INTRODUCTION

Ritonavir, [1] is chemically known as 2,4,7,12-tetra azatriazine-13-oxacid, 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 ordi-
narily 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 rito-
navir, 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 flags and sonicated for 15 min and then diluted to 1 ml of the above solu-
tion 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 6.5 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].

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 KH₂PO₄ 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 | 60.22 | 1.32 | 3.484 | 82.44 | 1.11 | 4.104 | 89.81 | 1.06 |
| 2      | 2.571 | 61.05 | 1.32 | 3.484 | 82.72 | 1.09 | 4.106 | 90.54 | 1.06 |
| 3      | 2.574 | 62.72 | 1.32 | 3.485 | 82.84 | 1.09 | 4.107 | 90.75 | 1.06 |
| 4      | 2.581 | 60.59 | 1.33 | 3.486 | 87.06 | 1.09 | 4.111 | 92.80 | 1.06 |
| 5      | 2.588 | 62.26 | 1.33 | 3.491 | 84.32 | 1.05 | 4.117 | 90.24 | 1.06 |
| 6      | 2.598 | 59.95 | 1.32 | 3.491 | 84.61 | 1.1  | 4.12  | 89.11 | 1.05 |

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: 

\[ y = 1694.2 \times x + 543.0 \quad (R^2 = 0.999) \] for ritonavir,

\[ y = 2923.9 \times x + 581.5 \quad (R^2 = 0.999) \] for ombitasvir,

\[ y = 3319.4 \times x + 605.2 \quad (R^2 = 0.999) \] for paritaprevir.

Hence the curves established were linear. The results were given in table 2.

Table 2: Linearity data for ritonavir, ombitasvir and paritaprevir

| Concentration (μg/ml) | Peak area (Ritonavir) | Peak area (Ombitasvir) | Peak area (Paritaprevir) |
|-----------------------|-----------------------|------------------------|--------------------------|
| 12.5                  | 207143                | 3.125                  | 18.75                    |
| 25                    | 434680                | 6.25                   | 37.5                     |
| 37.5                  | 632715                | 9.375                  | 56.25                    |
| 50                    | 849226                | 12.5                   | 75                       |
| 62.5                  | 1052389               | 15.625                 | 93.75                    |
| 75                    | 1274858               | 18.75                  | 112.5                    |
| Corr Coef             | 0.999                 | 0.999                  | 0.999                    |
| Slope                 | 1694.2                | 3.484                  | 581.5                    |
| Intercept             | 543.0                 | 82.44                  | 1.06                     |

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
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, 0.84 |
|         | 25                    | 25.17                    | 1274083     | 100.68     | 100.18, 0.84 |
|         | 25                    | 24.84                    | 1268467     | 99.36      | 99.54, 0.36 |
| 100%    | 50                    | 50.48                    | 1702847     | 99.70      | 99.86   |
|         | 50                    | 49.85                    | 1692222     | 99.96      | 99.96, 0.36 |
|         | 50                    | 49.94                    | 1693777     | 99.96      | 99.96, 0.36 |
| 150%    | 75                    | 74.37                    | 2107589     | 99.16      | 99.54, 0.36 |
|         | 75                    | 74.71                    | 2113333     | 99.61      | 99.86   |
|         | 75                    | 74.89                    | 2116459     |             |         |

*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, 0.39 |
|         | 6.25                  | 6.244                    | 548638      | 99.90      |          |
|         | 6.25                  | 6.224                    | 548057      | 99.59      |          |
| 100%    | 12.5                  | 12.573                   | 733683      | 100.58     | 100.33, 0.68 |
|         | 12.5                  | 12.606                   | 734655      | 100.38     |          |
|         | 12.5                  | 12.245                   | 729940      | 99.56      |          |
| 150%    | 18.75                 | 18.807                   | 915981      | 100.31     | 99.80, 0.64 |
|         | 18.75                 | 18.754                   | 914411      | 100.02     |          |
|         | 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                    | 3740935     | 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, 0.34 |
|         | 75                    | 75.16                    | 4985115     | 100.22     | 0.34    |
|         | 75                    | 75.34                    | 4990947     | 100.45     |          |
| 150%    | 112.5                 | 111.57                   | 6193620     | 99.17      | 99.66, 0.52 |
|         | 112.5                 | 112.74                   | 6232462     | 100.21     |          |
|         | 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
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 proposed 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 method 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                 | 250647                  |
| 2.     | 857139                | 357744                 | 250932                  |
| 3.     | 847451                | 357290                 | 250733                  |
| 4.     | 848792                | 353484                 | 246444                  |
| 5.     | 852392                | 353117                 | 249483                  |
| 6.     | 850073                | 354121                 | 248968                  |
| Mean   | 850753                | 355658                 | 249541                  |
| 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

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
LOD and LOQ

LOD and LOQ for ritonavir, ombitasvir and paritaprevir were 0.02, 0.019 and 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].

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

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

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.

Alkaline 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 7 days or 200 Watt-hours/m² 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 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
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. 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 inter-
vals of time at room temperature and the results were recorded. The % deviation should not be more than 5.0 %.

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 authors have contributed equally

CONFLICT OF INTERESTS
Declared none

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