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Original Article

RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF RITONAVIR, OMBITASVIR AND PARITAPREVIR IN TABLET DOSAGE FORMS AND THEIR STRESS DEGRADATION STUDIES

SYED IBRAHIM BAJE[1], B. JYOTHI[2], N. MADHAVI[3*]

[1,3]PG Department of Chemistry, JKC College, Acharya Nagarjuna University, Guntur, A. P., [2]Department of Chemistry, Swarna Bharathi Institute of Science and Technology, Khammam, Telangana
Email: madhavijkcchempg@gmail.com

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ABSTRACT

Objective: The objective of the present study was to develop and validate a novel reverse phase high performance liquid chromatographic (RP-HPLC) method, for simultaneous determination of ritonavir (RIT), ombitasvir (OMB) and paritaprevir (PAR) in bulk mixtures, and in tablets.

Methods: Determination of the drugs ritonavir (RIT), ombitasvir (OMB), and paritaprevir (PAR), was carried out applying Hypersil BDS C18 column (250 mm X 4.6 mm i.e., 5 µm particle size), with photodiode array detector at λ_{max} of 254 nm. The mobile phase applied for the current study composed of two solvents, i.e. A (0.01N % w/v potassium di-hydrogen orthophosphate buffer, pH 3.0 adjusted with dilute orthophosphoric acid) and B (acetonitrile). The mobile phase was pumped at a flow rate of 1.0 ml/min in the isocratic mode. The validation study with respect to specificity, linearity, precision, accuracy, and robustness, limit of detection (LOD) and limit of quantification (LOQ) was carried out employing the ICH guidelines.

Results: Ritonavir, ombitasvir, and paritaprevir showed linearity of response between 12.5-75 µg/ml for ritonavir, 3.125-18.75 µg/ml for ombitasvir and 18.75–112.5 µg/ml for paritaprevir, with a correlation coefficient (R^2) 0.999, 0.999,0.999 for RIT, OMB, and PAR respectively. The % recovery obtained was 99.82±0.14 % RIT, OMB 100.03±0.96 % and for 99.96±0.26 % PAR. The LOD and LOQ values for RIT, OMB, PAR were obtained to be 0.02, 0.019and0.02, µg/ml and 0.07, 0.06 and 0.07 µg/ml, respectively. The method also exhibits good robustness for different chromatographic conditions like wavelength, flow rate, mobile phase, and injection volume.

Conclusion: The method was successfully employed, for the quantification of RIT, OMB, and PAR, in the quality control of in-house developed tablets, and can be applied for the industrial use.

Keywords: Ombitasvir, Ritonavir, Paritaprevir, RP-HPLC, ICH guidelines

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INTRODUCTION

Ritonavir, [1] is chemically known as 2,4,7,12-tetra azatridecan-13-oicacid, 10-hydroxy-2-methyl-5-(1-methyl ethyl)-1-[2-(1-methyl ethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-5-thiazolmethyl ester. It is an antiretroviral drug [2], an inhibitor of HIV-1 (human immunodeficiency virus) protease [3-5] used to treat HIV infection and AIDS (acquired immune deficiency syndrome). As of now once in a while utilized for its own particular antiviral movement [6], yet remains generally utilized as a sponsor of other protease inhibitors. This prevents cleavage of the gag-pol polyprotein [7]. All the more particularly, ritonavir is utilized to restrain a specific liver catalyst that ordinarily processes protease inhibitors, CYP3A4 is a member of the cytochrome P450 family of oxidizing enzymes [8]. Ombitasvir is an antiviral medication for the treatment of hepatitis C [9] infection (HCV) due to hepatitis C virus. In the United States, it is affirmed by the Food and Drug Administration for use in the blend with paritaprevir, ritonavir and dasabuvir in Viekira Pak for the treatment of HCV genotype 1 [10] and with paritaprevir and ritonavir in Technivie for the treatment of HCV genotype 4 [11]. Paritaprevir is an acyl sulfonamide inhibitor that shows promising outcomes for the treatment of hepatitis C [12]. At the point when given in mix with ritonavir and ribavirin for 12 w, the rate of supported virological reaction at 24 w after treatment has been evaluated to be 95% for those with hepatitis C virus genotype 1 [13]. Resistance to treatment with paritaprevir is phenomenal, on the grounds that it focuses on the coupling site, however, has been believed to emerge because of transformations at positions 155 and 168 in NS3 [14]. Paritaprevir is available in three fixed-dose products: Viekira Pak (FDA), Technivie (FDA and Health Canada) and Holkira Pak (Health Canada) in Canada and the United States [15]. Different analytical methods are in like manner itemized in the written work for the estimation of ritonavir, ombitasvir and paritaprevir. As showed by composing study there is one specialized method for the estimation of ritonavir, ombitasvir and paritaprevir by RP-HPLC in tablet estimation [16, 17]. Thus, it has been proposed to make a method for estimation and endorsement of ritonavir, ombitasvir and paritaprevir in the arrangement according to the ICH rules [18].

MATERIALS AND METHODS

Instrumentation

Chromatography was performed with Alliance waters 2695 HPLC, autosampler, section stove, degasser, 2996 PDA locator and class empower-2 software.

Reagents and chemicals

Acetonitrile (HPLC grade), orthophosphoric acid (HPLC grade) and water (HPLC grade) were purchased from Merck (India) Ltd, Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of ritonavir, ombitasvir, and paritaprevir as reference standards were procured from Spectrum Pharma labs, Hyderabad, India.

Chromatographic condition

Chromatographic analysis was done using isocratic elution and by using acetonitrile and 0.01N potassium di-hydrogen phosphate, pH adjusted to 3.0 with OPA (65:35 by volume) as a mobile phase and was filtered through 0.45 µ membrane filter paper. The flow rate of mobile phase was monitored at 1 ml/min and eluents were detected at 254 nm. Operating pressure 2400 psi was maintained at room temperature by injecting the volume 10 µl with a runtime 7 min.

Preparation of standard solution

Accurately weighed 50 mg of ritonavir, 12.5 mg of ombitasvir and 75 mg of paritaprevir were taken and exchanged to three 100 ml volumetric flasks independently. 10 ml of methanol was added to flagons and sonicated for 15 min and then diluted to 1 ml of the above solution to 10 ml with the diluent.

Preparation of sample solution

5 tablets were weighed and calculated the average weight of each tablet. Then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 30 ml of diluent added and sonicated for 25

min, further, the volume made up with diluent and filtered. 1 ml of filtered sample stock solution was transferred to the 10 ml volumetric flask and made up with diluents.

Validation

The optimized chromatographic separation was aimed to obtain a resolution above 6.3 between all components, tailing factor is less than 2.0 and plate count will be more than 2000 with respect to the stationary, mobile phase compositions, flow rate, sample volume, detection wavelength and temperature.

Validation procedure

In the present method, validation was done with the aspect of system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ), forced degradation and stability according to the ICH guidelines [19, 20].

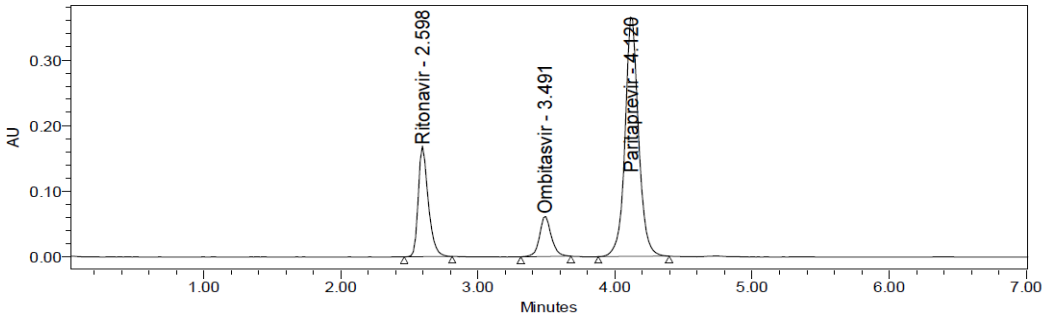

Fig. 1: Typical chromatogram for ritonavir, ombitasvir and paritaprevir

System suitability

As per the test method, the standard solutions were prepared and injected into HPLC system, from which the evaluated system suitability parameters were found to be within the limits [21, 22].

Specificity

The analyte was assessed unequivocally to know the components impurity which may be expected to be present with the help of specificity. As per test method blank was prepared and injected. No blank peak was eluted in the retention time of the analyte peak. Placebo solutions were prepared in duplicate and injected as per test method. It was found that no placebo peaks interfered at the retention time of the main peak [23].

Accuracy

Three different concentrations such as lower quantitation limit, medium quantitation limit, and higher quantitation limit were used to evaluate the accuracy of RP-HPLC method. The amount of drugs present, percentage recovery, and RSD were calculated by giving a minimum of three injections from each concentration.

Precision

The precision of test method was evaluated by considering six different concentrations. The amount of drugs present, percentage recovery, and RSD were calculated by giving a minimum of six preparations.

Linearity and range

Six series of standard solutions were selected for assessing linearity range, by using peak area versus concentration of the standard solution. Calibration curve was plotted and the regression equations were also calculated. The slope, intercept and correlation coefficient were calculated by the least squares method.

LOD and LOQ

By using optimized chromatographic conditions in accordance with 3.3 s/n and 10 s/n criteria, where s/n indicates signal-to-noise ratio, the LOD and LOQ were determined by injecting progressively lower concentrations of standard solutions into the HPLC column.

Forced degradation

In chromatogram of forced degradation there should be no interference between peaks and were well separated from each other with the resolution at least 1.0 and peak purity of the principal peaks should pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

Robustness

Small changes such as±10 % in the ratio of acetonitrile in the mobile phase,±0.1 ml/min in the flow rate and±5 °C in the temperature were made to demonstrate the robustness method. The separation factor, retention time and peak asymmetry were calculated.

Stability

Standard and the sample solutions were subjected to 24 h stability studies. The stability of these solutions was studied and observed for changes in the area and retention time of the peaks which were then compared with pattern of chromatogram of freshly prepared solution.

Statistical analysis

Wherever applicable, results were expressed as the mean±SD, % RSD and data were analyzed statistically by using t-test with aid of Microsoft Excel-2007 software and data was considered not significantly different at 5 % significance level of probability P ≤0.05**.**

RESULTS AND DISCUSSION

Method development

Initially, reverse phase liquid chromatography separation was tried to develop using various ratios of methanol and water, acetonitrile and water as mobile phases, in which drugs did not respond properly, and the resolution was also poor. The organic content of the mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes an important factor. Hypersil BDS 250 mm x 4.6 mm, i.e. 5 µm□with an isocratic mobile phase composed of 0.01N KH_2PO_4 buffer and acetonitrile (65:35A) at a flow rate of 1 ml/min. The column temperature was maintained at 30 °C and the detection was carried out using a PDA detector at 254 nm. The tailing of both peaks was reduced considerably and brought close to 1. Drug detections were tried at wavelength 254 nm. Ritonavir, ombitasvir and paritaprevir showed maximum absorption at 254 nm of wavelength and 254 nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.598 min, 3.491 min and 4.120 min for ritonavir, ombitasvir and paritaprevir. The chromatogram obtained was shown in the fig. 1

Method validation

System suitability and Specificity

10 µl of working standard solution (ritonavir 50 µg/ml, ombitasvir 12.5 µg/ml and paritaprevir 75 µg/ml) was prepared and injected into the system. It was determined by making six replicate injections and all the parameters were found to be within the limits. The results were given in table 1.

Table 1: System suitability parameters for ritonavir, ombitasvir and paritaprevir

S. No.	Ritonavir			Ombitasvir			Paritaprevir		
Inj	Rt(min)	Tp	Tailing	Rt(min)	Tp	Tailing	Rt(min)	Tp	Tailing
1	2.568	6022	1.32	3.484	8244	1.11	4.104	8981	1.06
2	2.571	6105	1.32	3.484	8272	1.09	4.106	9054	1.06
3	2.574	6272	1.32	3.485	8284	1.09	4.107	9075	1.06
4	2.581	6059	1.33	3.486	8706	1.09	4.111	9280	1.06
5	2.588	6226	1.33	3.491	8432	1.05	4.117	9024	1.06
6	2.598	5995	1.32	3.491	8461	1.1	4.12	8911	1.05

Linearity

The calibration curve was linear in the range of 12.5-75 µg/ml for ritonavir, 3.125-18.75 µg/ml for ombitasvir and 18.75-112.5 µg/ml for paritaprevir. These were represented in linear regression equation by as follows: y = 16942. x+543.0(R^2=0.999) for ritonavir, y=29239.x+581.5(R_2=0.999) for ombitasvir y= 33194.x+605.2 R_2=0.999) for paritaprevir and a regression line was established by the least squares method and correlation coefficient (R^2) for ritonavir, ombitasvir, and paritaprevir was found to be greater than 0.98. Hence the curves established were linear. The results were given in table 2.

Table 2: Linearity data for ritonavir, ombitasvir and paritaprevir

Ritonavir		Ombitasvir		Paritaprevir	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
12.5	207143	3.125	93230	18.75	621771
25	434680	6.25	185929	37.5	1252558
37.5	632715	9.375	269148	56.25	1865441
50	849226	12.5	365988	75	2474466
62.5	1052389	15.625	460995	93.75	3132183
75	1274858	18.75	547621	112.5	3728106
Corr Coef	0.999	Corr Coef	0.999	Corr Coef	0.999
Slope	16942	Slope	29239	Slope	33194
Intercept	543.0	Intercept	581.5	Intercept	605.2

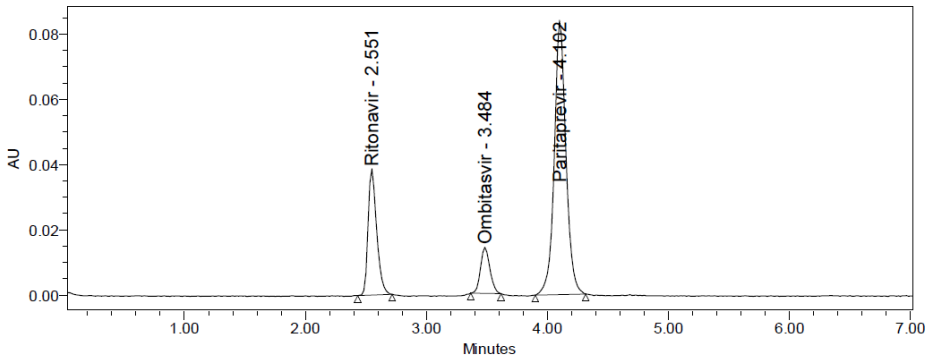

Fig. 2: Chromatogram for linearity-1

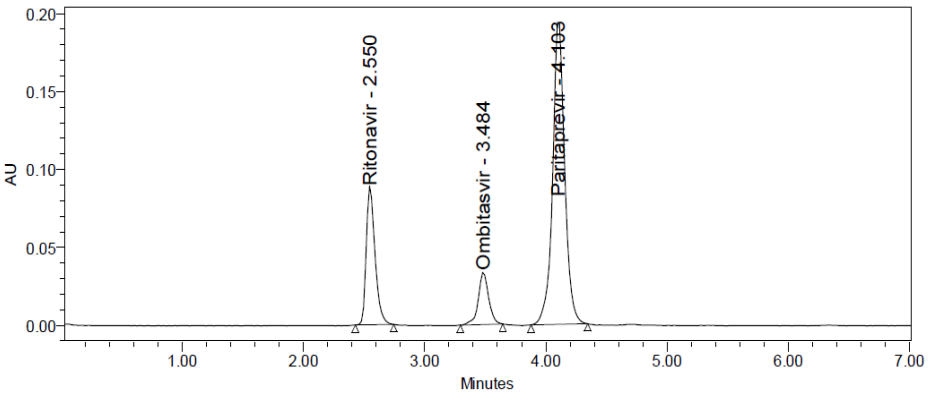

Fig. 3: Chromatogram for linearity-2

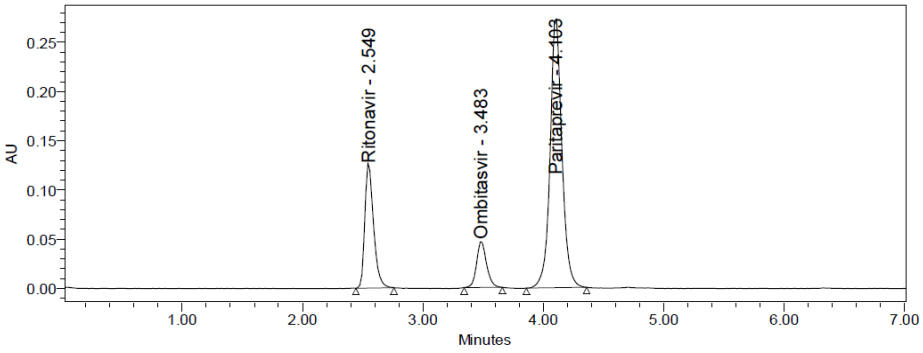

Fig. 4: Chromatogram for linearity-3

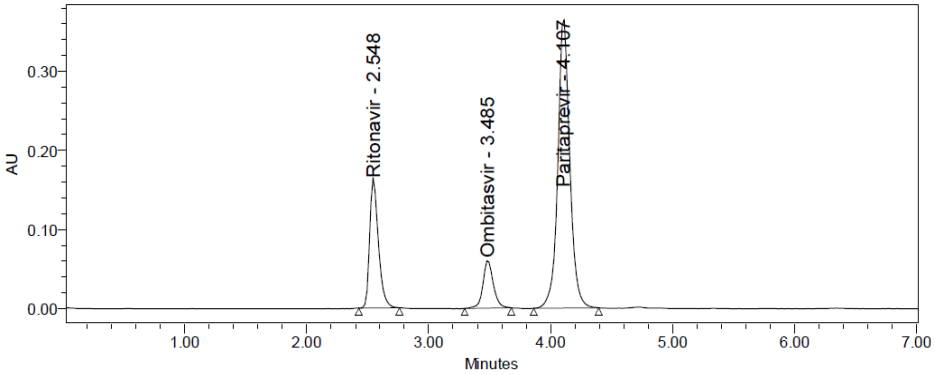

Fig. 5: Chromatogram for linearity-4

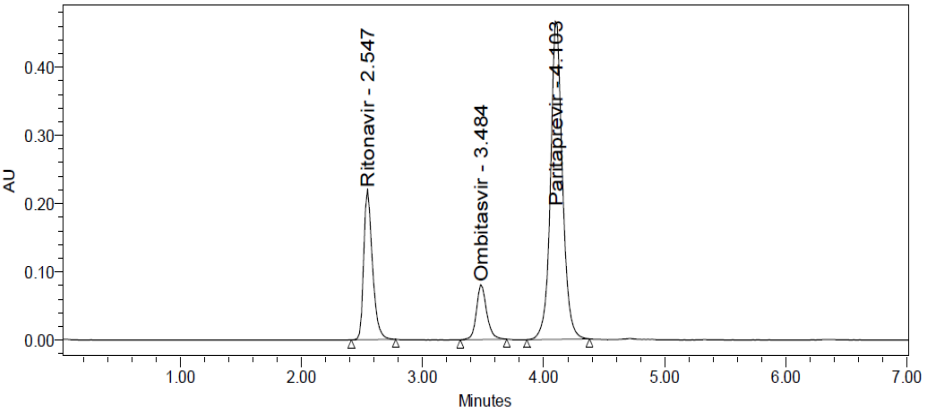

Fig. 6: Chromatogram for linearity-5

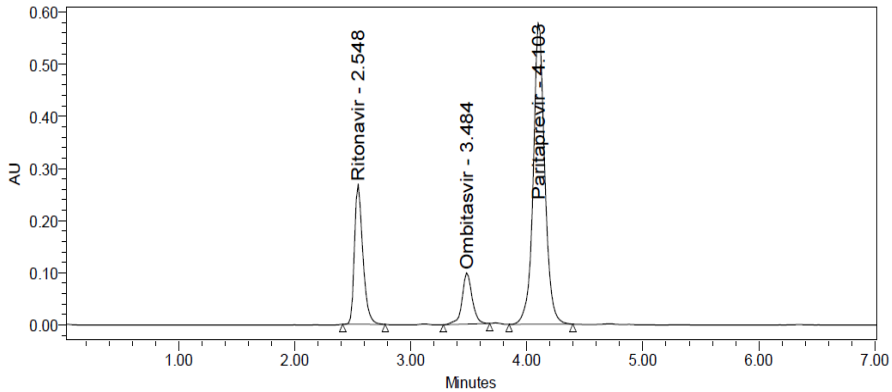

Fig. 7: Chromatogram for linearity-6

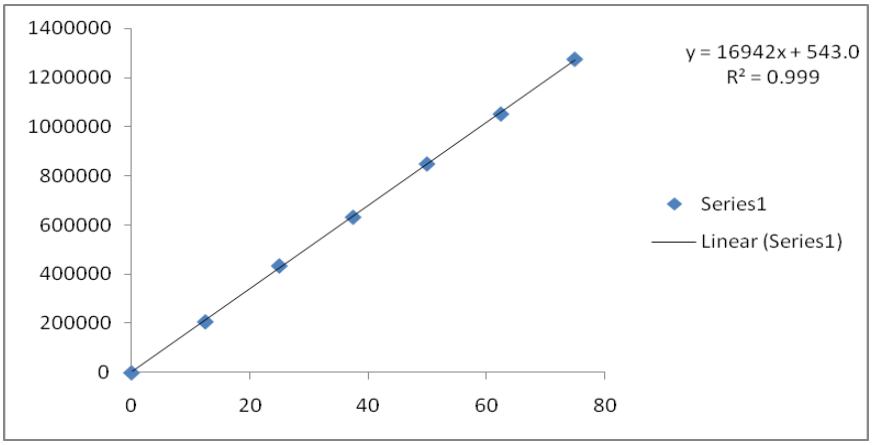

Fig. 8: Linearity plot for ritonavir

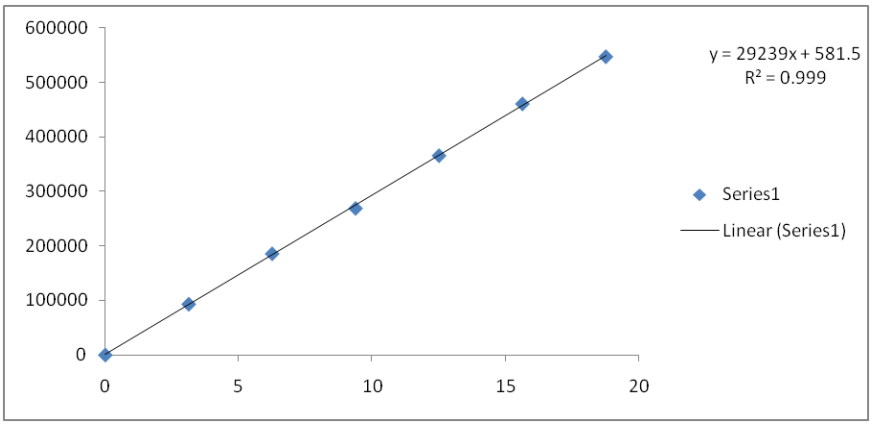

Fig. 9: Linearity plot for ombitasvir

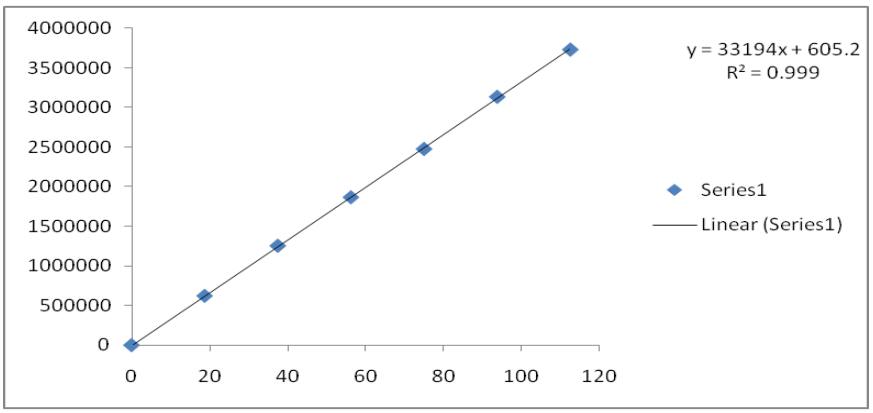

Fig. 10: Linearity plot for paritaprevir, X-axis = concentration, Y-axis = peak area

Table 3: Accuracy data for ritonavir

% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	Area counts	% Recovery	Mean±SD
50%	25	24.78	1267509	99.13	99.72,
	25	25.17	1274083	100.68	0.84
	25	24.84	1268467	99.36	
100%	50	50.48	1702847	100.96	100.18,
	50	49.85	1692222	99.70	0.68
	50	49.94	1693777	99.89	
150%	75	74.37	2107589	99.16	99.54,
	75	74.71	2113333	99.61	0.36
	75	74.89	2116459	99.86	

#SD: Standard deviation, result expressed in mean±SD and n=3

Table 4: Accuracy data for ombitasvir

% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	Area counts	% Recovery	Mean±SD
50%	6.25	6.273	549483	100.37	99.95,
	6.25	6.244	548638	99.90	0.39
	6.25	6.224	548057	99.59	
100%	12.5	12.573	733683	100.58	100.33,
	12.5	12.606	734655	100.85	0.68
	12.5	12.445	729940	99.56	
150%	18.75	18.807	915981	100.31	99.80,
	18.75	18.754	914411	100.02	0.64
	18.75	18.579	909292	99.09	

#SD: Standard deviation, result expressed in mean±SD and n=3

Table 5: Accuracy data for paritaprevir

% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	Area counts	% Recovery	Mean±SD
50%	37.5	37.92	3748935	101.13	100.06
	37.5	37.36	3730192	99.62	0.92
	37.5	37.29	3728023	99.45	
100%	75	74.84	4974242	99.78	100.15,
	75	75.16	4985115	100.22	0.34
	75	75.34	4990947	100.45	
150%	112.5	111.57	6193620	99.17	99.66,
	112.5	112.74	6232462	100.21	0.52
	112.5	112.05	6209539	99.60	

#SD: Standard deviation, result expressed in mean±SD and n=3

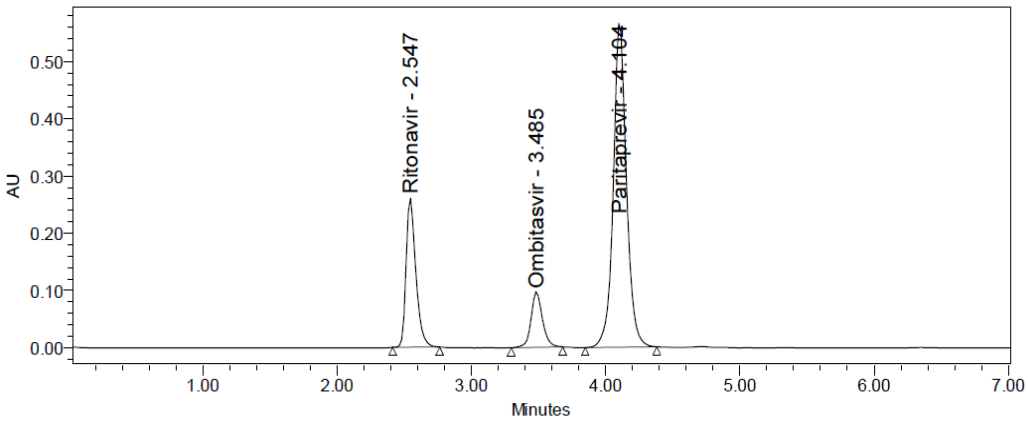

Fig. 11: Chromatogram for accuracy 50%-1

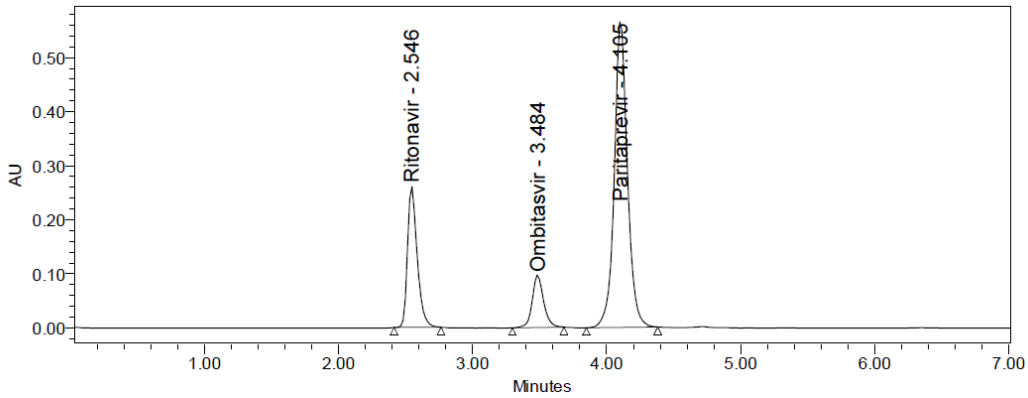

Fig. 12: Chromatogram for accuracy 50%-2

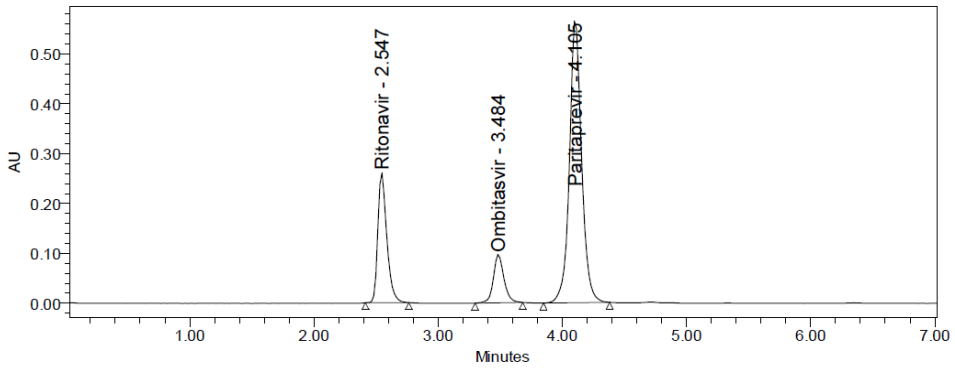

Fig. 13: Chromatogram for accuracy 50%-3

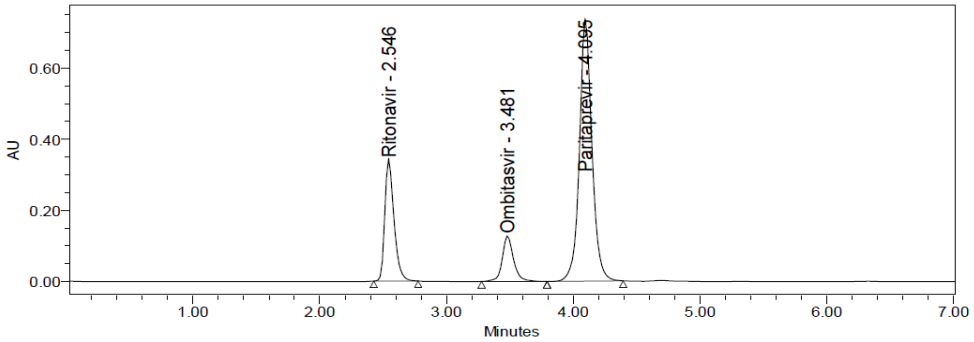

Fig. 14: Chromatogram for accuracy 100%-1

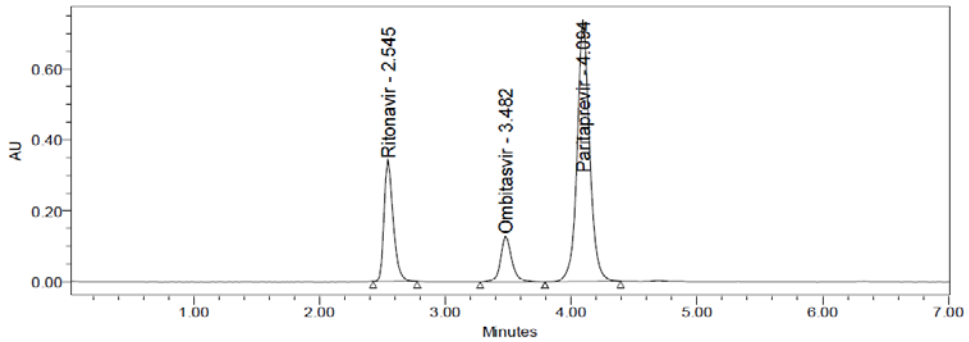

Fig. 15: Chromatogram for accuracy 100%-2

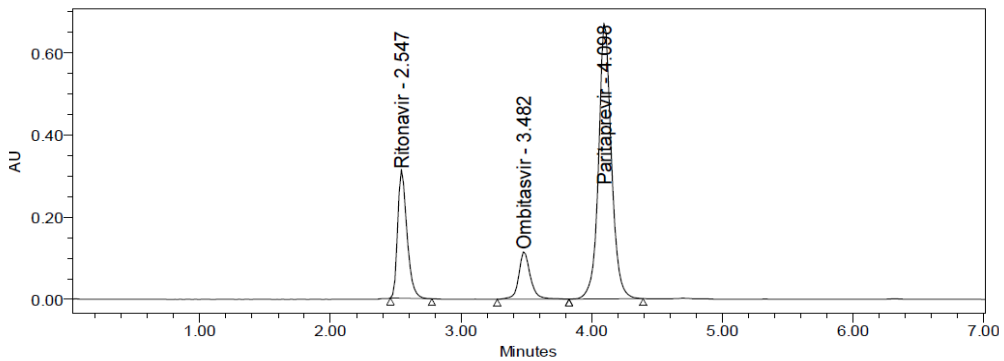

Fig. 16: Chromatogram for accuracy 100%-3

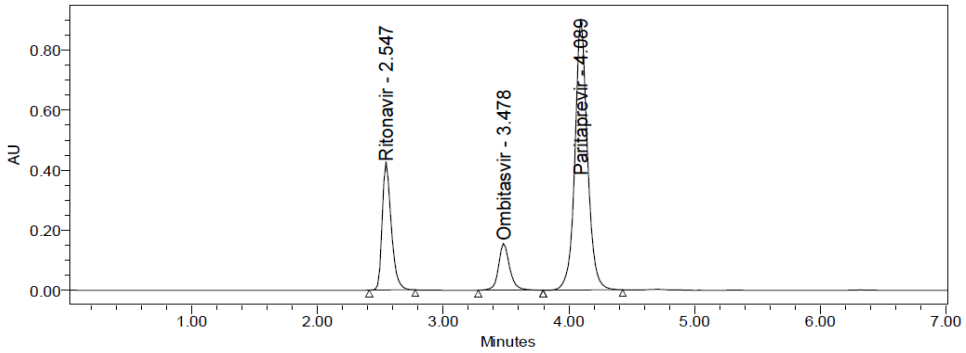

Fig. 17: Chromatogram for accuracy 150%-1

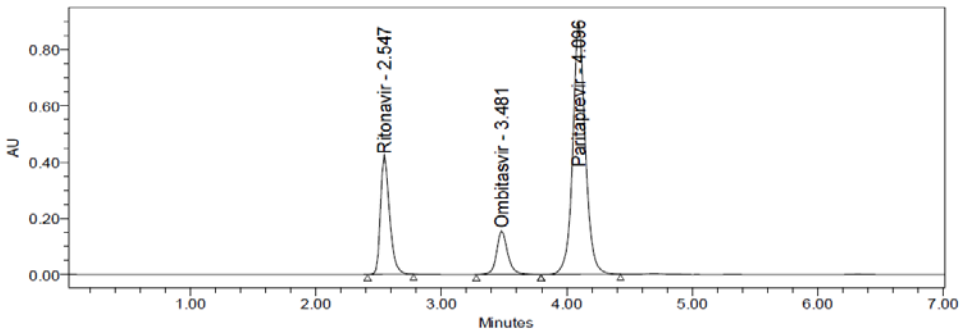

Fig. 18: Chromatogram for accuracy 150%-2

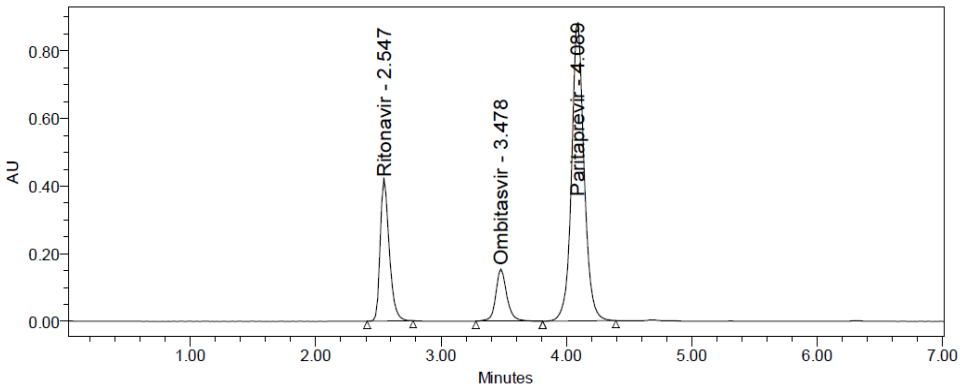

Fig. 19: Chromatogram for accuracy 150%-3

Table 6: Repeatability data for ritonavir, ombitasvir and paritaprevir

S. No.	Area of ritonavir n=6	Area of ombitasvir n=6	Area of paritaprevir n=6
1.	853526	367465	2520129
2.	863014	363235	2514709
3.	862364	364103	2578495
4.	851521	366719	2514428
5.	856136	363687	2508742
6.	852367	363628	2502558
Mean	856488	364806	2523177
SD	5053.1	1807.3	27753.0
%RSD	0.6	0.5	1.1

#n: number of injections (n=6), # %RSD: percent relative standard deviation

Accuracy

These results were within the acceptable limit of 98-102. The % RSD for ritonavir, ombitasvir and paritaprevir were 0.7, 1.0 and 0.6 and it is within the limit of≤2, hence the pr oposed method was accurate and the results were summarized in table 3, 4 and 5.

Precision

Repeatability

The % RSD found to be 0.6, 0.5 and 1.1 respectively, the obtained results were within an acceptable limit of≤2 and hence this m e-thod was reproducible and the results were shown in table 6.

Table 7: Intermediate precision data for ritonavir, ombitasvir and paritaprevir

S. No.	Area of ritonavir n=6	Area of ombitasvir n=6	Area of paritaprevir n=6
1.	848671	358194	2506847
2.	857139	357744	2509322
3.	847451	357290	2507333
4.	848792	353484	2464440
5.	852392	353117	2494836
6.	850073	354121	2489685
Mean	850753	355658	2495411
SD	3550.0	2323.2	17080.7
%RSD	0.4	0.7	0.7

#n: number of injections (n=6), # %RSD: percent relative standard deviation

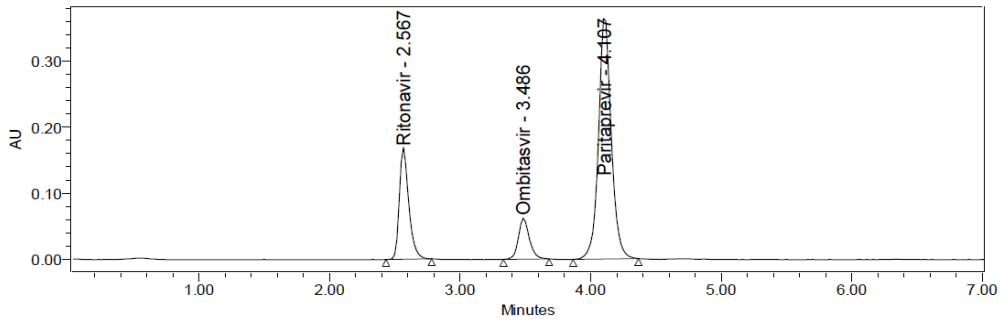

Fig. 20: Chromatogram for method precision-1

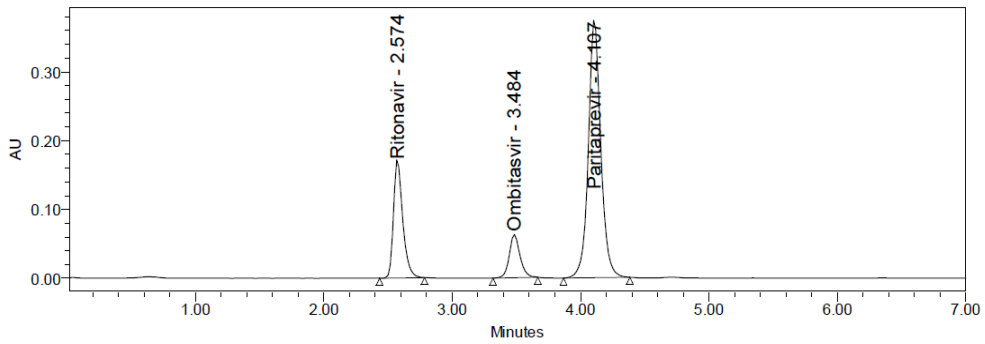

Fig. 21: Chromatogram for method precision-2

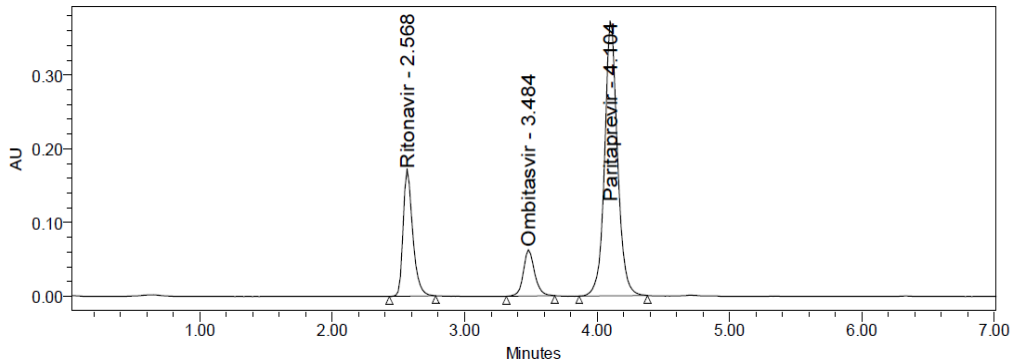

Fig. 22: Chromatogram for method precision-3

Intermediate precision

The % RSD for ritonavir, ombitasvir and paritaprevir were found to be 0.4, 0.7 and 0.7 and it was within an acceptable limit of ≤2.

Hence the method is reproducible on different days with different analyst and column. This indicates that the method was precise and the results were as shown in table 7.

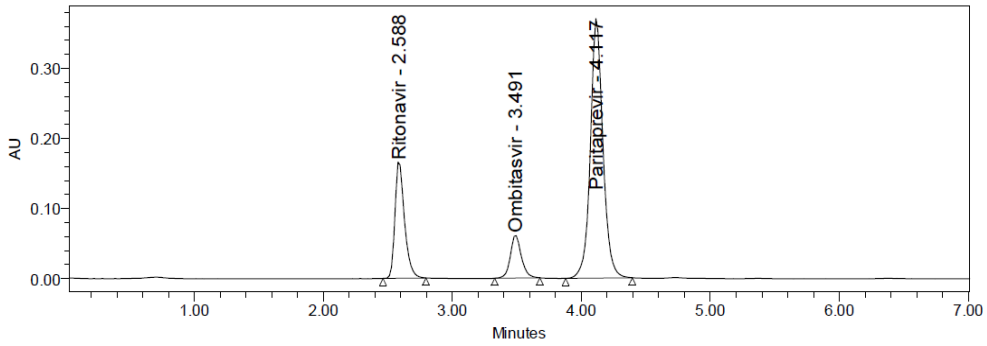

Fig. 23: Chromatogram for method precision-4

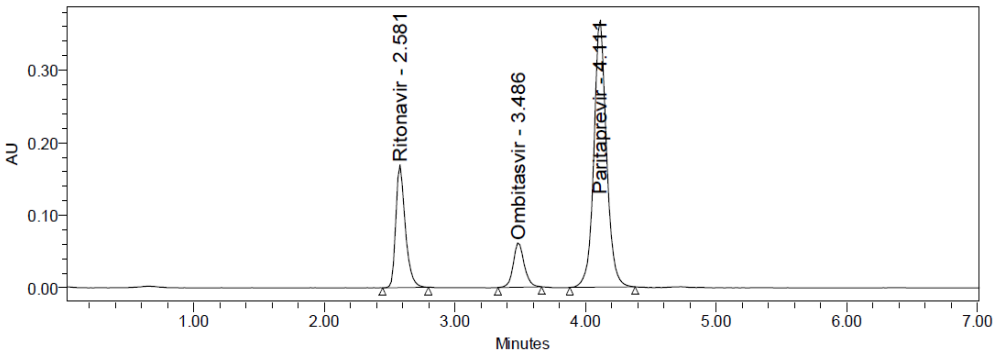

Fig. 24: Chromatogram for method precision-5

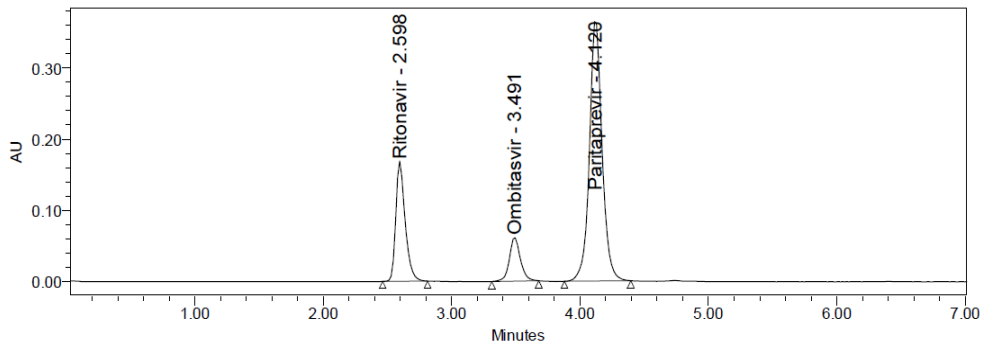

Fig. 25: Chromatogram for method precision-6

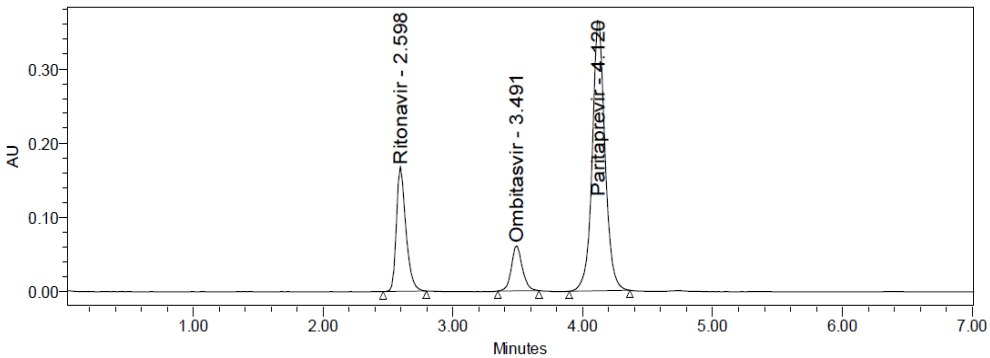

Fig. 26: Chromatogram for intermediate precision-1

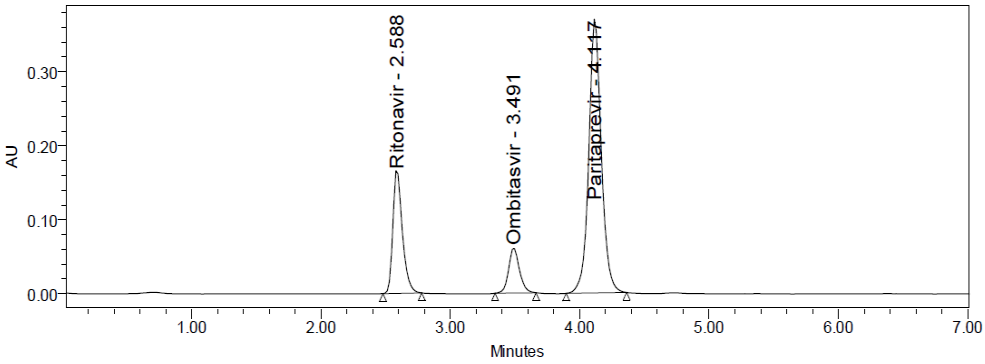

Fig. 27: Chromatogram for intermediate precision-2

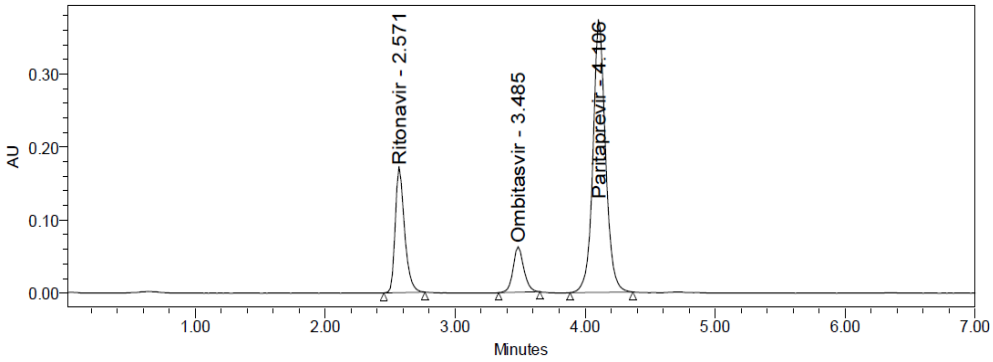

Fig. 28: Chromatogram for intermediate precision-3

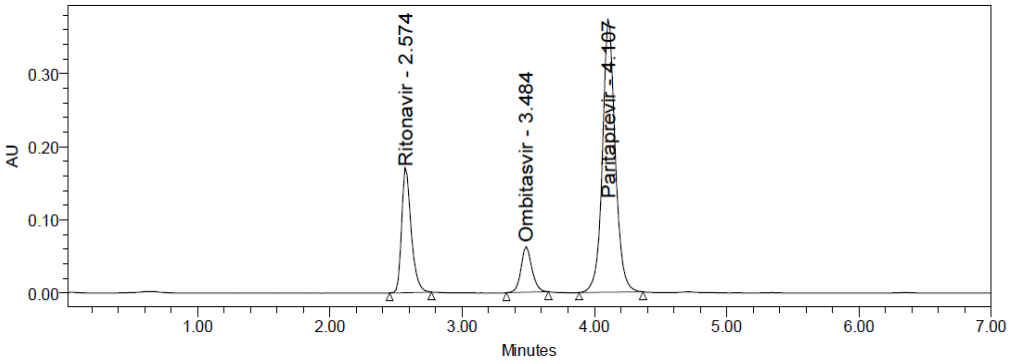

Fig. 29: Chromatogram for intermediate precision-4

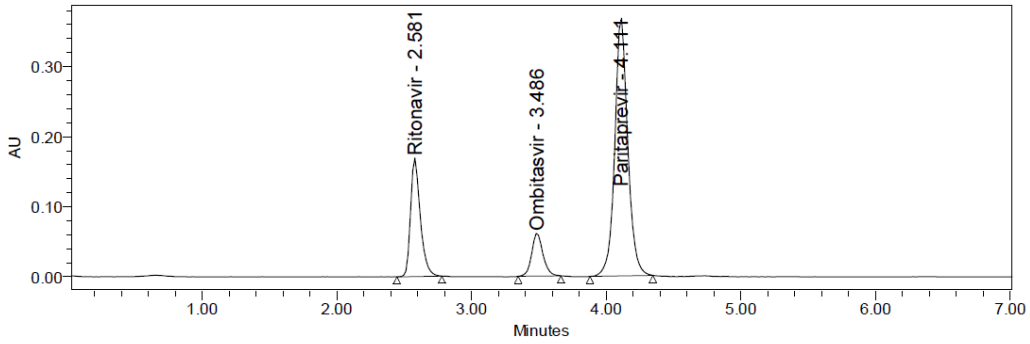

Fig. 30: Chromatogram for intermediate precision-5

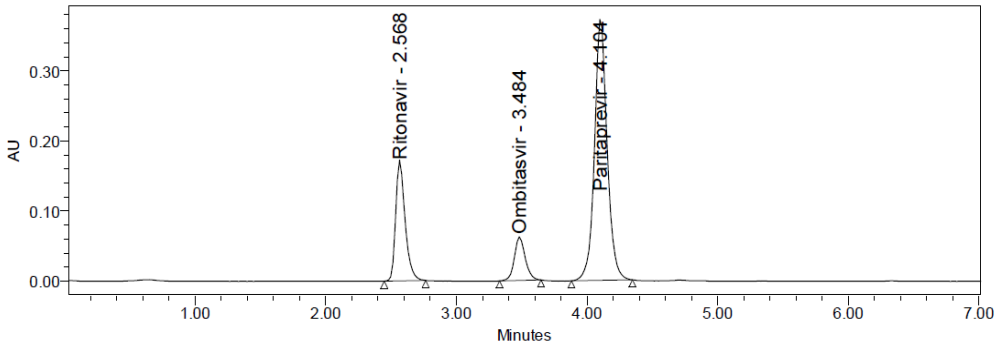

Fig. 31: Chromatogram for intermediate precision-6

LOD and LOQ

LOD and LOQ for ritonavir, ombitasvir and paritaprevir were 0.02, 0.019and 0.02 µg/ml and 0.07, 0.06 and 0.07 µg/ml respectively. The lowest value of LOD and LOQ as obtained by the proposed method indicates that the method was sensitive [24].

Table 8: Results of LOD and LOQ

Drug	LOD(µg/ml)	LOQ(µg/ml)
Ritonavir	0.02 µg/ml	0.07 µg/ml
Ombitasvir	0.019 µg/ml	0.06µg/ml
Paritaprevir	0.02 µg/ml	0.07 µg/ml

#LOD: limit of detection, # LOQ: limit of quantization

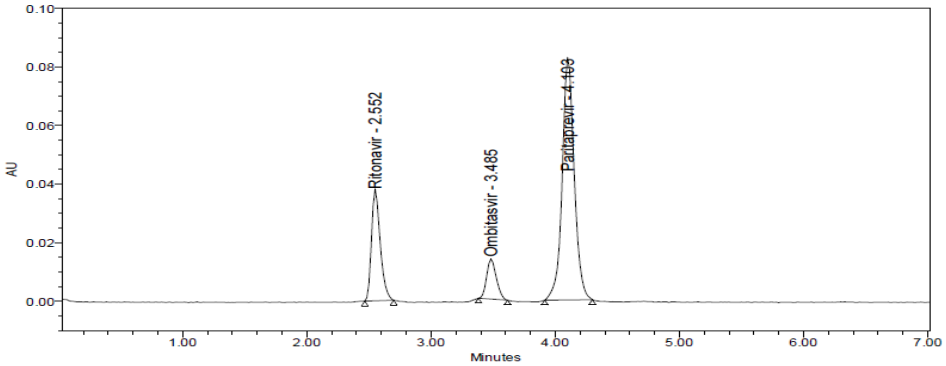

Fig. 32: Chromatogram for LOD

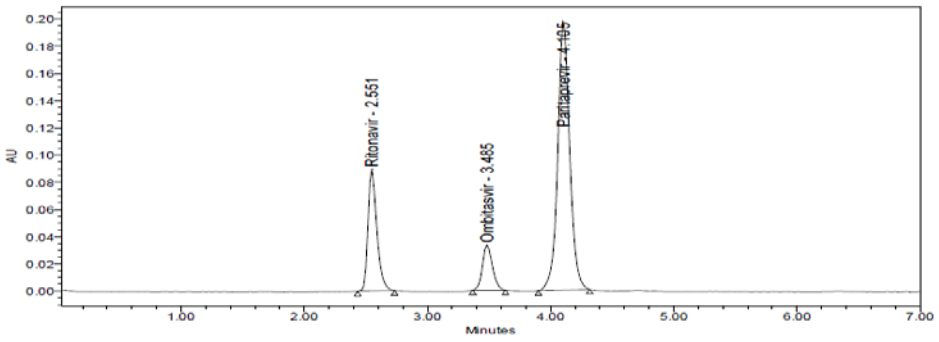

Fig. 33: Chromatogram for LOQ

Degradation studies

The degradation studies for ritonavir, ombitasvir and paritaprevir were performed by various conditions like acid, alkali, oxidation, thermal photolytic and neutral degradation and their limits like purity angle and purity threshold values were mentioned. It is observed that the purity angle<purity threshold and the results were shown in table 9, 10 and 11.

Oxidation

To 1 ml of stock solution of ritonavir, ombitasvir, and paritaprevir, 1 ml of 20 % hydrogen peroxide was added separately. The solutions were kept for 30 min at 60 °C. For HPLC study, the resultant solution was diluted to obtain 50 µg/ml, 12.5 µg/ml and 75 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Acid degradation studies

1 ml of 2N Hydrochloric acid was added to 1 ml of stock solution of ritonavir, ombitasvir and paritaprevir. Then it was refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 50 µg/ml, 12.5 µg/ml and 75 µg/ml solutions and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Alkali degradation studies

To 1 ml of stock solution of ritonavir, ombitasvir and paritaprevir, 1 ml of 2N sodium hydroxide was added and it was refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 50 µg/ml, 12.5 µg/ml and 75 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to know the stability of the sample.

Dry heat degradation studies

The standard drug solution was placed in an oven at 105 °C for 6 h to study dry heat degradation. For HPLC study, the resultant solutions was diluted to 150 µg/ml, 12.5 µg/ml and 75 µg/ml solution and10 µl were injected into the system and the chromatograms were recorded to measure the stability of the sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 500 µg/ml, 125 µg/ml and 750 µg/ml solutions to UV light by keeping the beaker in UV Chamber for 7days or 200 Watt-hours/m^2 in photostability chamber. For HPLC study, the resultant solution was diluted to obtain 50 µg/ml, 12.5 µg/ml and 75 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded in order to the stability of the sample.

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60 °C. For HPLC study, the resultant solution was diluted to 50 µg/ml, 12.5 µg/ml and 75 µg/ml solutions and 10 µl were injected into the system and to assess the stability of the sample, the chromatograms were recorded.

Table 9: Results of forced degradation studies of ritonavir

S. No.	Degradation condition	% Drug degraded	Purity angle	Purity threshold
1	Acid	4.00	0.199	0.346
2	Alkali	2.58	0.165	0.310
3	Oxidation	2.70	0.165	0.310
4	Thermal	1.91	0.184	0.316
5	UV	1.28	0.195	0.311
6	Water	0.26	0.165	0.310

Table 10: Results of forced degradation studies of ombitasvir

S. No.	Degradation condition	% Drug degraded	Purity angle	Purity threshold
1	Acid	4.12	0.209	0.361
2	Alkali	3.31	0.253	0.321
3	Oxidation	3.26	0.253	0.321
4	Thermal	2.33	0.259	0.345
5	UV	1.87	0.175	0.327
6	Water	0.56	0.253	0.321

Table 11: Results of forced degradation studies of paritaprevir

S. No.	Degradation condition	% Drug degraded	Purity angle	Purity threshold
1	Acid	3.97	0.103	0.303
2	Alkali	2.81	0.106	0.302
3	Oxidation	2.86	0.106	0.302
4	Thermal	2.09	0.109	0.305
5	UV	1.41	0.104	0.305
6	Water	0.42	0.106	0.302

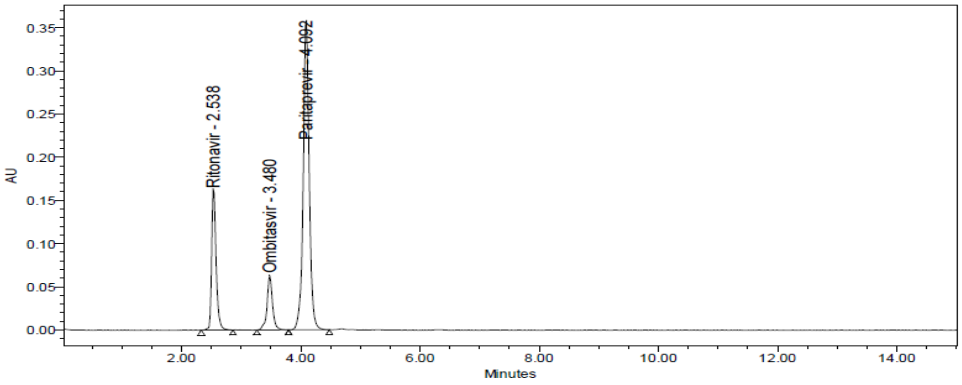

Fig. 34: Chromatogram for acid degradation

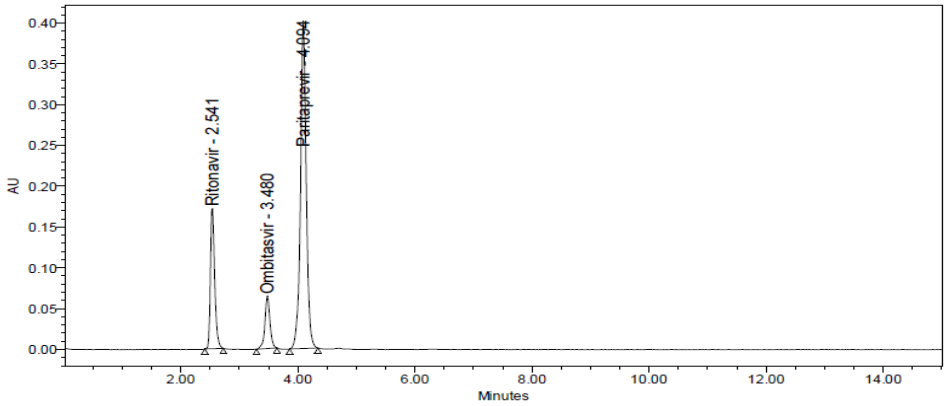

Fig. 35: Chromatogram for base degradation

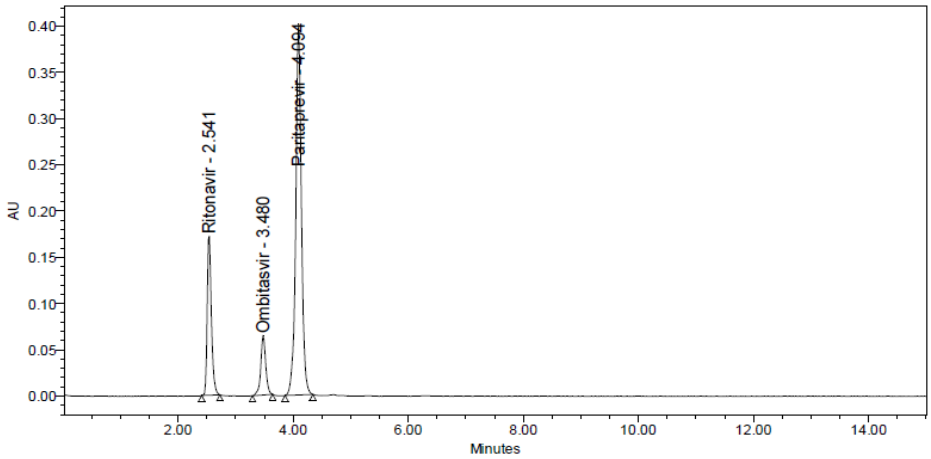

Fig. 36: Chromatogram for peroxide degradation

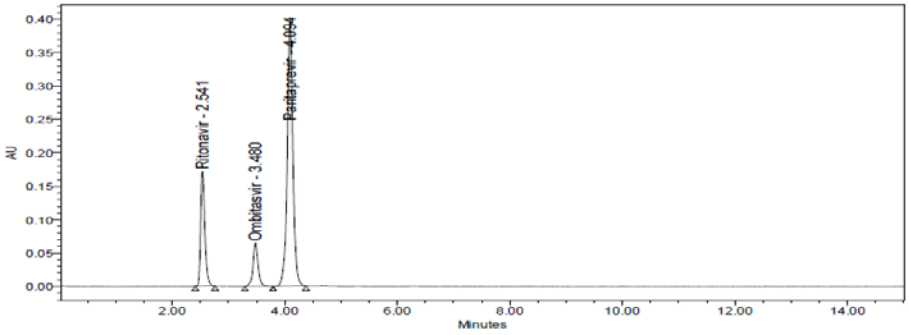

Fig. 37: Chromatogram for thermal degradation

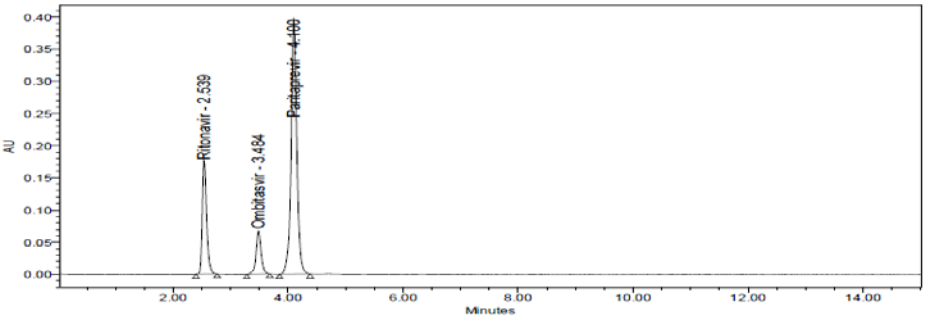

Fig. 38: Chromatogram for photolytic degradation

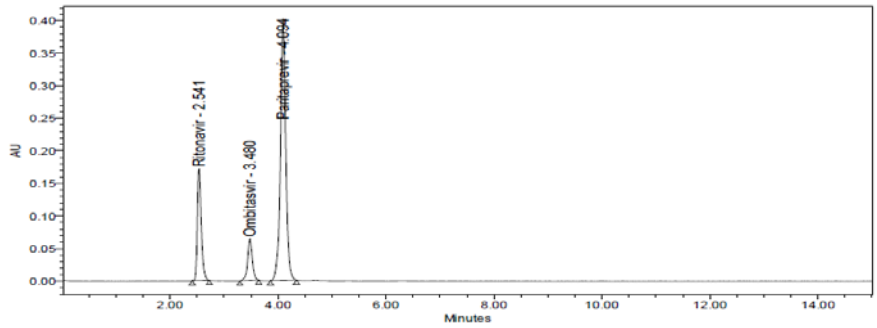

Fig. 39: Chromatogram for hydrolysis degradation

Robustness

It was observed that there was no marked change in mean Rt and % RSD was within a limit of ≤2. The tailing factor, resolution factor and no. of theoretical plates were found to be in acceptable limits for ritonavir, ombitasvir and paritaprevir. Hence this method was reliable with variations in the analytical conditions and the results of ritonavir, ombitasvir and paritaprevir were shown in table 12.

Table 12: Results for robustness

S. No.	Condition	% RSD of ritonavir	% RSD of ombitasvir	% RSD of paritaprevir
1	Flow rate (-) 0.9 ml/min	1.5	0.87	1.5
2	Flow rate (+) 1.1 ml/min	0.2	1.4	0.1
3	Mobile phase (-) 33B: 67A	0.7	0.79	0.6
4	Mobile phase (+) 27B: 73A	1.0	1.0	1.1
5	Temperature (-) 25 °C	1.1	1.3	1.1
6	Temperature (+) 35 °C	1.2	0.64	0.6

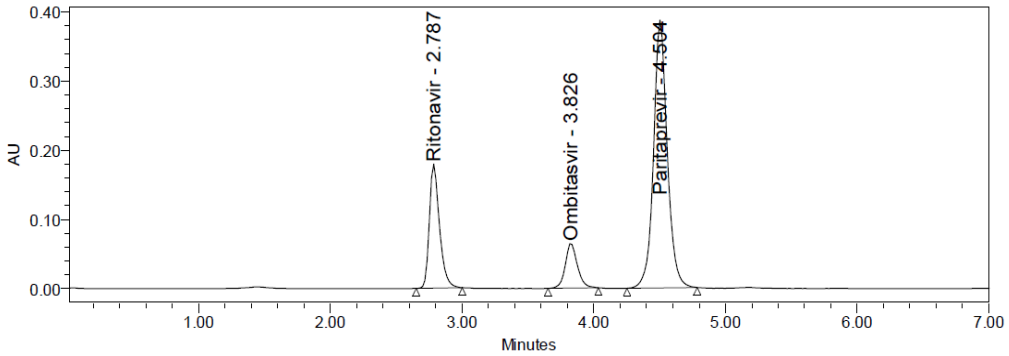

Fig. 40: Chromatogram for flow min

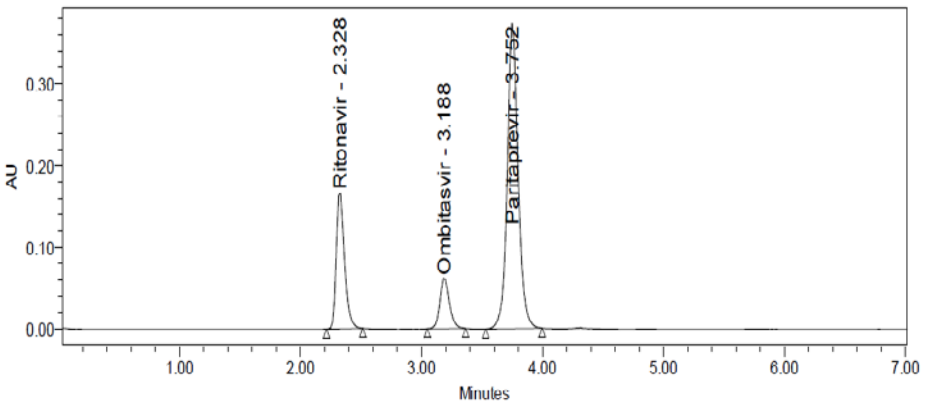

Fig. 41: Chromatogram for flow plus

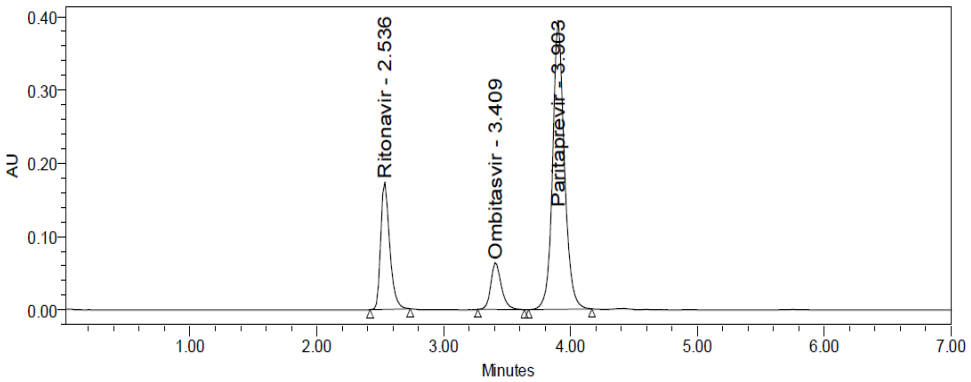

Fig. 42: Chromatogram for organic phase min

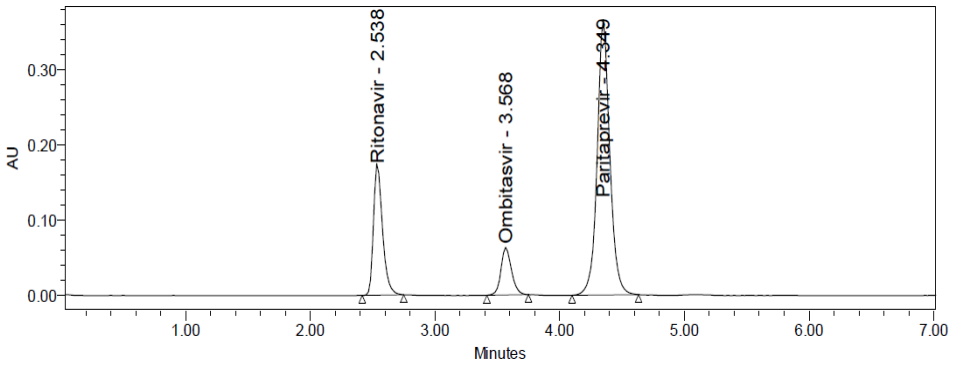

Fig. 43: Chromatogram for organic phase plus

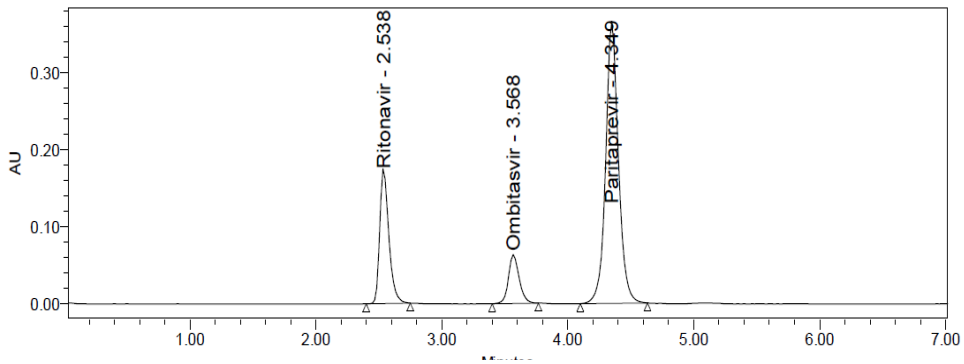

Fig. 44: Chromatogram for temperature min

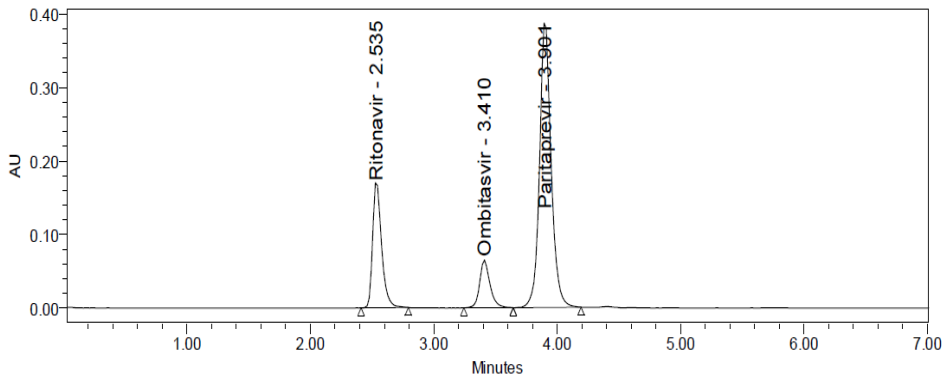

Fig. 45: Chromatogram for temperature plus

Solution stability

Sample solutions were analyzed initially for 24 h at different intervals of time at room temperature and the results were recorded. The % deviation should not be more than 5.0 %.

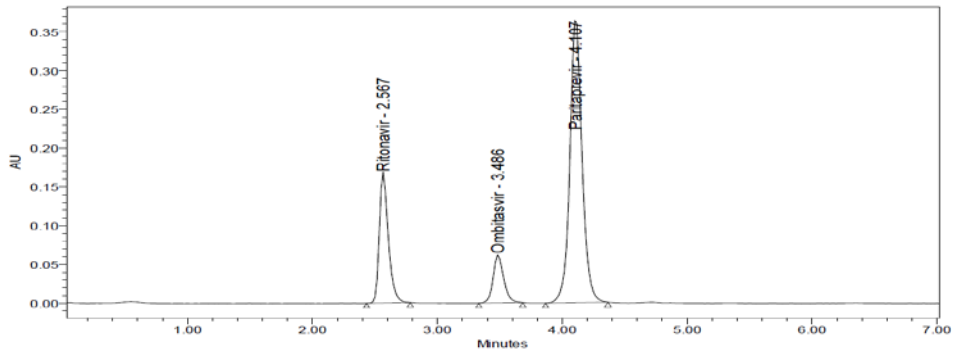

Fig. 46: Chromatogram for stability initial

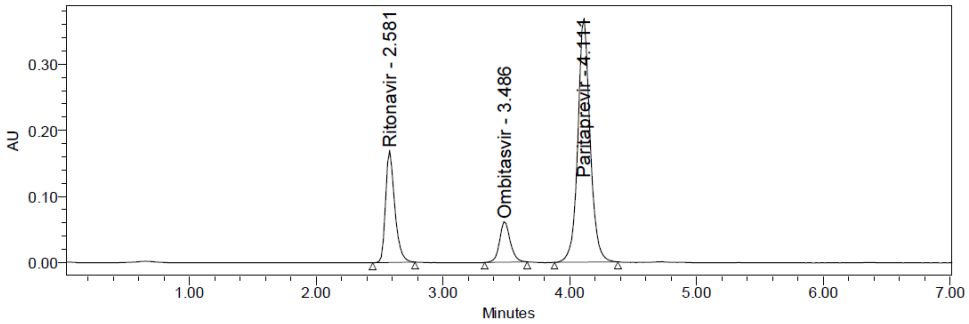

Fig. 47: Chromatogram for stability 24 h

CONCLUSION

Stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of ritonavir, ombitasvir and paritaprevir in pharmaceutical formulations as per ICH guidelines. The developed method was found to be accurate, precise and reliable with % RSD less than 2 %. Therefore, the developed method was simple, accurate, precise and robust. The present method was found to be stability indicating as the degradation of the drug substance was between 0.25-5 percent. Finally, this method can be used for better analysis of pharmaceutical formulations of ritonavir, ombitasvir and paritaprevir.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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