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ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF GLECAPREVIR IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Glecaprevir is an antiviral drug used in combination with other drugs includes sofosbuvir, ribavirin, and interferon, depending on the virus type to treat cirrhosis caused by hepatitis C (HCV). Several methods have been found for quantification, but those are not cost-effective, and they are time-consuming. The present study developed a simple, precise, accurate and cost-effective UPLC method to determine Glecaprevir quantity in tablet dosage forms. A simple and selective UPLC method is described for the determination of Glecaprevir Chromatographic separation was achieved on a Acquity BEH C18 (50 × 3.0mm. 1.7 µm) using a mobile phase consisting 0.1% of Orthophosphoric acid: Acetonitrile in a ratio of 60:40 v/v with detection of 248 nm. Linearity was observed in the range 50-150 µg/ml for Glecaprevir (r^2 = 1.000). The amount of drugs estimated by the proposed method was in good agreement with the label claim. The proposed method was validated as per ICH guidelines and applied for the determination of the cited drug in the dosage form.

KEYWORDS: *Glecaprevir,UPLC,Hepatitis (HCV) virus*

INTRODUCTION

Glecaprevir is chemically dimethyl N, N'-([1,1'-biphenyl]-4, 4'-diylbis{1H- imidazole-5,2-diyl-[(2S)-pyrrolidine-2,1-diyl][(2S)- 3- methyl- 1- oxobutane-1, 2-diyl]}) dicarbamate. Glecaprevir has molecular weight: 738.89 g/mol and molecular formula: $C_{40}H_{50}N_8O_6$. It is an antiviral drug used in combination with other medicaments to treat hepatitis C (HCV). The other medicines used in combination include interferon, sofosbuvir, and ribavirin, depending on the virus type 1. The dose of Glecaprevir present in the formulation was determined by using the Ultra Performance Liquid Chromatography method. UPLC has greater sensitivity, resolution, and speed of analysis.

UPLC operates at high pressure than HPLC, and fine particles, *i.e.*, less than 2.5 µm are used, and mobile phases at high linear velocities decrease the length of the column, reduces solvent consumption, and save time [2].

The UPLC is based on the use of a stationary phase consisting of particles less than 2.5 µm whereas the HPLC column is typically filled with 3-5 µm particles. The principle of this evolution is governed by the Van Deemeter equation, which is an empirical formula that describes the relationship between the linear velocity of flow rate and plate height [3, 4].

$$H = A + B / v + Cv$$

Where; *A*, *B* and *C* are constants, *v* is the linear velocity, the carrier gas flow rate.

*The *A* term is independent of velocity and represents "eddy" mixing. It is the smallest when the packed column particles are small and uniform.

The *B* term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates, and so this term is divided by *v*.

*The *C* term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the stationary packing phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus the term is proportional to *v*.

Therefore it is possible to increase throughput and thus the speed of analysis without affecting the chromatographic performance.

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The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC). Efficiency is proportional to column length and inversely proportional to the particle size [5, 6].

This technology has the advantage of chromatographic principles to run separations using a packed column with similar particle sizes less than 2.5 µm are used with high flow rates speed gives superior resolution and sensitivity.

MATERIALS AND METHODS

Chemicals and Reagents: The drug standard of Glecaprevir was kindly supplied by Madras Pharmaceuticals, Chennai, with certified purity of 99.97 ± 0.512. Daklinza 10 mg Tablets were purchased from apollo pharmacy, Hyderabad. HPLC grade acetonitrile, water, and methanol were obtained from Rankem. Analytical grade Potassium Dihydrogen orthophosphate, Dipotassium hydrogen orthophosphate and O-Phosphoric acid were obtained from Merck.

A Shimadzu (UV-1800) double beam UV-Vis spectrophotometer with 1cm quartz cuvette connected to a personal computer loaded with UV probe 2.21 software was used.

Chromatographic Method: [7, 8] Chromatographic separations were achieved by UPLC-agilent 1290 infinity with a quaternary solvent manager, with autosampler injector and photodiode array detector, coupled with Empower software for data acquisition. Acquity BEH C18 (50 × 3.0 mm. 1.7 µm) was used as the stationary phase for the development of the chromatographic separation, optimization, and method validation. Isocratic elution was conducted using a mobile phase 0.1% Orthophosphoric acid: Acetonitrile (60:40) v/v.

The flow rate was set at 0.5 mL/min. Column temperature was adjusted at 30 °C, and samples were injected at 10 µL injection volume with a run time of 5 min at a temperature 10 °C and determined at a wavelength of 248 nm. Glecaprevir 1 mg/mL stock solution was prepared for the UPLC method by dissolving 100mg of Glecaprevir in 100 mL of the mobile phase.

Preparation of 0.1% Ortho Phosphoric Acid: Taken 1 mL of orthophosphoric acid and transferred in to a 1000 mL of water & filtered through 0.45 µm filters to remove all fine particles and gases.

RESULTS AND DISCUSSION

UPLC Method Development: The main target of the proposed UPLC method was to achieve separation of Glecaprevir within short runtime. To determine the stationary phase (Acquity BEH C18 (50 × 3.0 mm. 1.7 µm)) column was chosen because it provided better peak symmetry. For organic modifier, a different ratio of orthophosphoric acid and acetonitrile were checked. It was found that orthophosphoric acid found better resolution. Mobile phase ratio was found to be a mixture of orthophosphoric acid: acetonitrile 60:40 v/v.

Flow rate at 0.5 mL/min was selected as the optimum flow rate. The optimum wavelength for detection was 248 nm. The retention time was

1.190 min, respectively. According to the ICH guidelines, the system sustainability tests should be carried out prior to analysis. Several parameters were studied, including tailing factor, retention time, height equivalent to theoretical plates, and RSD% of peak area for repetitive injections were studied. In all deliberately varied chromatographic conditions, the chromatogram of solution showed satisfactory resolution, as shown in **Fig. 1** and results are shown in **Table 1**.

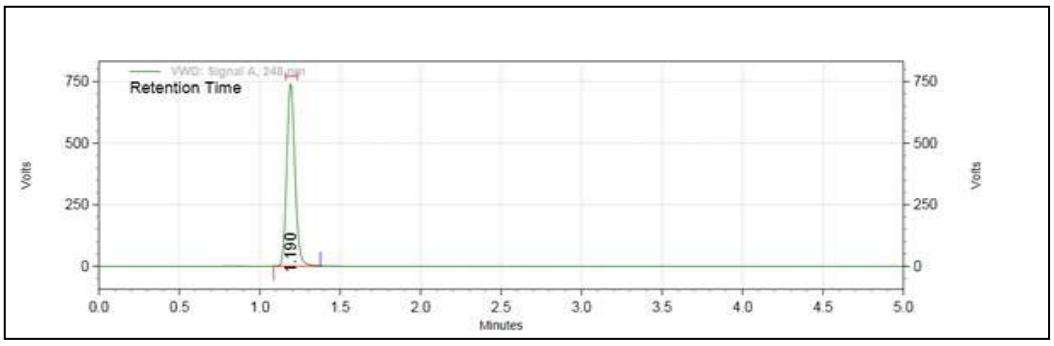

FIG. 1: UPLC CHROMATOGRAM OF GLECAPREVIR

TABLE 1: UPLC CHROMATOGRAM OF GLECAPREVIR

S. no.	Name	RT	Area	TP	TF
1	GLECAPREVIR	1.190	44113817	2652	1.2

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Validation of Proposed Methods: The developed method was validated as per ICH guidelines.

Linearity and Concentration Range: [9] Aliquots equivalent to 50-150 µg/mL of working solution (1mg/mL) of Glecaprevir were transferred into a 10 mL volumetric flask, and the volume was diluted with the mobile phase. The linearity values were summarized in **Table 2**.

TABLE 2: LINEARITY DATA OF GLECAPREVIR

S. no.	Concentration (µg/mL)	Area
1	50	21720461
2	80	34167231
3	100	44035624
4	120	52943892
5	150	67035271

The correlation coefficient R^2 was determined and was found to be 1.00 for GLECAPREVIR were given in **Table 3**. The linearity graph shown in **Fig. 2** and the chromatograms are shown in **Fig. 3-7**.

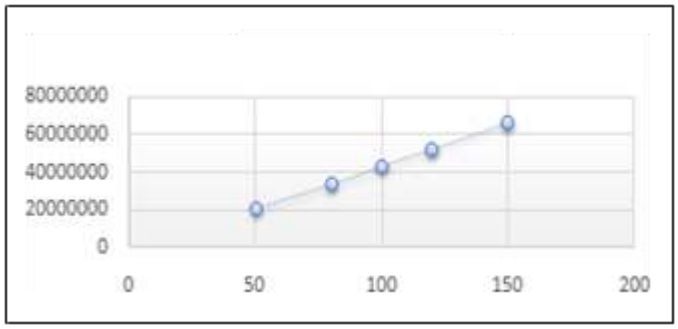

FIG.2: GRAPH FOR LINEARITY DATA OF GLECAPREVIR

TABLE 3: LINEARITY RESULTS OF GLECAPREVIR

S. no.	Parameter	Glecaprevir
1	Correlation coefficient	1.000
2	Slope	455392.02
3	Intercept	1558706.27

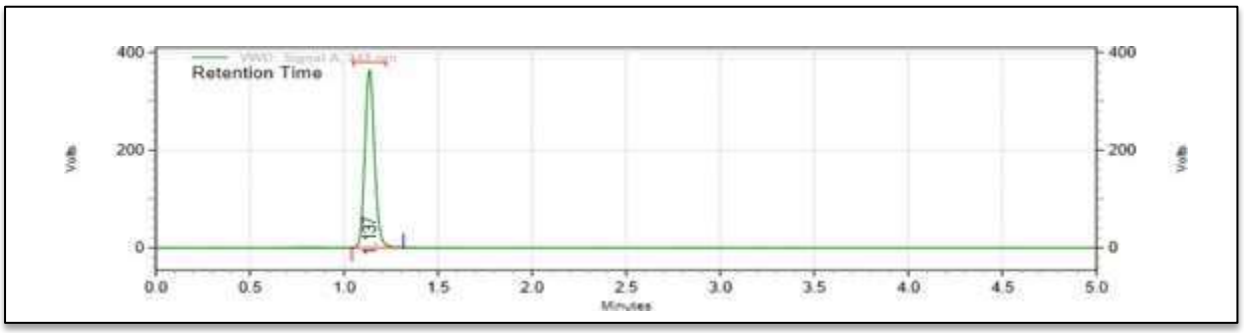

FIG. 3: CHROMATOGRAM OF LINEARITY FOR PREPARATION 1

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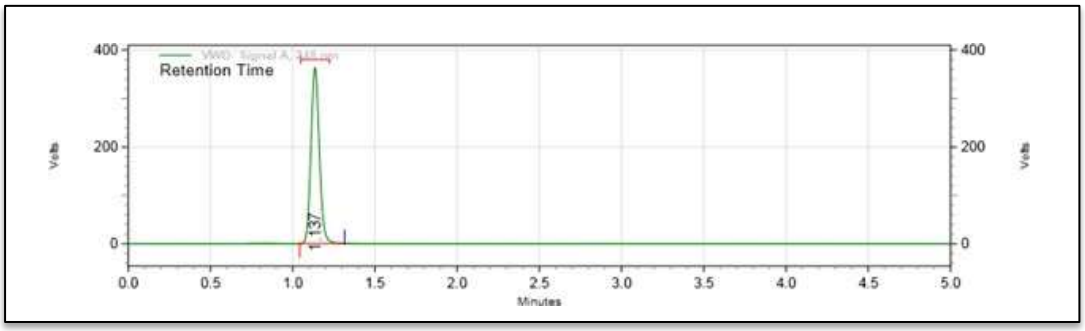

FIG. 4: CHROMATOGRAM OF LINEARITY FOR PREPARATION 2

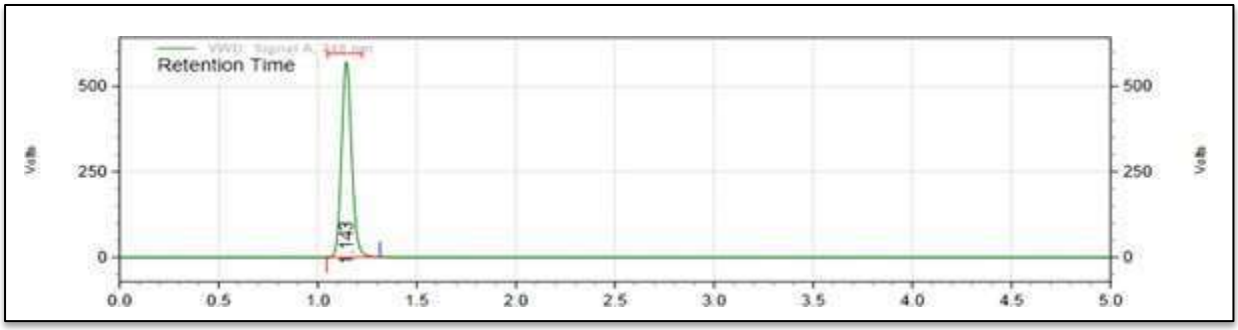

FIG. 5: CHROMATOGRAM OF LINEARITY FOR PREPARATION 3

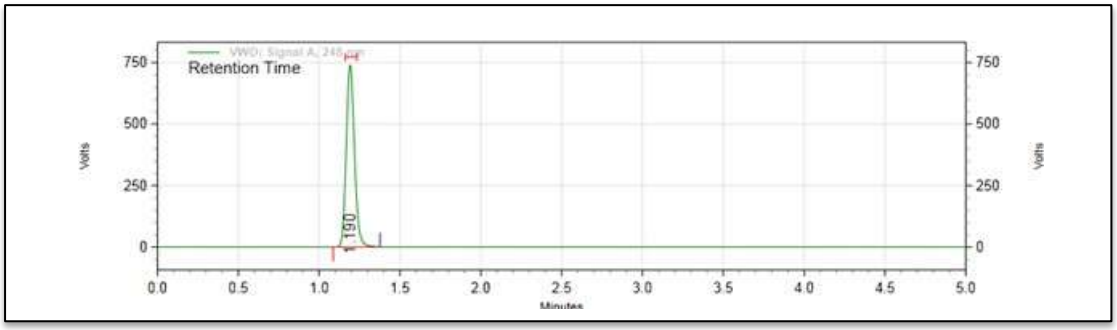

FIG. 6: CHROMATOGRAM OF LINEARITY FOR PREPARATION 4

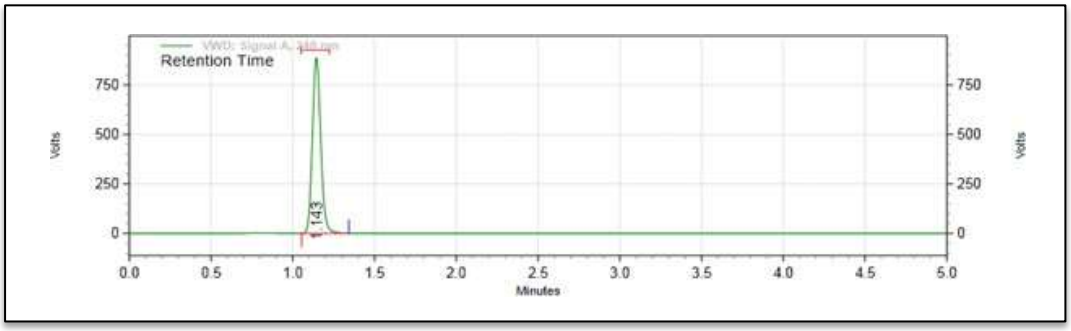

FIG. 7: CHROMATOGRAM OF LINEARITY FOR PREPARATION 5

System Suitability and Method Precision: [10] The system suitability was evaluated by giving Glecaprevir injection six times, and the chromatograms were recorded.

The results were summarised in **Table 4**. The plate count and tailing factor results were found to be within limits.

The method precision chromatograms were recorded, and the results were summarized in **Table 5**.

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TABLE 4: RESULTS FOR SYSTEM SUITABILITY OF GLECAPREVIR

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.192	44125114	2560	1.2
2	1.193	44176891	2421	1.1
3	1.193	44165637	2676	1.3
4	1.190	44147346	2706	1.2
5	1.187	44105682	2704	1.1
6	1.190	44102411	2786	1.2
Mean	1.191	44137180	-	-
SD	0.023	31102.11	-	-
% RSD	0.2	0.1	-	-

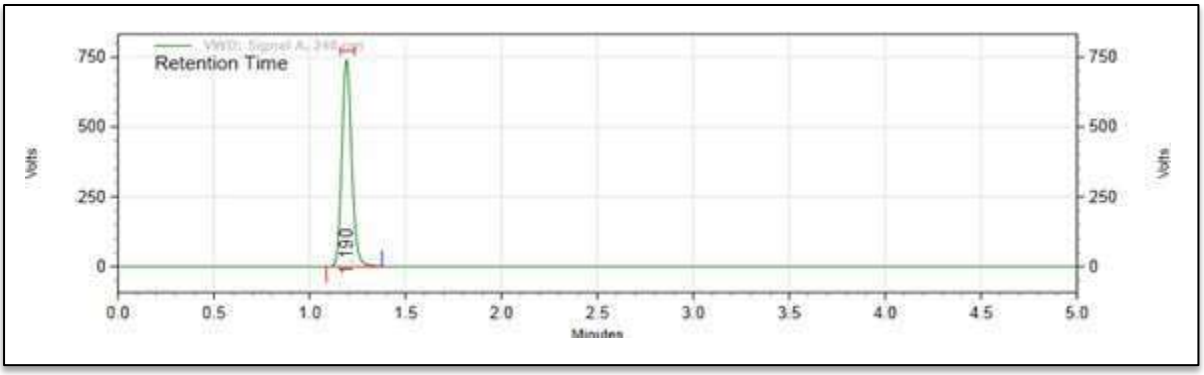

FIG. 8: CHROMATOGRAM OF METHOD PRECISION-01

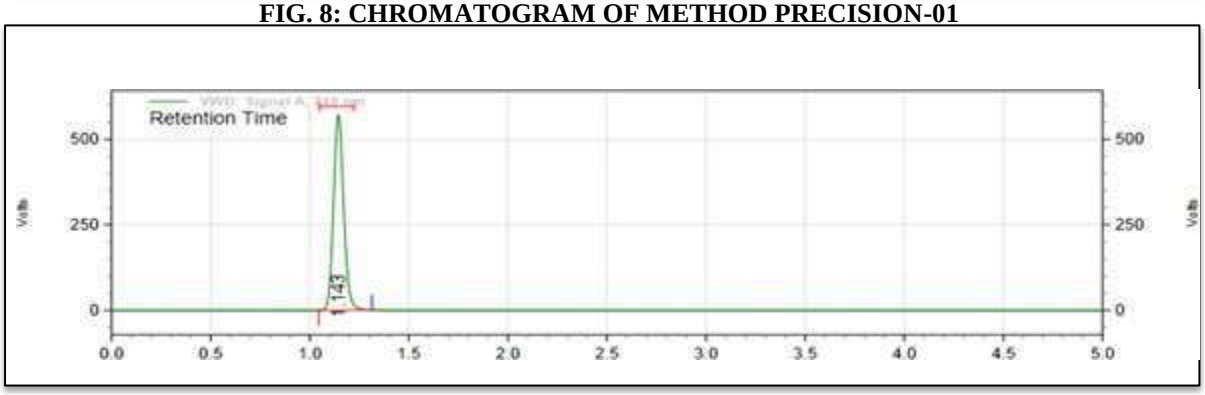

FIG. 9: CHROMATOGRAM OF METHOD PRECISION-02

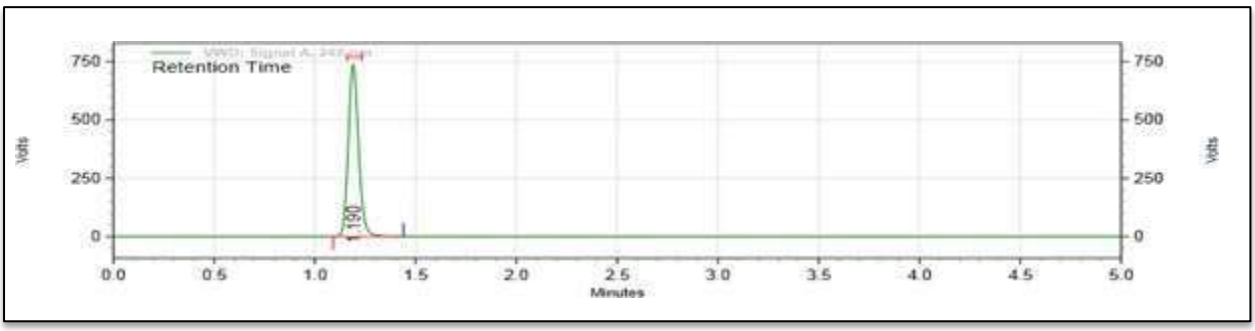

FIG. 10: CHROMATOGRAM OF METHOD PRECISION-03

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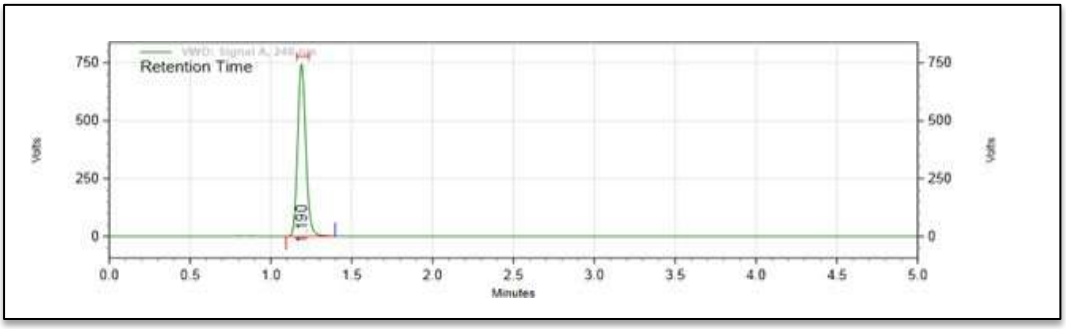

FIG. 11: CHROMATOGRAM OF METHOD PRECISION-04

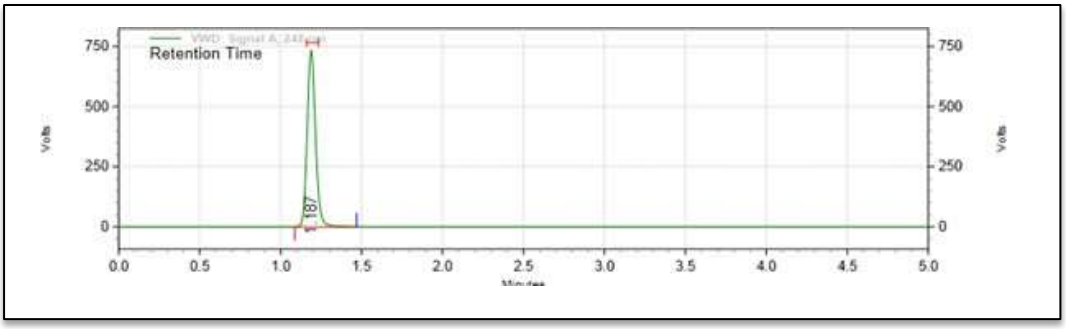

FIG. 12: CHROMATOGRAM OF METHOD PRECISION-05

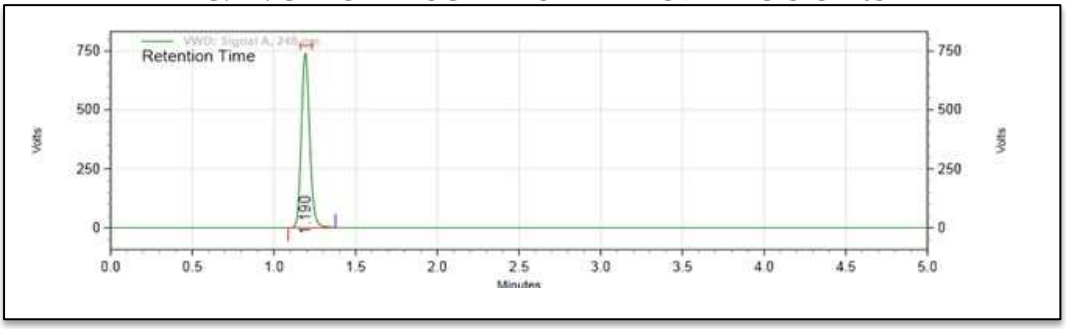

FIG. 13: CHROMATOGRAM OF METHOD PRECISION-06

TABLE 5: METHOD PRECISION RESULTS FOR GLECAPREVIR

Injection	Glecaprevir	
	Area	% Assay
1	44113817	98.9
2	44176366	98.7
3	44078346	98.4
4	44150181	98.7
5	44008775	98.9
6	44025521	98.0
Average		9666992
SD		2938.097
%RSD		0.030383

Specificity: A chromatogram of blank and placebo solutions had shown no peaks at the retention times of Glecaprevir. It indicates that diluent or excipient peaks do not interfere with the Glecaprevir peak. The chromatograms are shown in **Fig. 14** and **Fig. 15**.

Accuracy: The accuracy of the proposed method were determined by analyzing three different laboratory preparations of Glecaprevir in different ratios within the linearity range. The values of mean percentage recoveries were shown in **Table**

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6. The chromatograms are shown in **Fig. 16-24** [11].

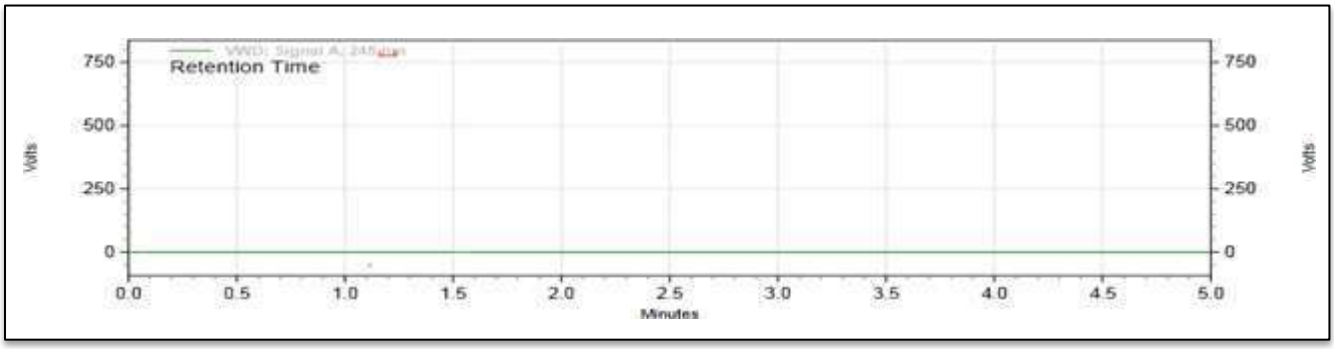

FIG. 14: CHROMATOGRAM OF PLACEBO

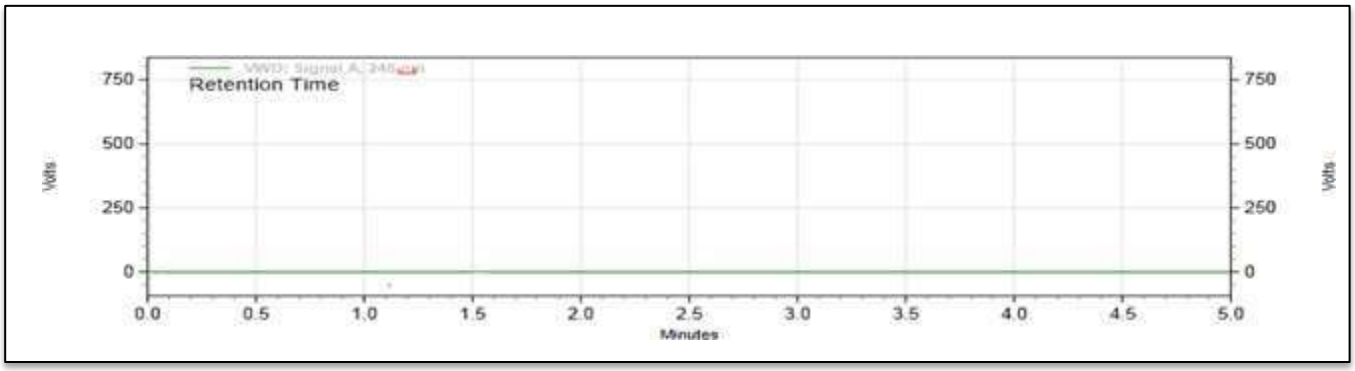

FIG. 15: CHROMATOGRAM OF BLANK

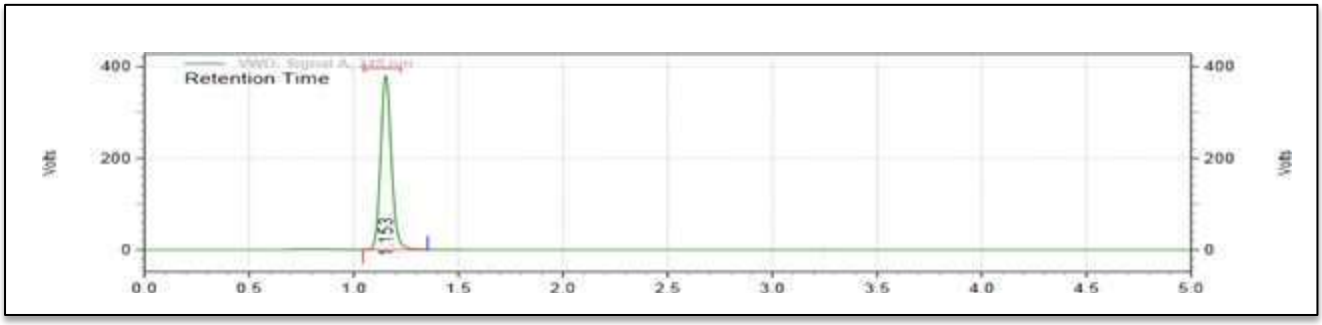

FIG. 16: CHROMATOGRAM OF 50% RECOVERY-1

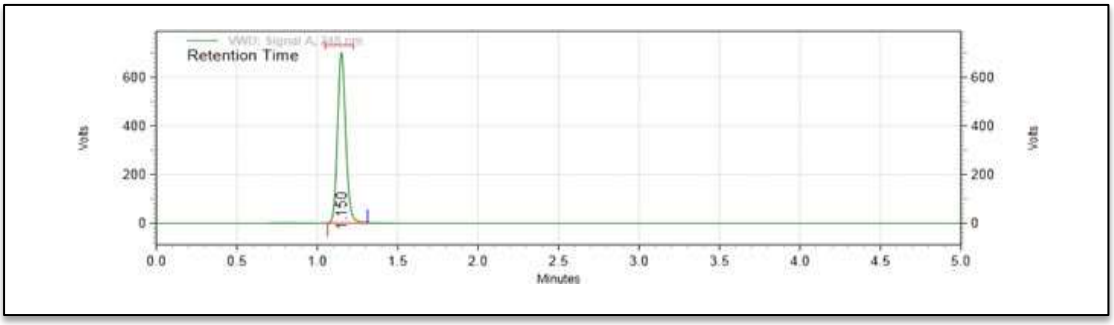

FIG. 17: CHROMATOGRAM OF 100% RECOVERY-1

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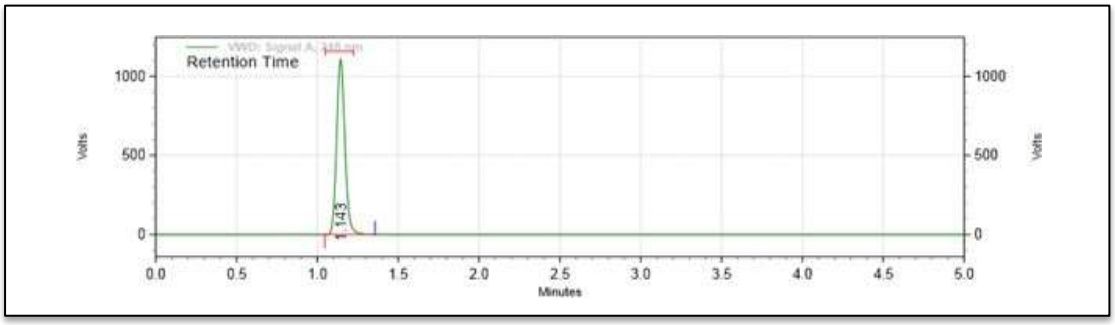

FIG. 18: CHROMATOGRAM OF 150% RECOVERY-1

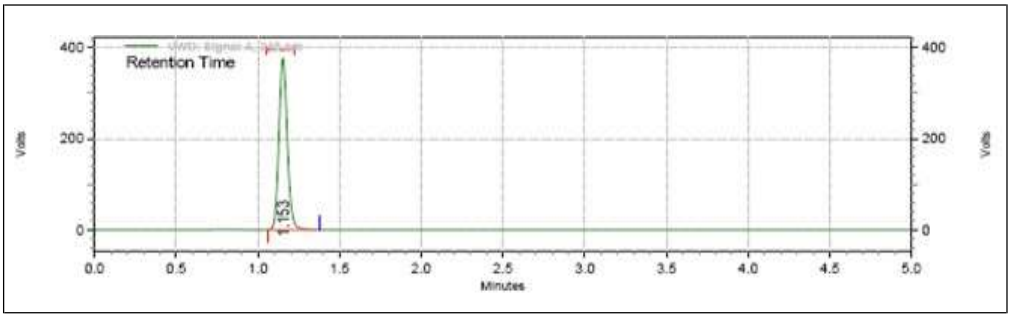

FIG. 19: CHROMATOGRAM OF 50% RECOVERY-2

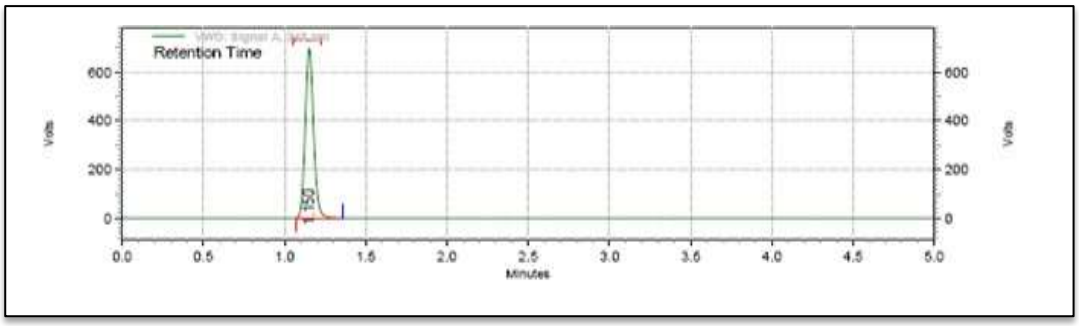

FIG. 20: CHROMATOGRAM OF 100% RECOVERY-2

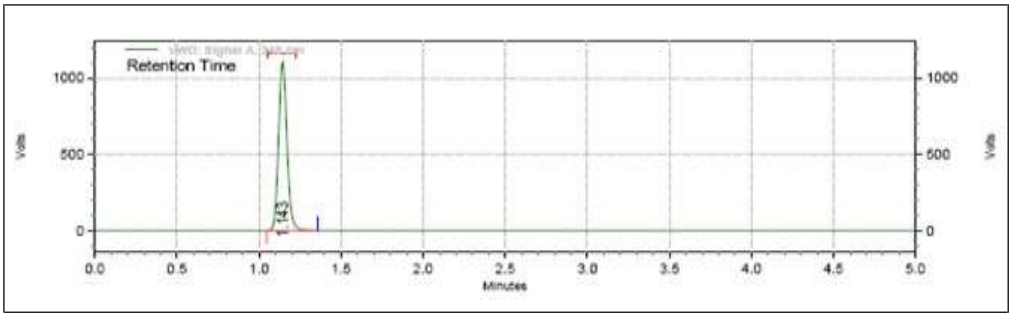

FIG. 21: CHROMATOGRAM OF 150% RECOVERY-2

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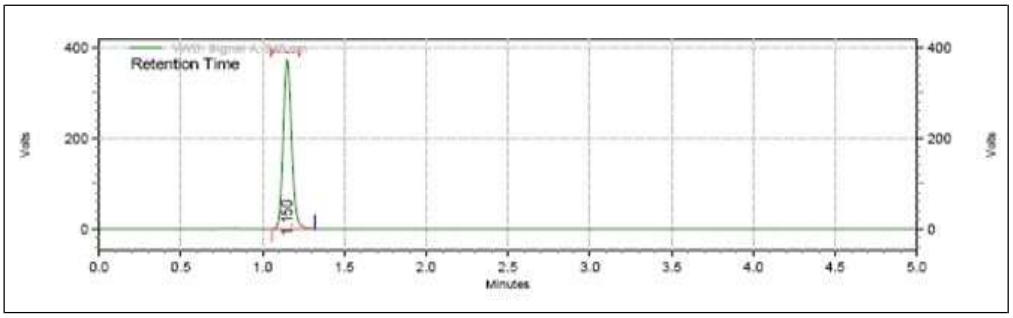

FIG. 22: CHROMATOGRAM OF 50% RECOVERY-3

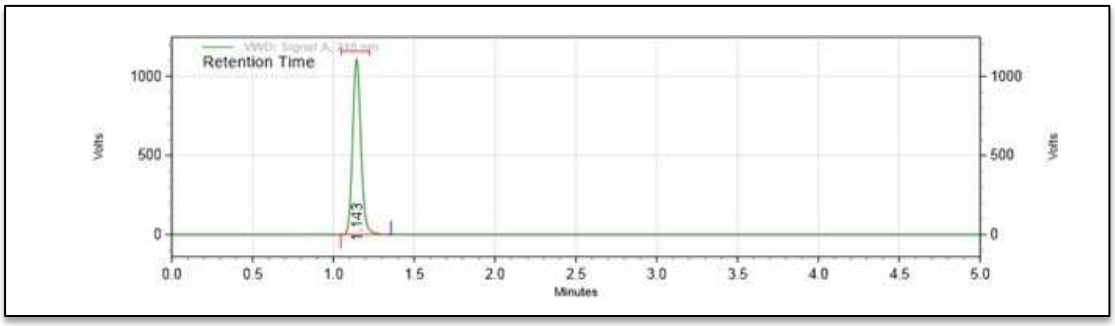

FIG. 23: CHROMATOGRAM OF 100% RECOVERY-3

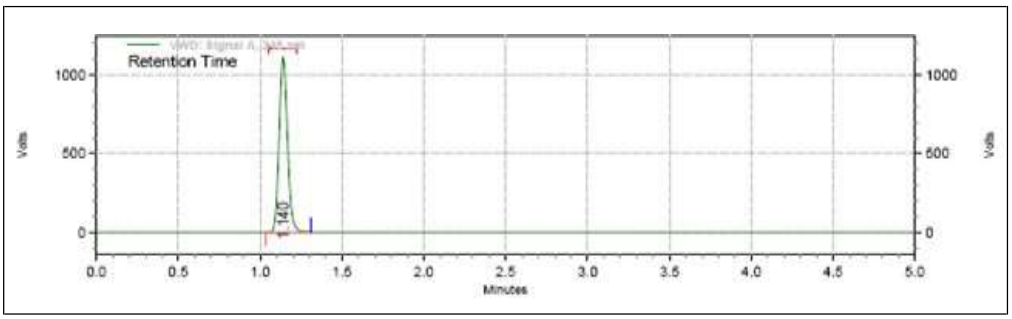

FIG. 24: CHROMATOGRAM OF 150% RECOVERY-3

TABLE 6: RESULTS FOR RECOVERY OF GLECAPREVIR

% Recovered	Area	Concentration Added	Concentration Recovered	% Recovery	Average
50% _01	7004575	250	252.18	100.9	100.5
50% _02	7020900	250	252.77	101.1	
50% _03	7002470	250	252.11	100.8	
100% _01	13910853	500	500.83	100.2	
100% _02	13902676	500	500.53	100.1	
100% _03	13701006	500	493.27	98.7	
150% _01	21010188	750	756.42	100.9	
150% _02	21026894	750	757.02	100.9	
150% _03	21021825	750	756.84	100.9	

Limit of Detection (LOD) & Quantitation (LOQ): [12, 13] According to ICH guidelines LOD and LOQ can be calculated using the standard deviation of the response and the slope. LOD = 3.3 × σ/S and LOQ = 10 × σ/S. Where, σ = the standard deviation of the response and S = the slope of the calibration curve. LOD and LOQ of the drug were found to be 35.14µg/mL and 106.48 µg/mL, respectively.

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Robustness: [14] The Robustness of the method was determined. The chromatograms are shown in **Fig. 25-28**. The results obtained by deliberate variation in method parameters are summarized below in **Table 7**.

TABLE 7: RESULTS FOR ROBUSTNESS OF GLECAPREVIR

Chromatographic changes		Retention time(min)	Tailing Factor	Theoretical Plates
Flow rate (mL/min)	0.4	1.473	1.1	2942
	0.6	0.933	1.2	2047
Temperature (°C)	25	1.143	1.2	2467
	35	1.137	1.1	2496

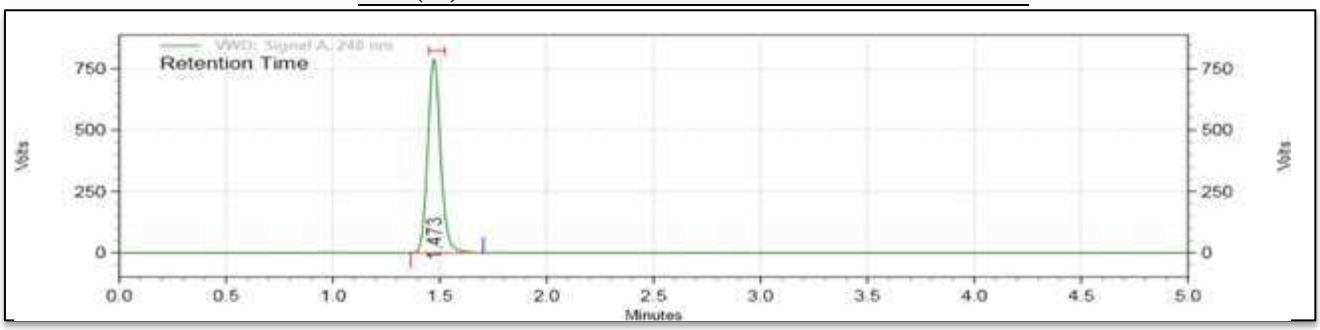

FIG. 25: CHROMATOGRAM OF FLOW RATE FROM 0.5mL/min TO 0.4mL/min

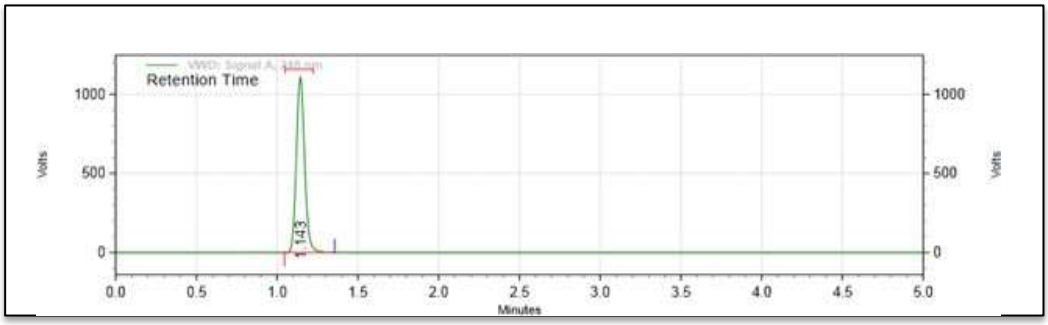

FIG. 26: CHROMATOGRAM OF FLOW RATE FROM 0.5mL/min TO 0.6mL/min 0.6mL/min

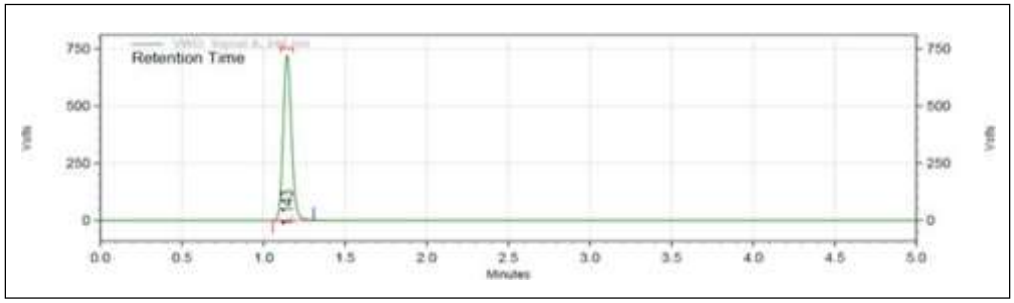

FIG. 27: CHROMATOGRAM OF TEMPERATURE FROM 30 TO 25°C

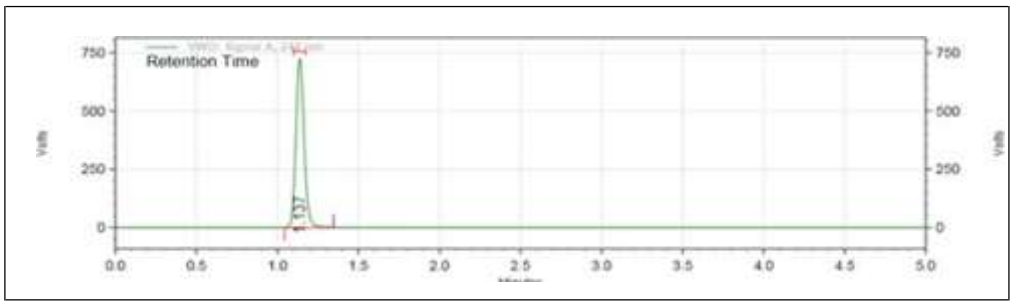

FIG. 28: CHROMATOGRAM OF TEMPERATURE FROM 30 TO 35°C

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Ruggedness: The ruggedness of the method was studied by determining the analyst-to-analyst variation by performing the Assay by two different analysts. The chromatograms are shown in **Fig. 29-32.**The method is rugged, and the results are summarized in **Table 8**

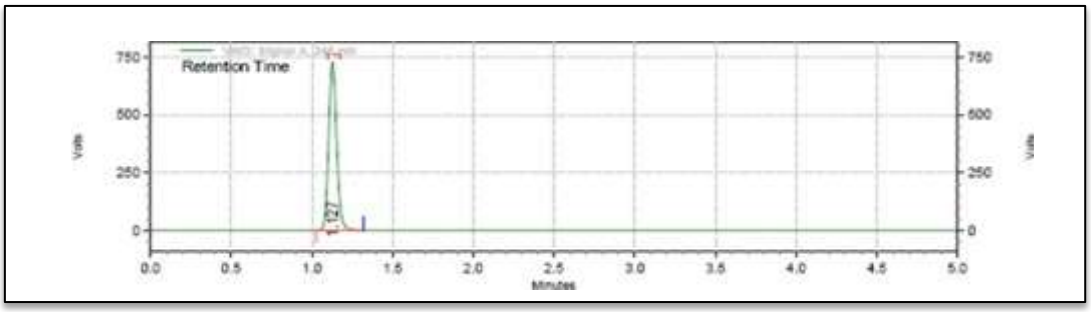

FIG. 29: CHROMATOGRAM OF ANALYST-01 STANDARD

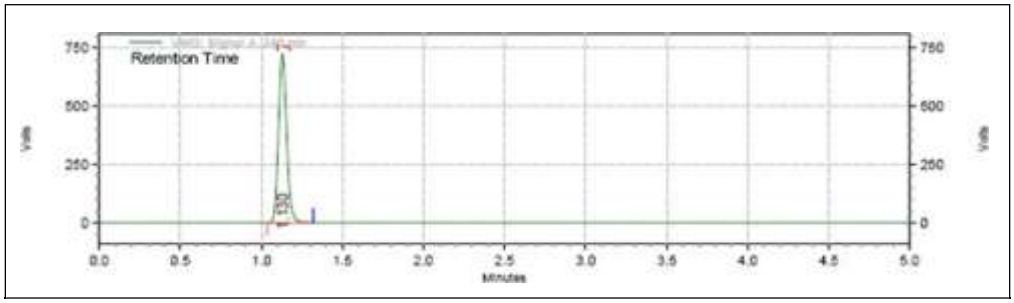

FIG. 30: CHROMATOGRAM OF ANALYST-01 SAMPLE

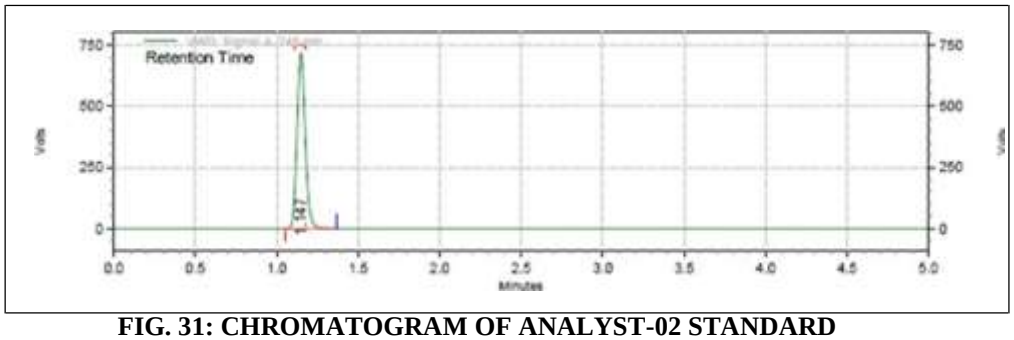

FIG. 31: CHROMATOGRAM OF ANALYST-02 STANDARD

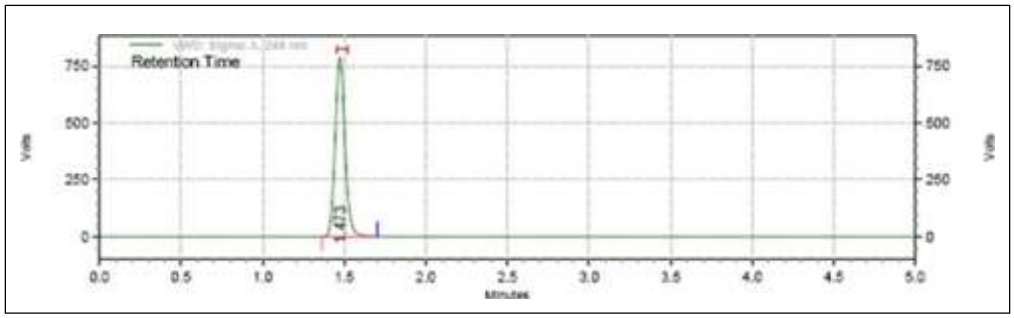

FIG. 32: CHROMATOGRAM OF ANALYST-02 SAMPLE

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TABLE 8: RUGGEDNESS RESULTS OF GLECAPREVIR

Glecaprevir	%Assay	Glecaprevir	%Assay
Analyst 01	98.8	Analyst 01	98.9
Analyst 02	98.9	Analyst 02	98.1
%RSD	0.18	%RSD	0.28

Analysis of Pharmaceutical Dosage Forms: [15] The proposed method was applied for the determination of Glecaprevir in pharmaceutical dosage form Daklinza 10 mg tablets, without interference from the excipients, and good recoveries were obtained by applying the standard addition technique. The results were summarized in **Table 9** and **10**.

TABLE 9: RESULTS OF DAKLINZA DOSAGE FORM

	Glecaprevir	
	Standard Area	Sample Area
Injection-1	44049957	43312224
Injection-2	44176366	43309599
Injection-3	43965547	43398270
Injection-4	44027772	44329833
Injection-5	43915825	44358634
Average Area	44027093.4	43741712
Standard deviation	551274.21	
%RSD	0.22	
Assay (%purity)	99.35	

TABLE 10: RESULTS OF ASSAY

Drug	Label claim (mg)	Amount found (mg)	% Assay
DACLATASVIR	10	9.87	98.7

CONCLUSION

A new precise, accurate, rapid method has been developed for the estimation of Glecaprevir pharmaceutical dosage form by UPLC. The cited drug was analyzed without any interference from excipients indicates the selectivity of the method. The proposed method is highly sensitive, precise, and accurate, as indicated by % recovery, % mean recovery and % RSD values.

From the results, it indicates that the UPLC method is applicable to assay of this antiviral drug with minimum sample preparation, cost, and time effectiveness with a satisfactory level of accuracy and precision. Hence, it is successfully applied for the quantification of API content in the commercial formulations of Glecaprevir in educational institutions and Quality control laboratories. UPLC method is economical, faster, consumes less mobile phase than HPLC; it indicates it is faster and eco-friendly.

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