STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SEPARATION AND ASSAY OF PIBRENTASVIR AND GLECAPREVIR

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ABSTRACT

Objective:

To develop and validate (using International Conference on Harmonization guidelines) a simple, fast and efficient stability indicating high performance liquid chromatographic method for simultaneous determination of pibrentasvir (PBVR) and glecaprevir (GCVR) in bulk and pharmaceutical formulation.

Methods:

PBVR and GCVR were separated within 6 Min on Cosmicsil C_{18} analytical column with 0.1M Na_2HPO_4: acetonitrile (60:40 v/v) with pH 4.5 at a flow rate of 1.0 Ml/min. Validation was performed with respect to system suitability, linearity, sensitivity, selectivity, precision, accuracy and robustness. PBVR and GCVR combined tablet sample was subjected to stress degradation in 0.1N HCl for 30 min, 0.1 N NaOH for 30 min, 30% H_2O_2 for 30 min, water for 30 min, in oven at 105 °C for 30 min and in sunlight for 24 hr.

Results:

The linearity for PBVR and GCVR were 5-80 and 12.5-200 µg/ml with regression coefficients of 0.9995 and 0.9997, respectively. Stability indicating ability of the developed method was shown by stress degradation studies. The proposed method was effectively applied for simultaneous determination of PBVR and GCVR in available tablet dosage form with good accuracy and precision.

Conclusion:

The developed method is apt for the assay of PBVR and GCVR in the presence of their degradation products.

Key words: Pibrentasvir, Glecaprevir, Antiviral combination, Hepatitis C virus, Method development, Validation

INTRODUCTION

Amalgamation of two direct acting antiviral drugs, pibrentasvir (PBVR) and glecaprevir (GCVR), was given approval by the food and Drug Administration in 2017 [1-3]. PBVR is chemically known as methyl N-[(2S,3R)-1-[(2S)-2-[6-[(2R,5R)-1-[3,5-difluoro-4-[4-(4-fluorophenyl)piperidin-1-yl] phenyl]-5-[6-fluoro-2-[(2S)-1-[(2S,3R)-3-methoxy-2-(methoxycarbonylamino) butanoyl] pyrrolidin-2-yl]-3H-benzimidazol-5-yl]pyrrolidin-2-yl]-5-fluoro-1H-benzimidazol-2-yl] pyrrolidin-1-yl]-3-methoxy-1-oxobutan-2-yl]carbamate (Fig.1).PBVR has molecular formula $C_{57}H_{65}F_5N_{10}O_8$ with molecular weight 1113.201 g/mol [4]. Chemically, GCVR is termed as (3aR,7S,10S,12R,21E,24aR)-7-tert-Butyl-N-{(1R,2R)-2-(difluoromethyl)-1-[(1-methylcyclopropane-1-sulfonyl)carbamoyl]cyclopropyl}-20,20-difluoro-5,8-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]trioxadiazacyclononadecino[11,12-b]quinoxaline-10-carboxamide (Fig.2). GCVR has molecular formula $C_{38}H_{46}F_4N_6O_9S$ with molecular weight 838.873 g/mol [5].

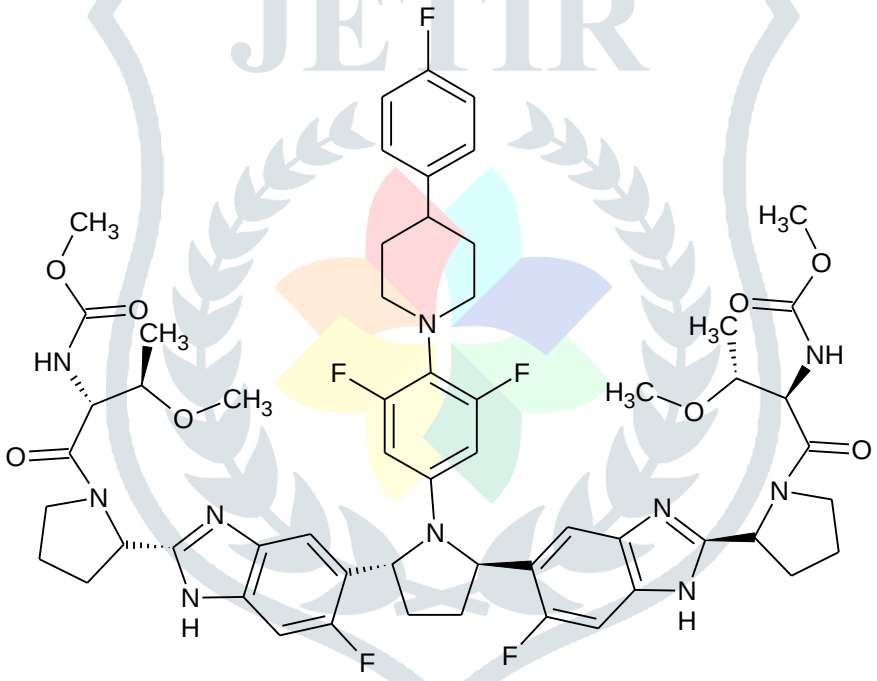

Fig. 1: Structure of PBVR

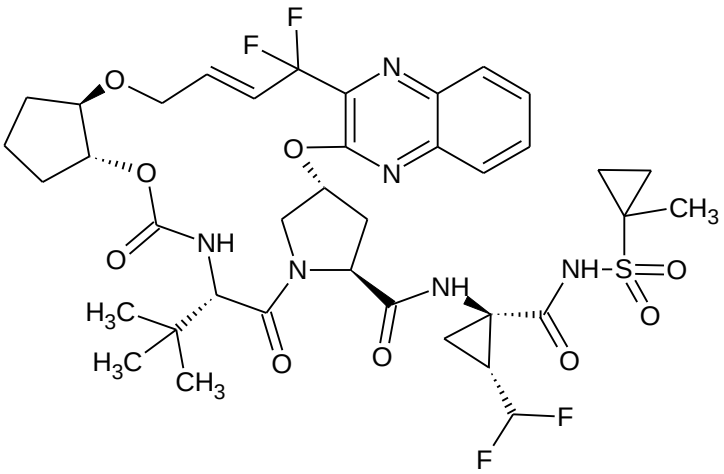

Fig. 2: Structure of GCVR

PBVR acts as an inhibitor for NS5A protease of hepatitis C virus while GCVR acts as an inhibitor for NS3/4A protease of hepatitis C virus. The NS3A, NS4A and NS5A proteases are essential for the hepatitis C virus RNA replication and virus assembly [6]. Hence, replication of RNA and assembly of virus and growth of virus is repressed in the existence of PBVR and GVCR. Mavyret™ tablets, labeled to contain 100mg GVCR and 40 mg PVCR, are available.This combination is recommended to treat patients (with mild or no cirrhosis) infected with hepatitis C virus genotypes 1a, 2a, 3a, 4, 5 and 6 [7-9]. The combination is also beneficialto patients who were treated in the past with NS5A inhibitor alone or NS3/4A protease inhibitor alone, but not both [7-9].

GVCR and PBVR combination is not certified in any major pharmacopeia and only two reports, to the best of our knowledge, proposed the use of HPLC and UPLC for the assessment of pibrentasvir and glecaprevir. The HPLC method, proposed by Hemalatha et al. is based on the separation and analysis of GVCR and PBVRon Altima C18 analytical column with a mixture of methanol, triethylamine buffer (pH 4.5) and acetonitrile (50:25:25, v/v/v) as mobile phase [10]. The mobile phase was set with flow rate 1.0 ml/min and detection was done at 225nm. In UPLC method reported Sridevi et al. GVCR and PBVRseparation and analysis was performed using BEH C18 column using a mobile phase consists of a phosphate buffer (pH 3.0): acetonitrile (45:55, *v/v*) [11]. Flow rate and detector were set 0.3 ml/min and 260nm, respectively. Hemalatha et al., and Sridevi et al.,methods are applied to bulk and tablets.

Previously, to the best of our literature survey knowledge, not testified work on the stability indicating RP-HPLC method for the simultaneous determination of GVCR and PBVR combination in bulk and in their tablet dosage forms was found. So this work aimed at developing and authorizing a stability indicating RP-HPLC method for the simultaneous quantification of GVCR and PBVR. The developed method was applied efficiently to tablet dosage forms.

MATERIALS AND METHODS

Instrumentation

HPLC was analyzed by using aWaters alliance HPLC apparatus consists of a quaternary pump, column heater, auto sampler injector equipped with waters photo diode array (PDA) detector. Data was acquired by a Waters software Empower2 version.

Materials used

GCVR and PBVR reference standards were procured as gift samples from Rainbow Pharma Training Lab, Hyderabad, India. HPLC grade acetonitrile (Merck India Ltd, Mumbai, India) is used. Analytical reagent grade chemicals were from SD. Fine Chemicals Ltd., Mumbai, India. They include Na_2HPO_4, HCl, NaOH, H_3PO_4 and H_2O_2.Milli-Q water used is from Millipore system. Mavyret tablets (AbbVie Limited, Berkshire, UK) containing GCVR (100mg) and PBVR (40mg) in combination was obtained from the local chemist.

HPLC conditions

HPLC separation and analysis of GCVR and PBVRwere done using a Cosmicsil C18 column (4.6mm × 150mm, 5.0µm) with temperature 25 ^oC. 10µlsample was introduced using an auto sampler injector. GCVR and PBVR were detected at 228 nm. Acetonitrile-0.1M Na_2HPO_4(pH 4.5) in 40:60 (v/v) ratio was used as mobile phase and isocratic mode flow rate was set at 1.0 ml/min.

Standard solutions of GCVR and PBVR1

Precisely weighed GCVR (100mg) and PBVR (40mg) were transferred to a 100 ml volumetric flask, dissolved and made equal to 100 ml with mobile phase giving a concentration of 1000 µg/ml of GCVR and 400 µg/ml of PBVR.In order to make working standard solutions, appropriate aliquots of standard stock solution were transferred to 10ml volumetric flasks and diluted to 10 ml with mobile phase to get solutions with concentration range 5-80 µg/ml (PBVR) and 12.5-200 µg/ml(GCVR).

Tablet sample solution

In a mortar, ten tablets were crushed into fine powder and a sample corresponding to 100 mg and 40mg of GCVR and PBVR, respectively was transferred to a 100 ml volumetric flask. About 30 ml mobile phase was added and sonicated for 20 min. The volume was completed to 100 ml with the same solvent. The GCVR and PBVR concentration in the resultant is 1000 µg/ml and 400 µg/ml, respectively. The solution was filtered using 0.45 µm pore size membrane filter.

Calibration curve construction

Working standard solutions are 10 µl each of with concentration range 5-80 µg/ml (PBVR) and 12.5-200 µg/ml (GCVR) were injected into the HPLC system. The analysis was done using the described HPLC conditions. The drug peak areas were plotted against their respective concentrations to get the calibration curve. The regression equations for GCVR and PBVR were also compute.

Procedure for analysis of GVCR and PVCR in tablets

Accurately measured 1.0 ml of the stock tablet sample solution (1000 µg/ml - GCVR and 400 µg/ml - PBVR) was diluted to 10 ml with mobile phase to get concentration corresponding to 100 µg/ml of GCVR and 40 µg/ml of PBVR. An aliquot of 10 µl of prepared solution was injected into the system and analyzed by the proposed method. The content of GCVR and PBVR was determined either using the corresponding calibration curve or regression equate.

Stress degradation study of GVCR and PVCR

Stress degradation study was performed according to InternationalConference on Harmonization guidelines Q1A (R2) [12]. Degradation was initiated by preparation of tablet sample solution with concentration 1000 µg/ml of GCVR and 400 µg/ml of PBVR. 10 ml of tablet sample solution is mixedwith 10 ml of various solvents: water (neutral degradation), 0.1 NHCl (acid degradation), 0.1 N NaOH (base degradation) and 30% H_2O_2 (oxidative degradation). All samples were exposed to selected stress conditions for 30 min at 25°C with sonication. After the degradation treatments, the samples were cooled, neutralized with 0.1N NaOH (acid degradation) or 0.1N HCl (base degradation). The resultant solutions were diluted to 100 ml with mobile phase, filtered and analyzed by the proposed method.

To study the effect of sunlight (photo degradation) and dry heat (thermal degradation) on GCVR and PBVR, tablet powder corresponding to 100mg and 40mg of GVCR and PVCR, respectively was placed in petri dish. The contents of petri dish were exposed to direct sunlight for 24 hours (photo degradation) and 105°C for 30 min in hot air oven (thermal degradation). Later the powder was cooled, and dissolved in and diluted to 100 ml with the mobile phase. A 10 ml aliquot was further diluted to 100 ml with the mobile phase. These solutions were filtered and analyzed by the proposed method. Assay and degradation studies were performed for stress samples against GCVR and PBVR standard solution. Peak purity tool was applied to check the purity of GVCR and PVCR peaks in stress conditions applied using PDA detector.

RESULTS

Method development

Trials were executed with the aim of selecting an appropriate analytical column and in mobile phase system for accurate determination of selected drugs and also for attainingoptimum resolution and good peak shapes for selected analytes. The GCVR and PBVR were elected using different stationary phases such as Inertsil C18, Waters C18, Sunsil C18 and Cosmicsil C18 with column dimension 250mm × 4.6mm, 5 µm particles. The good chromatographic separation was observed with Cosmicsil C18, hence the same column was chosen. Mobile phase systems such as 0.1% orthophosphoric acid: methanol and 0.1M NaH_2PO_4: acetonitrilewith different pH, ratios and flow rate were tried. Finally, mobile phase of composition 0.1M NaH_2PO_4: acetonitrile 60:40 (*v/v*) with pH 4.5 and flow rate of 1.0 ml/min resulted in a symmetrical, sharp and well resolved peaks at retention time values of 1.445 and 2.858 for GCVR and PBVR, respectively (Fig.

3).Better method sensitivity was achieved with 228nm as a detection wavelength, therefore the name was chosen as the analytical wavelength.

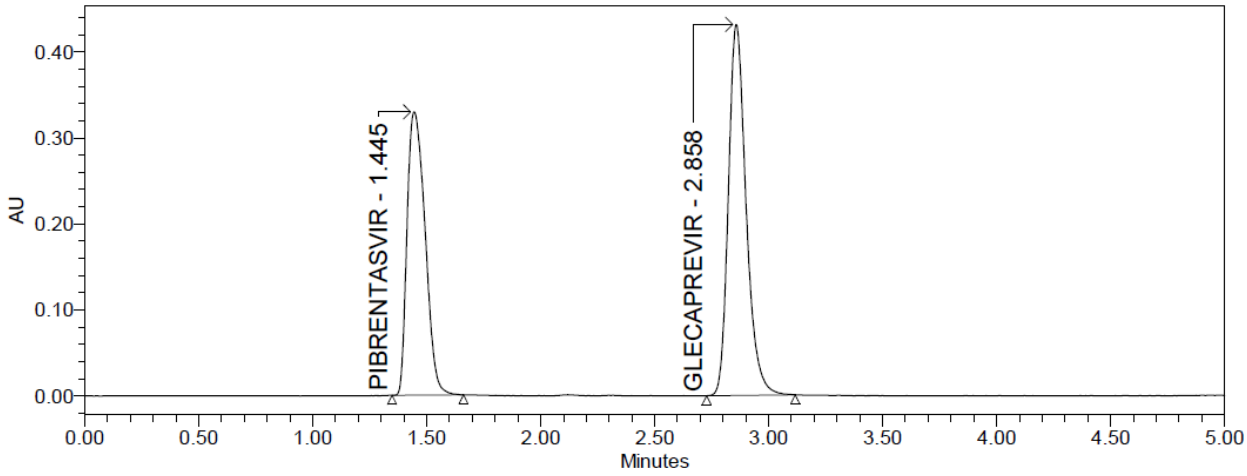

Fig. 3: HPLC chromatogram of GCVR and PBVR

Validation of method

The validation of the method was performed with recommendations established by International conference on harmonization [13].

System suitability test

Method system suitability is to validate the performance of the method developed. System suitability was checked with standard drug solution (100 µg/ml GCVR and 40 µg/ml PBVR) by calculating parameters including relative standard deviation of drug peaks, relative standard deviation of drug retention time, resolution, peak tailing factor and plate count. The results shown in Table 1, indicated the suitability of the system for analysis of GCVR and PBVR simultaneously.

Table 1: Summarized data of system suitability test

Drug	RT* (RSD)	PA* (RSD)	PC* (RSD)	PT* (RSD)	Rs* (RSD)
PVCR	1.445 (0.062)	2406290 (0.406)	9549 (0.109)	1.374 (0.322)	-
GVCR	2.856 (0.043)	6330680 (0.201)	6660 (0.557)	9.792 (0.151)	1.264 (0.433)
Accepted limits [13]	RSD ≤2	RSD ≤2	> 2000	≤2	> 1.5

RT-retention time; PA- peak area; PC-plate count; PT-peak tailing; Rs-Resolution;RSD –relative standard deviation; * Average five values

GCVR and PBVRstress degradation study

GVCR and PVCR were subjected to different conditions of degradation prescribed by the International Conference on Harmonization. The forced degradation study showed that GCVR and PBVR

are liable to acidic, basic, neutral, oxidative, thermal and photolytic conditions. The chromatograms of the treated samples showed wellseparated peaks of GCVR, PBVR and some degradation peaks at distinct retention time (Fig. 4a – 4f). Content of GCVR and PBVR remained and degraded was calculated in percentages and were summarized in Table 2.As the degradation product did not interfere with the assay of GCVR and PBVR, the method was found as specific. Purity of GCVR and PBVR peaks was confirmed using the photodiode array detector. Of all the degraded samples, the purity angle of GCVR and PBVR was lesser than their purity threshold values, indicated the purity of GCVR and PBVR peaks. Hence, the method was stability-indicating.

Table 2: Summary of data in GCVR and PBVRforced degradation study

Degraded with	GCVR				PBVR			
	Remained (%)	Degraded (%)	Purity Angle	Purity Threshold	Remained (%)	Degraded (%)	Purity Angle	Purity Threshold
Acid	82.53	17.47	0.239	0.751	80.84	19.16	0.335	0.896
Base	80.84	19.16	0.216	0.755	82.70	17.30	0.335	0.806
Hydrogen peroxide	80.34	19.66	0.264	0.758	82.80	17.20	0.339	0.807
Dry heat	79.6	20.40	0.291	0.655	81.20	18.80	0.359	0.8
Sun light	81.22	18.78	0.264	0.661	79.74	20.26	0.245	0.81
Water	80.27	19.73	0.217	0.656	81.81	18.19	0.306	0.706

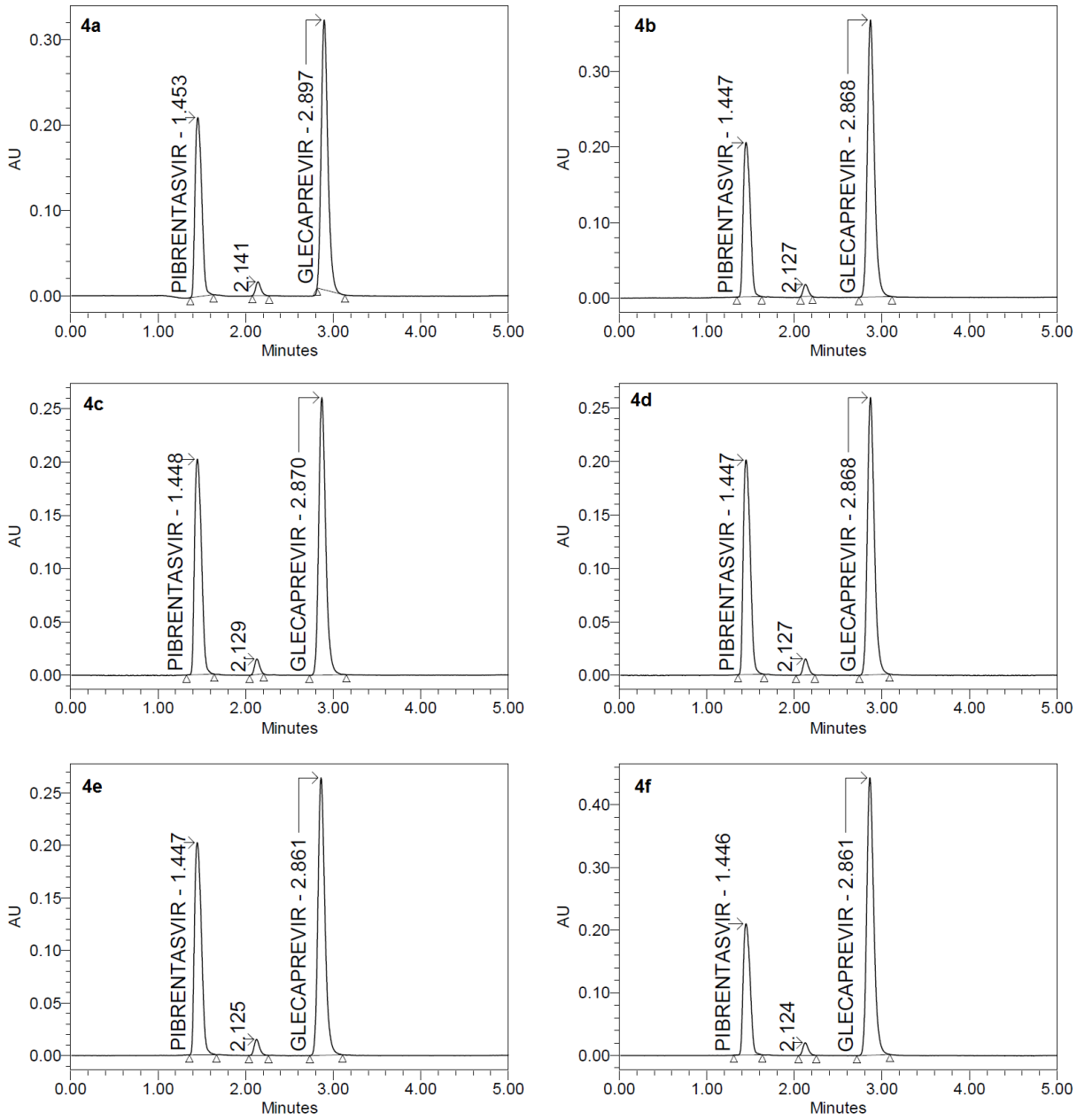

Fig. 4: Chromatograms of the sample treated with [a] Acid [b] Base [c] Hydrogen peroxide [d] Dry heat [e] Sun light [f] Water

Selectivity

The interference of peaks due to excipients and components of mobile with GCVR and PBVR peaks was examined to demonstrate the selectivity of method[14]. To demonstrate this, chromatograms of placebo blank, the mobile phase blank, standard drug and tablet sample were compared. The results are shown in Fig. 5. The chromatograms of selectivity test demonstrated the method selectivity.

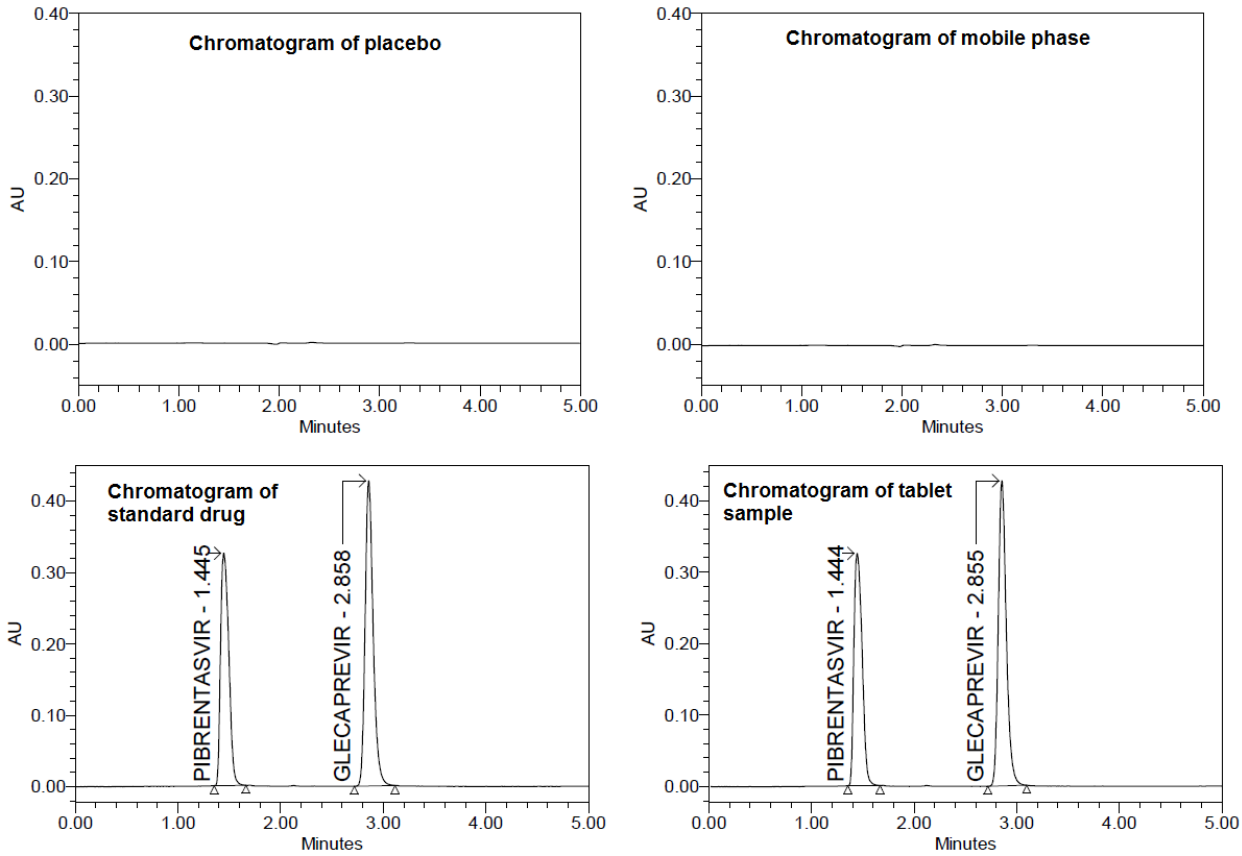

Fig. 5: Chromatograms showing absence of interfering peak in the region of retention time of GCVR and PBVR

Linearity, limit of detection and limit of quantification

The peak areas against the concentration of GCVR and PBVR were linear in the range of 12.5-200 and 5-80µg/ml, respectively. Linear regression data for GCVR and PBVR plots are presented in Table 3.The results (Table 3) indicated that peak area responses of GCVR and PBVR measured at 228nm shown good linearity over the concentration range studied.

Signal to noise ratio of 3:1 and 10:1 were used to determine the limits of detection (LOD) and quantification (LOQ), respectively. The results (Table 3) showed sufficient sensitivity of the method to quantify GCVR and PBVR.

Table 3: Linear Regression, LOD and LOQ data for GCVR and PBVR

Parameter	GCVR	PBVR
Linearity range (µg/ml)	12.5-200	5-80
Regression equation (A= mC+I)	A=63257C+ 1302	A=60197C-1016
Correlation coefficient (R^2)	0.9997	0.9995
LOD (µg/ml)	0.254	0.133
LOQ (µg/ml)	0.848	0.444

A=Peak area; m=Slope; C= Concentration of drug; I=Intercept

Precision and accuracy

Precision and accuracy was measured by executing six assays of standard GCVR (100 µg/ml) and PBVR (40 µg/ml) solution by the proposed method. The percentage relative standard of GCVR and PBVR peak area obtained was calculated to represent the precision (Table 4). The percentage of recoveries of GCVR and PBVR was determined to represent the accuracy (Table 4). RSD values for GCVR and PBVR peak areas were 0.055% and 0.311%, respectively. This indicated that the method was amply precise. The mean percentage values of recoveries for GCVR and PBVR are 99.47% and 99.34%. Good percentage values of recoveries showed that the method is sufficiently accurate.

Table 4: Data of method precision and accuracy

GCVR		PBVR	
Precision			
Concentration (µg/ml)	**Peak area (mAU)**	**Concentration (µg/ml)**	**Peak area (mAU)**
100	6332688	40	2420849
100	6336183	40	2401642
100	6339925	40	2401858
100	6330959	40	2401772
100	6333597	40	2409271
100	6338323	40	2407516
Mean*	6335279	**Mean***	2407151
%RSD	0.055	**%RSD**	0.311
Accuracy			
Concentration (µg/ml)	**Recovered (%)**	**Concentration (µg/ml)**	**Recovered (%)**
100	99.43	40	99.9
100	99.49	40	99.11
100	99.55	40	99.12
100	99.4	40	99.11
100	99.45	40	99.42
100	99.52	40	99.35
Mean*	99.47	**Mean***	99.34
%RSD	0.057	**%RSD**	0.310

***** Avaerage of six determinations; RSD – relative standard deviation

Recovery

The recovery of the method (through a standard addition technique) was determined three times by spiking pure GCVR and PBVR at different concentration levels, i.e. 50%, 100% and 150% of the labeled claim, to the pre-analyzed table sample. The percentage recovery of GCVR and PBVR at each level was calculated (Table 5). Satisfactory recoveries ranging from 99.20% to 99.48% for PBVR and 99.38% to 99.53% for GCVR were obtained by the proposed method. The results demonstrated the accuracy of the method and noninterference of tablet excipients in the assay of GCVR and PBVRsimultaneously.

Table 5: Data of recoveries of GCVR and PBVRby the proposed method

Labeled claim (mg)	Spiked (mg)	Found (mg)	Mean* found (mg)	Recovery (%)	Mean* recovery (%)
		PBVR			
40	20	59.64 59.58 59.85	59.69	99.40 99.30 99.75	99.48
40	40	79.38 79.44 79.34	79.39	99.23 99.30 99.18	99.23
40	60	99.28 99.25 99.07	99.20	99.28 99.25 99.07	99.20
		GCVR			
100	50	149.19 149.13 149.13	149.15	99.46 99.42 99.42	99.43
100	100	199.08 199.08 199.00	199.05	99.54 99.54 99.50	99.53
100	150	248.55 248.40 248.38	248.44	99.42 99.36 99.35	99.38

***** Avaerage of three determinations

Robustness

Method robustness was tested out by making deliberate changes in the HPLC parameters[15]:

- Flow rate (± 0.1 ml/min)
- Column temperature (± 2°C)
- Change in pH (± 0.2)
- Detection wavelength (± 2 nm)
- Percentage of acetonitrile (± 5%)

System suitability criteria were evaluated in all the conditions. The results are shown in Table 6. System suitability criteria for GCVR and PBVR were satisfied, indicated that the method is robust.

Table 6: Robustness Data for GCVR and PBVRby the proposed method

Condition	Investigated value	PBVR		GCVR		
		Plate count	Tailing factor	Plate count	Tailing factor	Resolution
Flow rate (ml/min)	0.9	8545	1.40	5975	1.25	9.61
	1.1	9657	1.34	7507	1.26	10.29
Column temperature (°C)	23	8545	1.40	5975	1.25	9.61
	27	9657	1.34	7507	1.26	10.29
pH	4.3	8289	1.56	7953	1.24	10.38
	4.7	8568	1.36	6762	1.25	9.91
Mobile phase	65:35	8514	1.38	6169	1.25	9.59

ratio (*v/v*)*	55:45	8573	1.38	6833	1.26	9.83
Detection	226	8533	1.38	6736	1.26	9.79
wavelength (nm)	230	8555	1.37	6667	1.27	9.80

*Na_2HPO_4 : Acetonitrile

Quantification of GCVR and PBVRin tablet formulations

The optimized and validated HPLC method was successfully applied for simultaneous quantification of GCVR and PBVR in their tablet product (Mavyret tablets, AbbVie Limited, Berkshire, UK). The amount of GCVR and PBVR in the tablet product was quantified either from the corresponding regression equation or calibration curve. The mean recovery percent ± RSD of three replicate determinations was 99.53% ± 0.380% for PBVR and 99.47% ± 0.066 for GCVR (Table 7). Satisfactory percent recovery values were obtained for GCVR and PBVR and the results were in good agreement with the labeled claim. Hence this method is suggested as suitable for the assay of GCVR and PBVR in tablet formulations.

Table 7: Determining the content of GCVR and PBVRin tablet product by the proposed method

Drug	Mg/ tablet	Drug content (mg)	Recovery (%)	Mean recovery ± SD	RSD (%)
PBVR	40	39.76 39.69 39.98	99.40 99.23 99.95	99.53 ± 0.378	0.380
GCVR	100	99.46 99.54 99.42	99.46 99.54 99.42	99.47 ± 0.061	0.061

SD-Standard deviation; RSD-Relative standard deviation

DISCUSSION:

This work aims at development and validation of a RP-HPLC method for the simultaneous quantification of GCVR and PBVR. A good chromatographic separation was observed with Cosmicsil C18, mobile phase of composition 0.1M NaH_2PO_4 (pH 4.5): acetonitrile 60:40 (*v/v*), flow rate of 1.0 ml/min and 228nm as detection wavelength. The system suitability parameters are within the acceptance limits. The obtained linearity curve shows a good relationship between drug concentration and peak area. The method was accurate, since the recovery percent value was near to 100%. The RSD values less than 1% indicating the precision of the method. The method was observed as specific, since no interference is observed as of retention time of drugs in placebo peak and blank mobile phase. Forced degradation studies indicated that GCVR and PBVR were degraded in stress conditions applied. Peak purity of GCVR and PBVR was detected through PDA tool.

CONCLUSIONS

The proposed stability indicating HPLC method provides simple, sensitive, selective, accurate and reproducible method for separation and simultaneous estimation of GCVR and PBVR. The method was validated following ICH guidelines. The method is specific and stable indicates as there is no interference from the degradation products produced in applied stress conditions. The proposed method can be applied to assay GCVR and PBVR simultaneously in tablet formulation with better accuracy.

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AUTHOR'S CONTRIBUTION

This paper is the research work of M.S. Swarna Pushpa under the guidance of Dr. T. Raja Rajeswari, Associate Professor, Dept. of Chemistry, Y.A. Govt. College for Women, Chirala, Andhra Pradesh.

CONFLICTS OF INTEREST

The authors declared that there are no conflicts of interest exists in the current study.

REFERENCES

1. FDA Approves Mavyret. Available at: https://www.drugs.com/newdrugs/fda-approves-mavyret-glecaprevir-pibrentasvir-hepatitis-c-4569.html
2. Mavyret drug trials snapshot. Available at: https://www.fda.gov/Drugs/InformationOnDrugs/ ucm571769.htm
3. Lamb YN. Glecaprevir/Pibrentasvir: First Global Approval. Drugs 2017;77:1797-804.
4. Pibrentasavir, Pubchem, 2018. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Pibrentasvir#section=Top
5. Glecaprevir, Pubchem, 2018. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Glecaprevir#section=Top
6. Ralf B, Francois P, Volker L, Patrice A. Assembly of infectious hepatitis C virus particles. Trends Microbiol 2011; 19: 95–103.
7. Aghemo A, Colombo M. Glecaprevir/Pibrentasvir: The final piece in the Hepatitis C Virus Treatment Puzzle?. Gastroenterol 2018;154:1195-6.

8. Carrion AF, Martin P. Glecaprevir + pibrentasvir for treatment of hepatitis C. Expert Opin Pharmacother 2018;19:413-9.
9. Tarik A, Patrick M, Raymond FS. Treatment of hepatitis C virus infection with direct-acting antiviral agents: 100% cure?.Liver Int 2018; 38, 7-13.
10. Hemalatha K, Kistayya C, Nizamuddhin ND, Dastiagiriamma D. Simultaneous estimation of new analytical method development and validation of glecaprevir and pibrentasvir by high performance liquid chromatography. Innovat Int J Med Pharma Sci 2018; 3, 5-8.
11. Sridevi M, Rao TS, Naidu CG. Development and validation for the simultaneous estimation of glecaprevir and pibrentasavir in drug product by UPLC. Eur J Biomed Pharma Sci2018;5: 473-80.
12. International Conference on Harmonization Q1B, Stability testing: Photostability Testing of New Drug Substances and Products, Proceeding of the International Conference on Harmonization, Geneva, 1996.
13. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1), ICH, 2005.
14. Bhavani T, GeethaSusmita A, Rajitha G. Method development, validation and stability studies for the determination of lurasidone hydrochloride in bulk and tablet dosage form by RP-HPLC. Int J Pharm Pharm Sci 2018; 10: 58-63.
15. Gorja A, Sumanta M. Stability indicating method development and validation for the estimation of panobinostat lactatein pharmaceutical dosage forms by UPLC. Int J Pharm Pharm Sci 2018; 11: 60-65.