**Journal of Neonatal Surgery**
*ISSN(Online): 2226-0439*
*Vol. 14, Issue 27s (2025)*
https://www.jneonatalsurg.com

# Analytical Method Development and Validation of Stability Indicating Rp-Hplc Method for The Simultaneous Estimation of Ombitasvir, Paritaprevir and Ritonavir

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Cite this paper as: G.Sai sri lakshmi, Somasundaram I, (2025) Analytical Method Development and Validation of Stability Indicating Rp-Hplc Method for The Simultaneous Estimation of Ombitasvir, Paritaprevir and Ritonavir, *Journal of Neonatal Surgery*, 14 (27s), 420-427

## ABSTRACT

Special, effective high pressure liquid chromatography method has been developed for the simultaneous quantification of Ombitasvir and Paritaprevir.By using Waters HPLC e-2695 quaternary pump with a PDA detector of 2998 instrument the chromatographic separation of Ombitasvir,Paritaprevir and Ritonavir was achieved on the column of X-bridge phenyl150x4.6mm, 3.5µ using an isocratic elution with a buffer containing 0.1percenttriethyl amine and acetonitrile at a rate of 50:50 as a mobile phase with a flow rate of 1 ml/min at ambient temperature. A detector wavelength of 254 nm utilizing the PDA detector were given in the instrumental settings. The linearity was studied between the concentration range of 3.13-18.75 µg/ml of Ombitasvir,18.75-112.5 µg/ml of Paritaprevir and 12.5-75 µg/ml of Ritonavir were injected.The plotted calibration curves were linear with a regression coefficient of $R^2$> 0.999, indicates that the linearity was with in the limit. As a part of method validation the parameters like specificity, linearity, accuracy, ruggedness, robustness were determined and the results were found to be within the allowable limit. The method developed was found to be applicable to routine analysis and to be used for the measurement of both active pharmaceutical ingredients (i.e, Ombitasvir,Paritaprevir and Ritonavir).Validation of the proposed method was carried out according to an International Conference on Harmonization (ICH) guidelines.

**Keywords**: *Ombitasvir, Paritaprevir, Ritonavir, HPLC, Validation*

## 1. INTRODUCTION

Ombitasvir is an antiviral [1,2, 3, 4] drug for the treatment of hepatitis [5, 6] C virus (HCV) infection by AbbVie. In the United States, it is approved by the Food and Drug Administration for use in combination with paritaprevir, ritonavir and dasabuvir in the product Viekira Pak for the treatment of HCV [7] genotype 1, and with paritaprevir and ritonavir in the product Technivie for the treatment of HCV genotype 4.

Paritaprevir (previously known as ABT-450) is an acylsulfonamide inhibitor of the NS3-4A serine protease manufactured by Abbott Laboratories that shows promising results as a treatment of hepatitis C [8, 9]. When given in combination with ritonavir [10] and ribavirin for 12 weeks, the rate of sustained virologic [11, 12] response at 24 weeks after treatment has been estimated to be 95% for those with hepatitis C virus genotype 1 [13]. Resistance to treatment with paritaprevir is uncommon, because it targets the binding site, but has been seen to arise due to mutations at positions 155 and 168 in NS3.

Ritonavir, sold under the brand name Norvir, is an antiretroviral [14, 15] medication used along with other medications to treat HIV/AIDS [16, 17]. This combination treatment is known as highly active antiretroviral therapy (HAART) [18]. Often a low dose is used with other protease inhibitors [19, 20]. It may also be used in combination with other medications for hepatitis C. It is taken by mouth. The tablets of ritonavir are not bioequivalent to capsules as tablets may result in higher peak plasma concentrations.Common side effects include nausea, vomiting, loss of appetite [21], diarrhea, and numbness [22] of the hands and feet. Serious side effects include liver problems, pancreatitis [23], allergic reactions, and arrythmias [24]. Serious interactions may occur with a number of other medications including amiodarone and simvastatin. At low doses it is considered to be acceptable for use during pregnancy. Ritonavir is of the protease inhibitor class. Typically, however, it is used to inhibit the enzyme that metabolizes other protease inhibitors. This inhibition allows lower doses of these latter medications to be used.

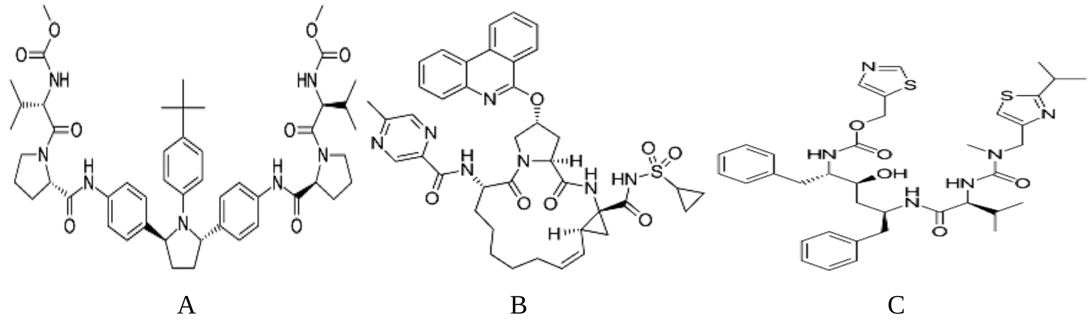


FIG. 1: CHEMICAL STRUCTURE OF (A) OMBITASVIR (B) PARITAPREVIR AND (C) RITONAVIR

## 2. MATERIALS AND METHOD

**Chemicals:** Acetonitrile, HPLC-grade triethyl amine, water were purchased from Merck India Ltd, Mumbai, India. APIs of Ombitasvir, Ritonavir and Paritaprevir standards were procured from Glenmark,Mumbai.

**The Instrumentation:** Waters alliance liquid chromatography (model e-2695) monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study.

**Preparation of buffer:** 1 ml of triethyl amine is dissolved in 1 lt of HPLC grade water and filter through 0.45 µ filter paper.

**Chromatographic conditions:** The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% triethyl amine, and X-bridge phenyl column150X4.6mm, 3.5µ with a flow rate of 1 ml /min.

**Diluent:**0.1% triethyl amine and Acetonitrile in the ratio (50:50) is used as diluent.

**Preparation of the standard stock solution:** For standard stock solution preparation, add 70ml of diluents to 12.5mg of Ombitasvir,75 mg of Paritaprevirand 50 mg of Ritonavir were taken in a 100 ml volumetric flask and sonicate for 10 minutes to fully dissolve the contents and then make up to the mark with diluent.

**Preparation of Standard solution:** 5 ml of solution is drawn from the above normal stock solution into a 50ml volumetric flask and diluted up to the level.

## 3. RESULTS AND DISCUSSION

The main analytical challenge during development of a new method was to separate active Pharma ingredients. In order to provide a good performance the chromatographic conditions were optimized.

**Method optimization:** To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally 0.1% triethyl aminebuffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method various stationary phases such as $C_8$, $C_{18}$ phenyl and amino,luna phenyl columns were tested. From these trials the peak shapes were relatively good with a X-bridge phenyl column150X4.6mm, 3.5µ with a PDA detector. The mobile phase flow rate has been done at 254nm in order to obtain enough sensitivity. According to ICH guidelines, the established method was validated.

### Method validation

The optimized RP-HPLC validated method according to ICH guidelines in terms of system suitability, linearity, accuracy, precision and robustness.

**System suitability:**Device suitability was performed by injecting standard solution containing 12.5 µg/ml of Ombitasvir, 75 µg/ml of Paritaprevir and 50 µg/ml of Ritonavir in six replicates. The results show that the machine fitness parameter is within the limit provided by ICH. The results were shown below table 1. Figure 2 represents the chromatogram of standard.

**TABLE 1: RESULTS OF SYSTEM SUITABILITY**

| System suitability parameter | Acceptance criteria | Drug name | | |
|---|---|---|---|---|
| | | Ombitasvir | Paritaprevir | Ritonavir |
| USP Plate count | NLT 2000 | 2936 | 8930 | 10944 |
| USP Tailing | NMT 2.0 | 1.07 | 1.09 | 1.09 |
| USP Resolution | NLT 2.0 | | 13.01 | 4.62 |
| % RSD | NMT 2.0 | 1.63 | 0.2 | 0.16 |
| Retention Time | NLT 2.0 | 2.496 | 5.159 | 6.229 |

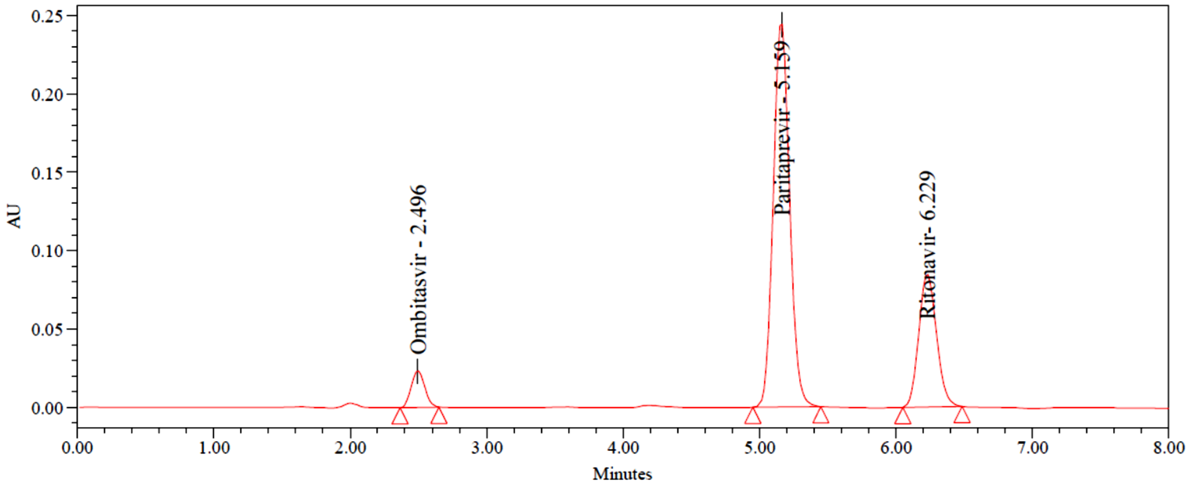


FIG. 2: CHROMATOGRAM OF STANDARD

**Specificity:** There was no interference from blank at the retention time of Ombitasvir and Paritaprevir. This proves the technique is specific. Figure 3 shows the chromatogram of blank.

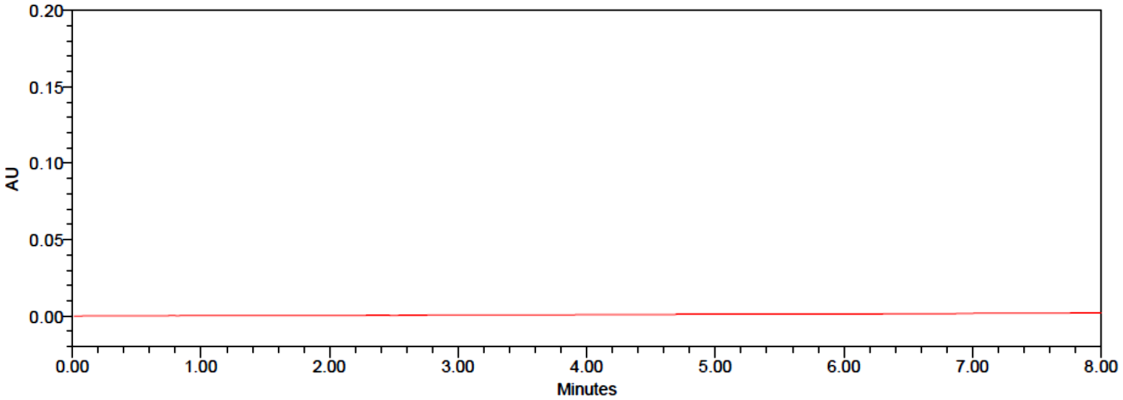
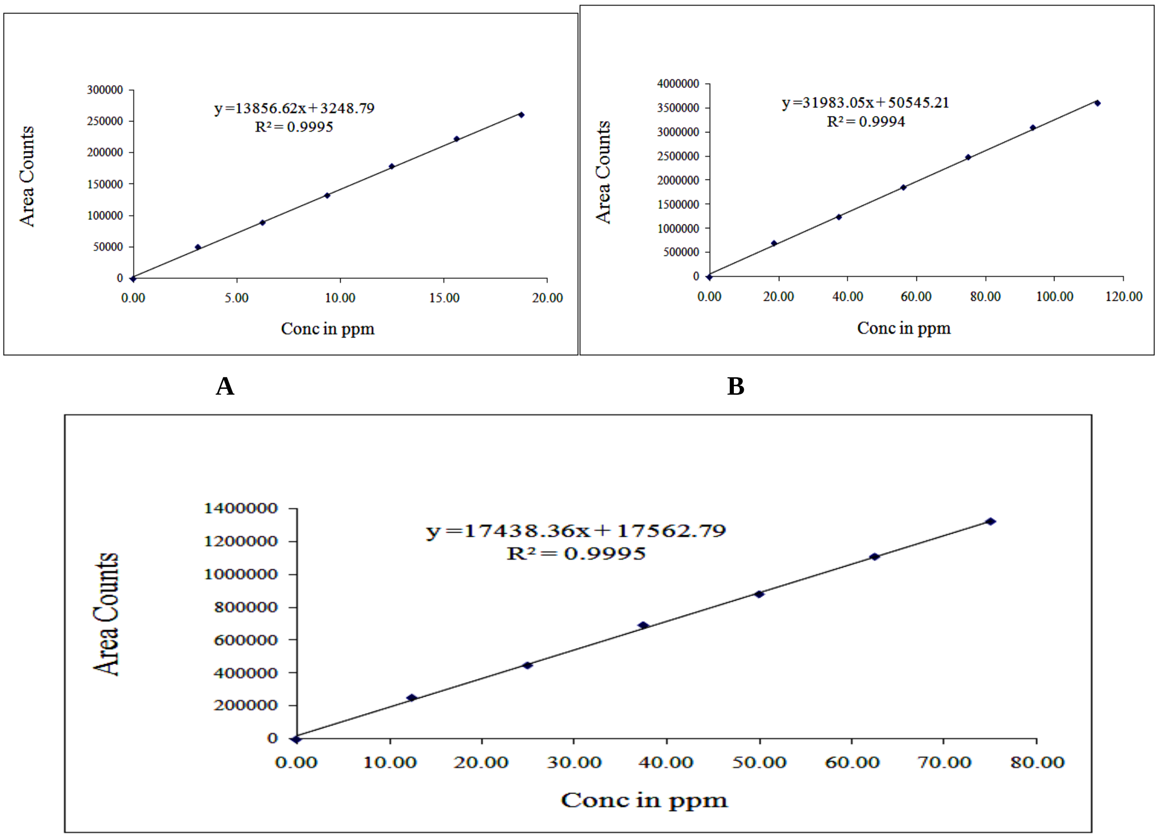


FIG. 3: CHROMATOGRAM OF BLANK

**Linearity:**Linearity was calculated by plotting a calibration curve of the peak area against its respective concentration, linearity was determined. From this calibration curve, it was noticed that the curve was linear between the range of 3.13-18.75 µg/ml of Ombitasvir, 18.75-112.5 µg/ml of Paritaprevir and 12.5-75 µg/ml of Ritonavir. The results of linearity were shown in table 2. Calibration plots were shown in figure 4.

**TABLE 2: LINEARITY RESULTS**

| Linearity | Ombitasvir | | Paritaprevir | | Ritonavir | |
|---|---|---|---|---|---|---|
| | Conc. (µg/ml) | Area | Conc. (µg/ml) | Area | Conc. (µg/ml) | Area |
| Linearity-1 | 3.13 | 50404 | 18.75 | 698237 | 12.50 | 252712 |
| Linearity-2 | 6.25 | 89176 | 37.50 | 1239993 | 25.00 | 448507 |
| Linearity-3 | 9.38 | 132054 | 56.25 | 1852364 | 37.50 | 691487 |
| Linearity-4 | 12.50 | 178464 | 75.00 | 2477387 | 50.00 | 879429 |
| Linearity-5 | 15.63 | 221978 | 93.75 | 3087304 | 62.50 | 1107194 |
| Linearity-6 | 18.75 | 260006 | 112.50 | 3591858 | 75.00 | 1321180 |
| Linearity-7 | 3.13 | 50404 | 18.75 | 698237 | 12.50 | 252712 |
| CC | 0.99957 | | 0.99948 | | 0.99957 | |
| Slope | 13856.62 | | 31983.05 | | 17438.36 | |
| Intercept | 3248.79 | | 50545.21 | | 17562.79 | |



**A** **B**

**FIG. 4: CALIBRATION PLOTS OF (A) OMBITASVIR (B) PARITAPREVIR AND (C) RITONAVIR**

**Accuracy:**The accuracy of the system was achieved by measuring the recovery experiments at three stages (50 percent, 100 percent and 150 percent). APIs with concentrations of6.25, 12.5,18.75 µg/ml of Ombitasvir and 37.5, 75,112.5 µg/ml of Paritaprevirand 25, 50, 75 µg/ml of Ritonavir were prepared. For each spike stage, the test solution was injected three times and the test was performed according to the test process. The recovery results were similar to 100% and also the RSD values were less than ±2%. The percentage recovery, mean and relative standard deviations were determined. Recovery values shown within the desired range were correct. The results are summarized below. Accuracy findings have been shown in table

3.

**TABLE 3: RESULTS OF ACCURACY**

| S. No. | % Level | Ombitasvir<br>% Recovery | Paritaprevir<br>% Recovery | Ritonavir<br>% Recovery |
|---|---|---|---|---|
| 1 | 50 | 99.6 | 100.5 | 99.6 |
| 2 | 100 | 101.5 | 100.1 | 99.2 |
| 3 | 150 | 100.7 | 99.8 | 98.6 |

**Precision:** The precision of the analytical technique is the degree of proximity of the sequence of measurements obtained from multiple homogeneous mixture samplings. The accuracy of the process of the drugs were calculated by injection of six individual determinations of Ombitasvir (12.5 µg/ml) and Paritaprevir (75 µg/ml) and Ritonavir (50 µg/ml). Method precision results were shown in table 4 and method precision chromatogram was shown in figure 5.

**TABLE 4: RESULTS OF INTRADAY PRECISION**

| S. No | Region of Ombitasvir | Region of Paritaprevir | Region of Ritonavir |
|---|---|---|---|
| 1 | 174538 | 2427194 | 874245 |
| 2 | 176309 | 2427149 | 877657 |
| 3 | 170367 | 2427755 | 875615 |
| 4 | 173466 | 2419468 | 872437 |
| 5 | 173762 | 2421265 | 873738 |
| 6 | 178523 | 2419468 | 873320 |
| Mean | 174494 | 2423716 | 874502 |
| % RSD | 1.58 | 0.17 | 0.21 |

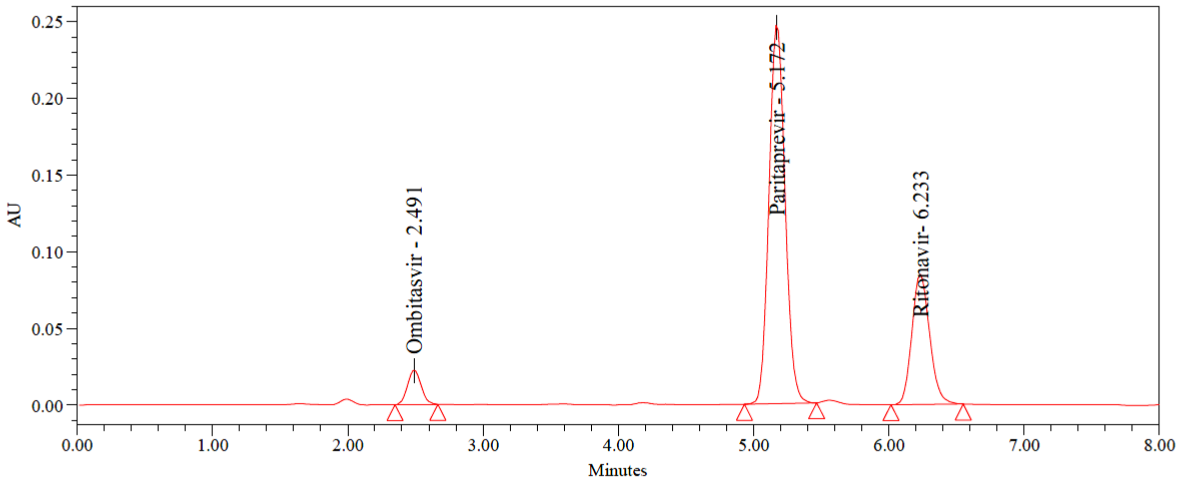


FIG. 5: CHROMATOGRAM OF METHOD PRECISION

**Intermediate Precision:** Six replicates of the sample solution were analyzed bydifferent researchers and different tools were checked on separate days. The peak regions used to assess the average percent of RSD values have been determined. The findings are shown in the table 5.

### TABLE 5: INTER-DAY PRECISION RESULTS

| Analyst | Retention period of OBT(minute) | Peak region of OBT | Retention period of PRP(minute) | Peak region of PRP | Retention period of RTV(minute) | Peak region of RTV |
|---|---|---|---|---|---|---|
| Analyst 1 | 2.494 | 172757 | 5.177 | 2418669 | 6.236 | 877657 |
| Analyst 2 | 2.491 | 172353 | 5.172 | 2426320 | 6.233 | 870554 |
| Mean | - | 172555 | - | 2422494 | - | 874105 |
| %RSD | - | 0.17 | - | 0.22 | - | 0.57 |

**LOD and LOQ:** LOD and LOQ were determined separately using the calibration curve technique. The LOD and LOQ of the compound were measured using the developed RP-HPLC method by injecting lower and lower concentrations of the standard solution. The LOD and LOQ concentrations and their s/n values of Ombitasvir and Paritaprevir and Ritonavir were represented in the following table 6.

### TABLE 6: LOD AND LOQ RESULTS

| Name of the medication | LOD | | LOQ | |
|---|---|---|---|---|
| | S/N | Conc. (µg/ml) | S/N | Conc. (µg/ml) |
| Ombitasvir | 3 | 0.379 | 22 | 1.25 |
| Paritaprevir | 8 | 2.273 | 29 | 7.5 |
| Ritonavir | 5 | 1.515 | 24 | 5 |

**Robustness:**The conditions of the experiment was designed to measure the robustness of the intentionally changed conditions such as flow rate, organic percentage in mobile phase. Results of robustness were shown in table 7.

### TABLE 7: ROBUSTNESS RESULTS

| **Parameter** | **% RSD of Ombitasvir** | **% RSD of Paritaprevir** | **% RSD of Ritonavir** |
|---|---|---|---|
| Flow (1.2 ml/minute) | 0.18 | 0.29 | 0.30 |
| Flow (0.8 ml/minute) | 1.82 | 0.26 | 0.32 |
| Org phase (55:45) | 1.76 | 0.11 | 0.13 |
| Org phase (45:55) | 0.71 | 0.46 | 0.49 |

**Degradation studies:** Ombitasvir and Paritaprevir and Ritonavirsample was subjected to various conditions of forced degradation in order to induce partial degradation of the compound. Forced degradation experiments have been performed to establish that the process is acceptable for degradation materials. In addition the studies include information on the condition under which the drug is unstable, such that the steps are also taken during formulation to prevent possible instabilities. Forced degradation results were shown in table 8.

**Acid degradation:**5 ml of sample stock solution was moved to a volumetric flask of 50 ml, add 1 ml of 1N HCl and left it for 15 min. After 15 min add 1 ml of 1N NaOH and make up to the diluent mark.

**Alkali degradation:** 5 ml ofsample stock solution was moved to a volumetric flask of 50 ml, add 1 ml of 1N NaOH and left it for 15 min. After 15 min add 1 ml of 1N HCl and make up to the mark.

**Peroxide degradation:**5 ml of sample stock solution was moved to a volumetric flask of 50 ml, add 1 ml of 30% hydrogen peroxide solution and make upto the mark with diluents.

**Reduction degradation:**5 ml of sample stock solution was moved to a volumetric flask of 50 ml and add 1 ml of 30% sodium bi sulphate solution and make upto the mark with diluents.

**Thermal degradation:** The sample solution was set in an oven at 110°C for 24 hrs. The resultant solution was injected into HPLC system.

**Photolytic degradation:** The sample solution was placed in sun light for 24 hrs. The resultant solution was injected into HPLC system.

**Hydrolysis degradation:** 5 ml of sample stock solution was moved to a volumetric flask of 50 ml and add 1 ml of HPLC grade water and make upto the mark with diluents.

**TABLE8: FORCED DEGRADATION RESULTS**

| Degradation condition | Ombitasvir % deg | Paritaprevir% deg | Ritonavir % deg |
|---|---|---|---|
| Control | 0.1 | 0 | 0.1 |
| Acid deg | 14.2 | 13.4 | 16.5 |
| Alkali deg | 13.5 | 13.1 | 15.2 |
| Peroxide deg | 15.7 | 14.8 | 14.3 |
| Reduction deg | 12.5 | 11.7 | 11.6 |
| Thermal deg | 9.5 | 10.4 | 7.7 |
| Photolytic deg | 3.6 | 2.9 | 3.1 |
| Hydrolysis deg | 2.4 | 2.2 | 1.8 |

## 4. CONCLUSION

This method described the quantification of Ombitasvir, Paritaprevir and Ritonavir in bulk and pharmaceutical formulation as per ICH guidelines. The evolved technique was found to be accurate, precise, linear and reliable. The advantage lies in the simplicity of standard preparation and reproducibility data are satisfactory. The evolved chromatographic method can be effectively applied for regular investigation in drug research.

## ACKNOWLEDGEMENT

I am thankful to my guide for encouragement and supporting to finish this research work.

## CONFLICTS OF INTEREST

Author declares that there has been no conflicts of interest.

## FUNDING SUPPORT

None

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