; 1.7 µm)using a mobile phase mixture of 10 mM potassium dihydrogen phosphate buffer (pH 3.5) and acetonitrile with the composition of 70:30v/v.The mobile phase was pumped at a flow rate of0.3 mL/min. The column temperature was maintained at 30°C. The analytes were detected at 260 nm. The retention times found for glecaprevir and pibrentasvir were 0.9 and 1.4 min respectively. The developed UPLC method was validated for parameters like specificity, linearity, accuracy, precision and robustness. The specificity of the method was demonstrated by thelack of interference from its placebo and by stress degradation studiesonthe respective drug molecules. The assay was linear in the concentration ranges of 5–30µg/mLfor glecaprevir and 2-12 µg/mL for pibrentasvir. The accuracy of the method was between 98–102%. The method was found to be sensitive, robust and fairly suitable for the simultaneous quantitation of glecaprevir and pibrentasvir in tablet formulations. Forced degradation studies were performed on the drugs and the resulting degradation products did not interfere with the estimation of glecaprevir and pibrentasvir, demonstrating that the proposed method is specific for theirestimation.

Keywords: Glecaprevir, Pibrentasvir, Forced Degradation, UPLC and Validation

INTRODUCTION

Genotypes of Hepatitis C Virus (HCV) 1 and 3 are collectively liable for about 70% of an estimated 71 to 80 million HCV infections, worldwide.In most HCV genotypes, direct-acting antiviral agents have been the preferred therapy for chronic HCV infection and exhibit elevated rates of sustained virologicreaction[1,2]. Glecaprevir, the HCV NS3/4A protease inhibitor, and pibrentasvir, the NS5A inhibitor, are DAAs used as combination therapy in adults with chronic infection with HCV[3.] Glecaprevir is an inhibitor of the HCV NS3/4A protease enzymewhich is responsible for viral replication. NS4A is a cofactor for NS3 which held responsible for the restriction of NS3 in order to balance its enzymatic activities. Glecaprevir blocks the intracellular viral life cycles by repressing the NS3/4A protease[4-6]

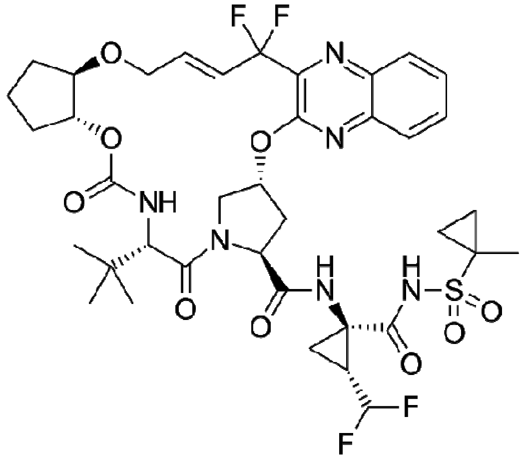

Fig. 1: Chemical structure of Glecaprevir

Pibrentasvir acts by inhibiting the assembly of NS5A phosphoprotein which is very important for its replication, assembly and maturation. The NS5A in its basal phosphorylated form which is maintained by C-terminal serine group is the essential factor in ensuring its attachment with the capsid viral protein. Pibrentasvir acts by inhibiting this interaction[7, 8].Based on the reported literature, it was found that some stability indicativehigh performance liquid chromatographic methods[4-11]were available for estimation of glecaprevir and pibrentasvir simultaneously. However, only a few UPLC methods[12-14] were published for concurrentquantitation of glecaprevir and pibrentasvir. Hence, there was a need to develop a sophisticated UPLC method required in terms of speed, sensitivity and cost effectiveness. Therefore, the author attempted to develop a suitable new UPLC stability indicativeassaytoquantitate both the drugs simultaneously in commercial pharmaceutical tablet dosage forms utilizing robust UPLC technique, which was more advantageous over HPLC in terms of speed and sensitivity. The developed method was validated according to Q2R1 ICH guidelines[15] in order to fit for its intended purpose. The structures of glecaprevir and pibrentasvir are shown in Figures 1 and 2.

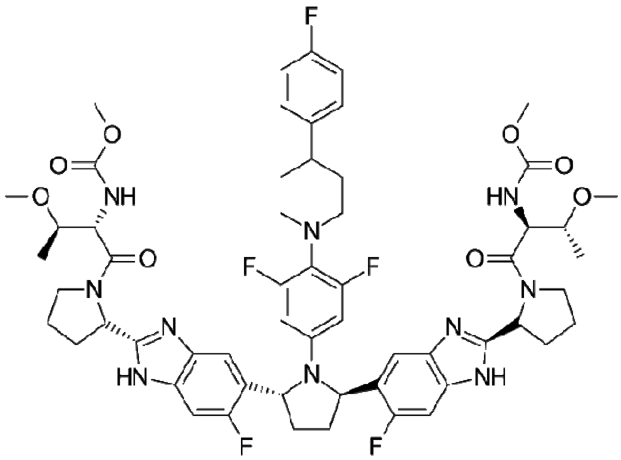

Fig. 2: Chemical structure of Pibrentasvir

Chemicals and equipment used in the study

Chemicals and solvents

The chemicals and the solvents used for the present study are listed in Table 1.

Table 1: Chemicals and Solvents

Chemical Name	Make	Grade
Water	Merck	HPLC
Acetonitrile	Merck	HPLC
Potassium dihydrogen phosphate	Sigma-Aldrich	ACS Reagent
Ortho-phosphoric acid	Sigma-Aldrich	ACS Reagent

Conc. Hydrochloric acid	S.D. Fine Chemicals	AR (35.4 %)
Sodium hydroxide	S.D. Fine Chemicals	AR
Hydrogen peroxide	S.D. Fine Chemicals	AR (30 % w/v)

Equipment

The equipment used for the present study is listed in Table 2.

Table 2: Equipment

Equipment	Make
UPLC: Waters Acquity 2996 with Empower 2 software	Waters Corporation, Milford, USA
Electronic balance	Mettler Toledo
pH meter	Mettler Toledo
Ultra sonicator	Lab India Instruments
Thermal oven	Thermostat
Micro pipettes	Brand and Eppendorf

Reference standards and commercial tablets

The reference drug substances of glecaprevir and pibrentasvir were obtained from Clearsynth Laboratories Ltd, India. The commercial pharmaceutical dosage forms "Mavyret" Tablets (100 mg Glecaprevir + 40 mg Pibrentasvir; Abbvie Inc.) was bought from the local pharmacy store.

Method development

Preparation of standard stock solutions (1.0 mg/mL)

Individualstock solutions of the analytes were prepared by transferring 10 mg of glecaprevir and 10 mg of pibrentasvir into separate 10 mL volumetric flasks and adding 7 mL of the diluent.The flasks were sonicated for 5 min to dissolve the drugs completely. The final volumeswere made up with the diluent to get 1.0 mg/mL concentration of the drugs.

Preparation of mixed working standard solution

The combinedworking standard solution was prepared by taking 0.2 mL of glecaprevirstock solution and 0.08 mL of pibrentasvirstock solution was pipetted into a 10 mL volumetric flask. The final volume was made up with the diluent to get concentrations of 20 µg/mL of glecaprevir and 8 µg/mL pibrentasvir.

Diluent

A mixture of water and acetonitrile in the ratio of 50:50 v/v was used as the diluent for the preparation and dilution of thedrug solutions.

Optimized chromatographic conditions

The acceptable resolution of the drugs glecaprevir and pibrentasvir was achieved on an Acquity UPLC CSH C18 column (100 x 2.1 mm; 1.7 µm) utilizing a70:30v/v mixture of 10 mM potassium dihydrogen phosphate buffer (pH 3.5) and acetonitrile. The mobile phase was optimized in order to get better peak shapes and resolution between the peaks with accepted peak tailing and reproducibility of the response. The flow rate was set at 0.3 mL/min. The column temperature was maintained at 30°C. The sample volume used for injection into the system was 2 µL. Run time was chosen as 3min. The drugs in the eluates were monitored at 260nm. Under the above optimized chromatographic conditions, the retention times obtained for Glecaprevir and Pibrentasvirwere 0.921 and 1.387 min respectively. The corresponding system suitability parameters are given in Table 3.

Table 3: System suitability parameters

Parameter	Glecaprevir	Pibrentasvir
Peak Area	1084794	382503
Retention Time (min)	0.921	1.387
USP Plate count	5678	3940
USP Tailing	1.37	1.26
% CV	0.9	0.5
Resolution	5.2	

Validation of the proposed method

The developed UPLC method was validated as per theQ2R1 ICH guidelines by evaluatingthe below parameters:

1. Specificity
2. Linearity
3. Accuracy
4. Precision
5. Robustness
6. Detection and Quantitation Limits

Specificity

The specificity of the method was established by analyzing the blank, placebo and standard solutions separately. No interfering peaks were observed at the retention times of glecaprevir and pibrentasvir (Fig. 3-4). Hence, the developed method was found to be specific for the simultaneous estimation of glecaprevir and pibrentasvir.

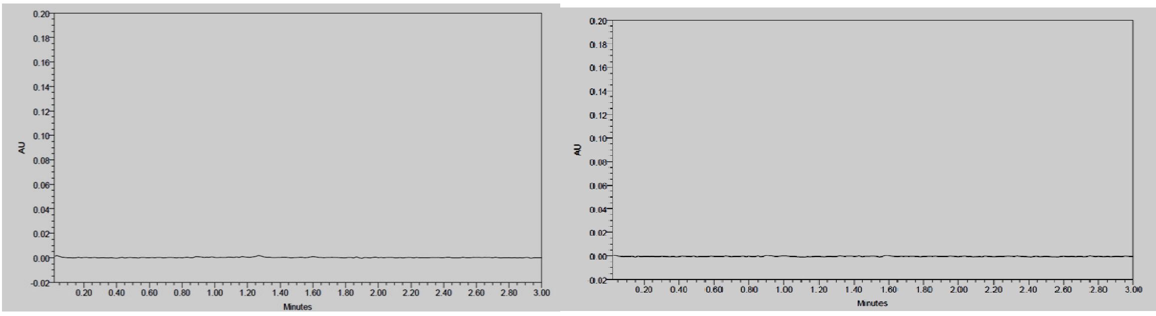

Fig. 3: Chromatograms of the blank and placebo

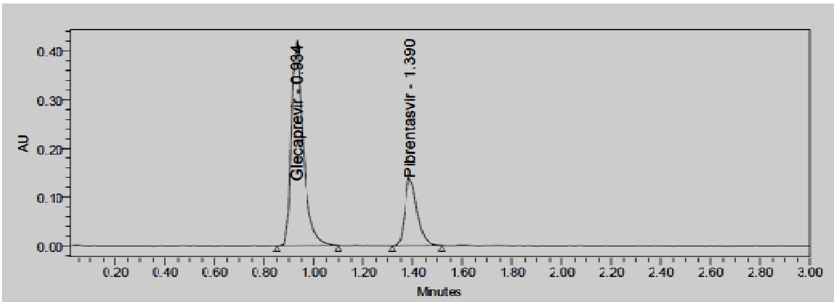

Fig. 4: Chromatogram of the mixed standard solution

Linearity

Linearity of the proposed analytical method was assessed by injecting three replicates of the mixed working standard solutions at six different concentration levels in the ranges of 5 to 30 µg/mL for glecaprevir and 2 to 12 µg/mL for pibrentasvir. The corresponding responses were found to be linear. Theresults are summarized in Table 4.

The linear regression equation for glecaprevir was found to be y = 53343x + 1296 (r^2=.0.9988) (Fig. 5). The linear regression equation for pibrentasvir was found to be y = 47108x + 3313 (r^2=0.9995) (Fig. 6).

Table 4: Linearity data of glecaprevir and pibrentasvir

Glecaprevir				Pibrentasvir			
Conc. (µg/mL)	Peak Area	Average Peak Area (n=3)	% RSD	Conc. (µg/mL)	Peak Area	Average Peak Area (n=3)	% RSD
5	255897	257323	0.5	2	95826	95502	0.4
	258281				95585		
	257792				95094		
10	528629	530857	0.4	4	193044	192277	1.1
	531169				189930		
	532774				193856		
15	803240	805389	0.4	6	286209	284354	0.8
	803886				284893		

	809041				281960		
20	1103248	1100483	0.3	8	385161	384610	0.1
	1097459				384586		
	1100743				384084		
25	1326270	1327346	0.1	10	479784	478378	0.3
	1328972				477137		
	1326795				478214		
30	1582000	1587429	0.4	12	560454	563304	0.4
	1586110				564478		
	1594178				564981		

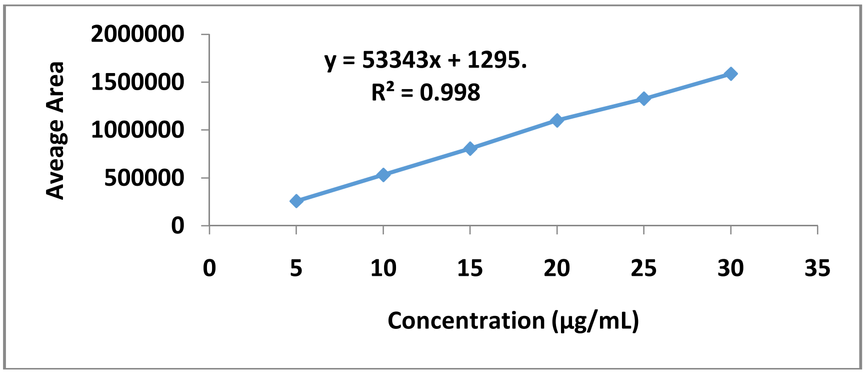

Fig. 5: Linearity curve for Glecaprevir

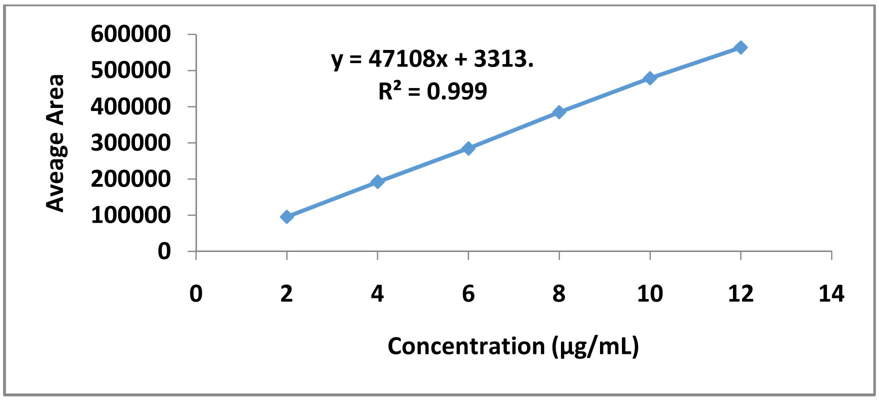

Fig. 6: Linearity curve for Pibrentasvir

Accuracy

The accuracy of the proposed UPLC method was evaluated by determining the recoveries of glecaprevir and pibrentasvir after adding pre-determined amounts to the mixed standard solution. Solutions ofglecaprevir and pibrentasvir at 50, 100 and 150% concentration levels were prepared, analyzed and the corresponding chromatograms obtained. The mean percent recoveries were calculated from the peak areasof the drugs. The corresponding resultsare shown in Table 5.

Table 5: Accuracy data of glecaprevir and pibrentasvir

Conc. Level	Glecaprevir				Pibrentasvir			
	Amount added (µg/mL)	Difference Amount recovered (µg/mL)	Mean % Recovery	% RSD	Amount added (µg/mL)	Difference Amount recovered (µg/mL)	Mean % Recovery	% RSD
50%	10.000	9.822	99.3	0.93	4.000	4.024	99.9	0.64
		9.993				3.986		
		9.969				3.975		
100%		20.021	99.7	0.41		7.926	99.6	0.54

	20.000	19.864			8.000	8.012		
		19.906				7.960		
150%	30.000	30.248	99.7	0.95	12.000	12.072	100.1	0.44
		29.731				11.997		
		29.788				11.969		

Precision

The precision of the proposed UPLC method was evaluated by conducting intra-day and inter-day precision studies.

Intra-day precision (Repeatability)

The precision of the method was assessed by performing six independent assays of the test sample, calculating the % RSD of the peak area values. The relevant results are tabulated in Table 6.

Table6: Intra-day precision of glecaprevir and pibrentasvir

S. No.	Glecaprevir			Pibrentasvir		
	Peak Area	USP Plate count	USP Tailing	Peak Area	USP Plate count	USP Tailing
1	1075536	5050	1.31	380735	4063	4063
2	1087510	5054	1.32	381267	3804	3804
3	1079258	4845	1.34	380705	3959	3959
4	1090527	4765	1.39	379293	3779	3779
5	1085000	5324	1.45	380442	3775	3775
6	1084453	5374	1.31	382317	3776	3776
Average (n=6)	1083714	-		380793	-	
SD	5473			993		
% RSD	0.5			0.3		

Intermediate Precision (Inter-day precision)

The inter-day precision of the method was checked by performing the above procedure on two consecutive days under the same experimental conditions, taking the overall average and calculating the % RSD.The corresponding resultsare shown in Table 7.

Table 7: Inter-day precision of glecaprevir and pibrentasvir

Day	Glecaprevir			Pibrentasvir		
	Average Peak Area (n=6)	USP Plate count	USP Tailing	Average Peak Area (n=6)	USP Plate count	USP Tailing
Day 1	1072048	5087	1.33	364378	3841	1.20
Day 2	1084765	5123	1.27	361741	3902	1.27
Overall Average	1078406	-		363060	-	
SD	8993			1865		
% RSD	0.8			0.5		

Robustness

Small deliberate changeswere made in the methodconditions like flow rate, mobile phase composition and column temperature and the corresponding chromatograms were obtained. No appreciabledifferenceswere observed in the system suitability results, which were within the acceptable limitsas per the Q2R1 ICH guidelines. The results are shown in Tables 8, 9 and 10.

Table8: Robustness data of change in flow rate

Flow rate (mL/min)		Drug	Retention time (min)	Asymmetry	Plate count	Resolution
Actual	Change					
0.3	**0.2**	Glecaprevir	0.974	1.4	5401	5.6
		Pibrentasvir	1.496	1.2	3979	
	0.4	Glecaprevir	0.833	1.4	4955	5.5
		Pibrentasvir	1.275	1.2	3767	

Table9: Robustness data of change in mobile phase composition

Mobile Phase (Buffer-ACN v/v)		Drug	Retention time (min)	Asymmetry	Plate count	Resolution
Actual	Change					
70:30	**65:35**	Glecaprevir	0.896	1.4	5169	5.3
		Pibrentasvir	1.343	1.2	3743	
	75:25	Glecaprevir	0.892	1.3	5342	5.9
		Pibrentasvir	1.416	1.2	3987	

Table10: Robustness data of change in column temperature

Column temperature (°C)		Drug	Retention time (min)	Asymmetry	Plate count	Resolution
Actual	Change					
30	**28**	Glecaprevir	0.892	1.3	5462	5.8
		Pibrentasvir	1.396	1.2	4048	
	32	Glecaprevir	0.885	1.3	4959	5.5
		Pibrentasvir	1.355	1.2	3669	

Detection and Quantitation Limits

The LOD values were found to be 0.010 µg/mL for glecaprevir and 0.060 µg/mL for pibrentasvir. The LOQ values were found to be 0.040µg/mL for glecaprevir and 0.180µg/mL for pibrentasvir. The relevant LOD and LOQ data are given in Table 11 and the corresponding chromatograms in Fig. 7.

Table 11: LOD and LOQ results

Drug	LOD (µg/mL)	LOQ (µg/mL)
Glecaprevir	0.010	0.040
Pibrentasvir	0.060	0.180

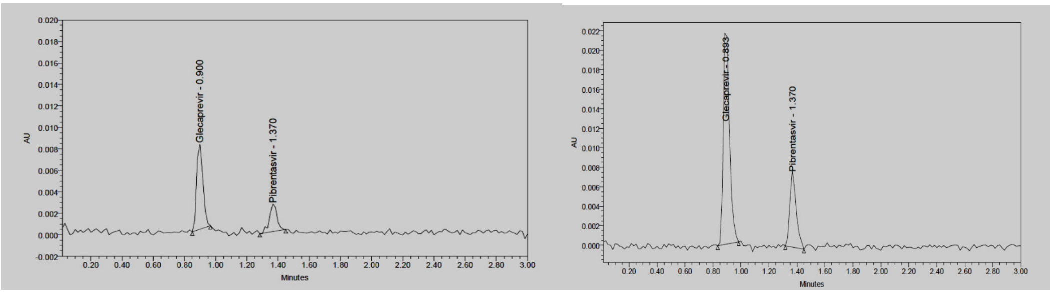

Fig. 7: LOD & LOQ Chromatograms

Simultaneous estimation of glecaprevir and pibrentasvir in tablet dosage form

Twenty tablets were weighed and finely powdered. An amount of powder equivalent to the weight of one tablet was accurately weighed and transferred into a 50 mL volumetric flask. 30 mL of the diluent was added to the flask and sonicated for 30 min.The contents were diluted to volume with the diluent and filtered through a 0.45 µ membrane filter. 100 µL of the filtrate was pipetted into a 10 mL volumetric flask and the volume was made up with the diluent. The resultant solution was chromatographed and analyzed.The average assayvalue obtained forglecaprevir was 98.56 % and that for pibrentasvir was 95.17 %. Theresults are given in Table 12 and the corresponding chromatogram in Fig. 8.

Table12: Assay ofglecaprevir and pibrentasvir

S. No.	Glecaprevir			Pibrentasvir		
	WSS Peak Area	Peak Area from formulation	% Assay	WSS Peak Area	Peak Area from formulation	% Assay
1	1092688	1076750	98.84	381172	367952	96.10
2	1074279	1073529	98.55	380967	369894	96.61
3	1093043	1076591	98.83	380375	363867	95.03
4	1087541	1071685	98.38	385494	363847	95.03
5	1074237	1071230	98.33	383674	362222	94.60
6	1094869	1062501	98.45	383335	358488	93.63
Average	1084794	1072048	98.56	382503	364378	95.17
SD	9497.8	5232.3	0.222	1983.4	4076.4	1.065
% RSD	0.9	0.5	0.2	0.5	1.1	1.1

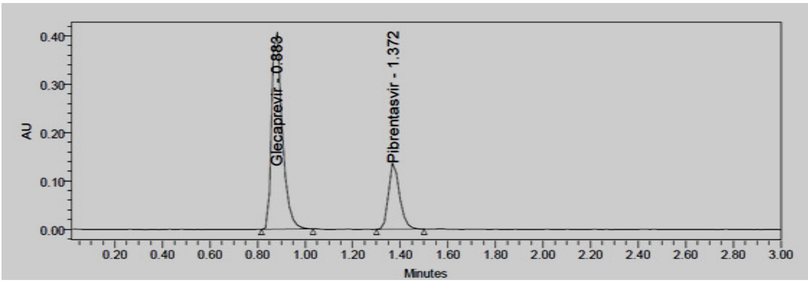

Fig. 8: Chromatogram of marketed formulation

Forced degradation studies on the drug substances

The forced degradation studies were conducted on the mixed working standard solution of glecaprevir and pibrentasvir. The drugs were subjected to degradation with acidic, alkaline, peroxide, dry heat, photolytic and neutral stress conditions. The results of the study are given in Table 13 and the corresponding chromatograms are shown in Fig 10.

Table 13: Forced degradation study data

Type of degradation	Stress conditions	Glecaprevir		Pibrentasvir	
		% Assay	% Degradation	% Assay	% Degradation
Acid	2N HCl refluxed at 60 °C for 30 min	93.37	6.6	94.24	5.8
Base	2N NaoH Refluxed at 60 °C for 30 min	95.62	4.4	95.54	4.5
Peroxide	20 % H_2O_2 at 60 °C for 30 min	96.16	3.8	96.60	3.4
Dry heat	Heat at 105 °C for 6 hrs	98.48	1.5	98.36	1.6
Photolytic	UV chamber at 200 Watts hours/$m^2$for 7 days	98.13	1.9	98.89	1.1
Neutral	In water at 60 °C for 6 Hrs	99.34	0.7	99.27	0.7

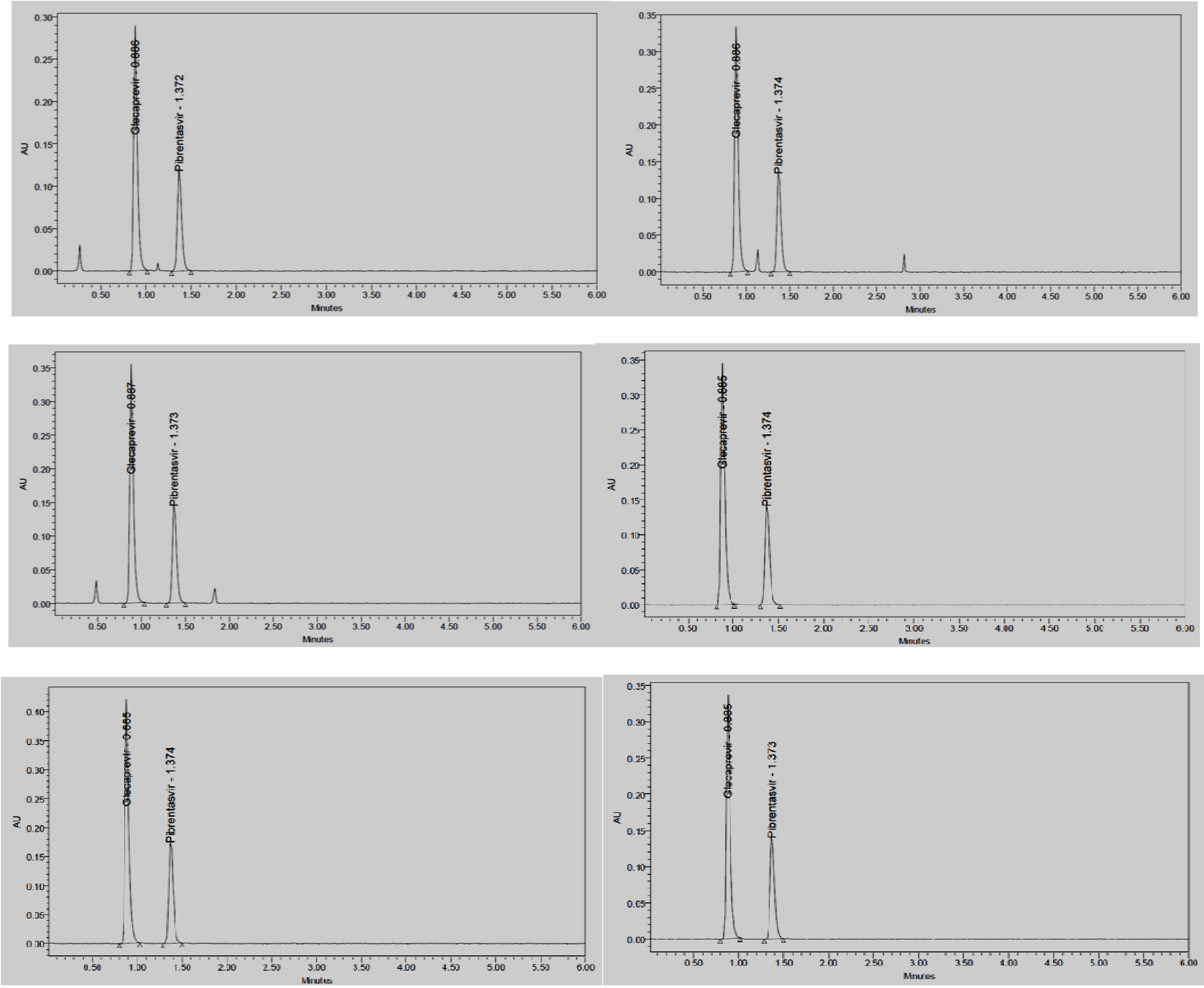

Fig. 8: Forced degradation chromatograms in the presence of acid, base, peroxide, thermal, photolytic and neutral conditions

DISCUSSION

The UPLC method established for thesimultaneous quantitation of glecaprevir and pibrentasvirwas sensitive, accurate and reproducible and can be employed for estimation of the drugs simultaneouslyin their pharmaceuticalformulations. The chromatographic conditions were optimized prior to validation in terms of stationary phase, mobile phase composition, flow rate and column oven temperature, which gave good system suitability.The developed method, was validated as per ICH Q2A (R1) guidelines. The developed method was found to be specific because there was no interference from the placebo, matrix, and degradation products at the retention times of the analytes. Hence, the developed method can be used for routine quality control analysis of glecaprevir and pibrentasvir in their combined tablet dosage forms.

REFERENCES

[1] Zeuzem S, FosterGR, Wang S, Asatryan A, Gane E, FeldJJ, Asselah T, Bourlière M, Ruane PJ, Wedemeyer H, Pol S, Flisiak R, Poordad F, Chuang WL, Stedman CA, Flamm S, Kwo P, Dore GJ, Sepulveda-Arzola G, Roberts SK, Soto-Malave R, Kaita K, Puoti M, Vierling J, Tam E, Vargas HE, Bruck R, Fuster F, Paik SW, Felizarta F, Kort J, Fu B, Liu R, Ng TI, Pilot-Matias T, Lin CW, Trinh R, Mensa FJ.Glecaprevir–Pibrentasvir for 8 or 12 weeks in HCVGenotype 1 or 3infections. NEnglJ Med.2018; 378(4):354-69. doi: 10.1056/NEJMoa1702417, PMID 29365309.

[2] Chayama K, Suzuki F, Karino Y, Kawakami Y, Sato K, Atarashi T, Naganuma A, Watanabe T, Eguchi Y, Yoshiji H, Seike M, Takei Y, Kato K, Alves K, Burroughs M, Redman R, Pugatch DL, Pilot-Matias TJ, Krishnan P, Oberoi RK, Xie W, Kumada H.Efficacy and safety of glecaprevir/pibrentasvir in Japanese patients with chronic genotype 1 hepatitis C virus infection with and without cirrhosis. J Gastroenterol.2018; 53(4):557-65. doi: 10.1007/s00535-017-1391-5, PMID 28948366.

[3] Maureen M, Robert H, Susan M, LinCW.Pharmacokinetics, safety, and efficacy of Glecaprevir/Pibrentasvir in adolescents with chronichepatitis C virus: Part 1of the DORA Study, *Hepatology*, 2019.
[4] Hemalatha K, Kistayya C, Nizamuddhin N, Dastiagiriamma D. Simultaneous estimation of newanalyticalmethoddevelopment and validation of glecaprevir and pibrentasvir by high-performanceliquidchromatography. InnovatIntJ Med PharmSci.2018; 3:5-8.
[5] Saradhi N, KumarR, Venkata Reddy M.A newtechnicalmethod for glecaprevir and pibrentasvirincombineddosageformsusingNonpolarHPLC*WJPMR*.2018; 4(12):173-8.
[6] Sreeram V, Venkateswarlu Ch.Stability indicatingRP-HPLCmethod for the simultaneousestimation of glecaprevir and pibrentasvir in drugproduct. JPharmSci Res.2018; 10(11):2757-61.
[7] Babu DC, Chetty CM, SK. Mastanamma M.A New ForceindicatingRP-HPLCmethoddevelopment and validation for the simultaneousestimation of pibrentasvir and glecaprevir in bulk and its tabletdosageform. Pharm Methods.2018; 9(2):79-86. doi: 10.5530/phm.2018.2.14.
[8] Sunitha N, Bhargav S, Subash C, Appa Rao B.Method development and validation of glecaprevir and pibrentasvirinpure and pharmaceuticaldosageformsbyRP-HPLCmethod. Indian J Res Pharm Biotechnol.2019; 7(2):67-83.
[9] KumarR, Raja S.Simultaneous assay of twoantiviralagents, pibrentasvir and glecaprevir, usingstabilityindicatingRP-HPLCmethod in bulk and tablets.PharmLett.2018; 10(8):33-47.
[10] ReddyYN, Sreeramulu J, Balaswami B.A New Stability-Indicating RP-HPLC-Pda Method for Simultaneous Estimation of Glecaprevir and Pibrentasvir in tablet dosage form.ResJ Pharm Technol.2019; 12(2):625-31. doi: 10.5958/0974-360X.2019.00111.2.
[11] Saroja J, Anantha Lakshmi P, Rammohan Y, Divya D.Quantification of antiviral drug combination, glecaprevir and pibrentasvir, in bulk and tablet formulation by stability indicating RP-HPLC method. The PharmInnovJ*, l*.2019; 8(1):163-9.
[12] Raju SDVVSSN, Manikandan A, Rao SV.Validation of Simple Isocratic RP-UPLC Method for Glecaprevir and Pibrentasvir determination and its Application in the Study of Stress Degradation. ResJ Pharm Technol.2019; 12(9):4299-304. doi: 10.5958/0974-360X.2019.00739.X.
[13] Deepthi R, GowriSankar D.Stability-indicatingmethoddevelopment and validation for the simultaneousestimation of glecaprevir and pibrentasvir in pharmaceuticaldosageform by UPLC.JPharmSci Res.2019; 11(8):2838-43.
[14] Poojitha D.Ultra performanceliquidchromatographicmethoddevelopment and validation for the determination of glecaprevir and pibrentasvir in pharmaceuticaldosageforms.Asian J Biomed PharmaceutSci.2019; 9.
[15] International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use: Validation of Analytical procedures, Text and Methodology. Vol. Q2;2005. (p. R1).