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Stability indicating HPLC Method Development and Validation for the Simultaneous Estimation of Ritonavir, Ombitasvir and Paritaprevir in combined Tablet Dosage Form

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ABSTRACT
A simple, Accurate and precise method was developed for the simultaneous estimation of the Ritonavir, Ombitasvir and Paritaprevir in combined tablet dosage form. Chromatogram was run through BDS C18 (150 x 4.6 mm, 5µ). Mobile phase containing 0.1% OPA and Acetonitrie in the proportion of 60:40 A was pumped through column at a flow rate of 1.0ml/min. Temperature was maintained at 30°C. Optimized wavelength for Ritonavir Ombitasvir and Paritaprevir was 260nm. Retention time of Paritaprevir , Ritonavir and Ombitasvir Were found to be 2.315 min; 3.114 min and 3.650 min. %RSD of system precision for Paritaprevir , Ritonavir and Ombitasvir were and found to be 0.4, 1.3 and 1.0 respectively. %RSD of method precision for Paritaprevir , Ritonavir and Ombitasvir were and found to be 0.2, 0.4 and 0.9 respectively.

Key Words: Ritonavir, Ombitasvir, Paritaprevir, RP-HPLC

I. INTRODUCTION :

Ritonavir[1] is an HIV protease inhibitor that interferes with the reproductive cycle of HIV. Although it was initially developed as an independent antiviral agent, it has been shown to possess advantageous properties in combination regimens with low-dose ritonavir and other protease inhibitors. It is now more commonly used as a booster of other protease inhibitors and is available in both liquid formulation and as capsules protease inhibitors. It is now more commonly used as a booster of other protease inhibitors and is available in both liquid formulation and as capsules. Ritonavir is chemically described as 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl]carbamate

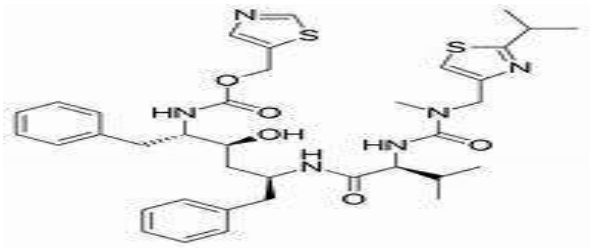

Fig.1:Chemical structure of Ritonavir

Ombitasvir[2] is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV).. Ombitasvir is chemically described as methyl N-[(2S)-1-[(2S)-2-({4-[(2S,5S)-1-(4-tert-butylphenyl)-5-{4-[(2S)-1-[(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl]pyrrolidine-2-amido]phenyl}pyrrolidin-2-yl]phenyl}carbamoyl)pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate.

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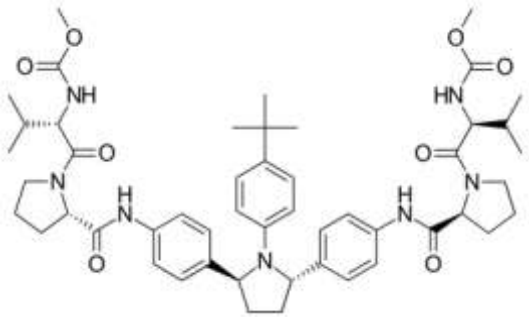

Fig.2: Chemical structure of Ombitasvir Paritaprevir[3] is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus.Paritaprevir is chemically described as(1S,4R,6R,7Z,14S,18R)-N-(cyclopropanesulfonyl)-14-(5-methylpyrazine-2-amido)-2,15-dioxo-18-(phenanthridin-6-yloxy)-3,16-diazatricyclo[14.3.0.0^{4},^{6}]nonadec-7-ene-4-carboxamide

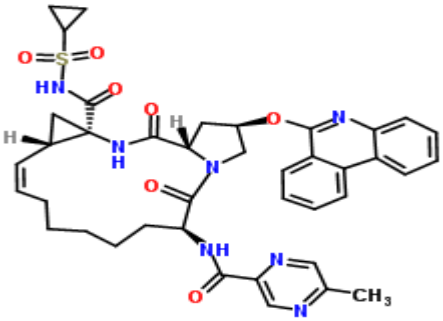

Fig.3: Chemical structure of Paritaprevir

According to the literature search, there are few high performance liquid chromatography (HPLC) methods for estimation of selected drugs in single or in combination with other drugs[4, 5] were reported.

II. METHODS

CHEMICAS AND REAGENTS

All the reagents used in the experimental work were of analytical grade.HPLC grade water was prepared by Milli Q reverse Osmosis (Millipore, Bedford, USA) and meets European Pharmacopoeia requirements. CAN (Sigma–Aldrich, Merk and Rankem) were used for preparing the mobile phase. Mobile Phase was used as solvent. Working standards of Ritonavir, Ombitasvir and Paritaprevir were provided by Glenmark Pharmaceuticals (Mahape, NaviMumbai).Biktarvy®(containing 200 mg of ritonavir, 50mg of Ombitasvir and 25mg of Pritaprevir) were purchased from local market.

Chramatographic conditions (instrumentation and analytical conditions)

Instruments

An Alliance 2695 (Waters, USA) chromatographic system was used, equipped with a Quaternary pump, and waters 2996 photo diode array detector, Agilent C18 (150×4.6mm,5µ), auto sampler thermostat and degasser. Chromatographic software Empower was used for data collection and processing Separations were performed using Agilent C18 (150×4.6mm,5µ) packed with 5 µm particle size. A 1m long steel capillary with 0.25 mm internal diameter, was inserted between the injection system and the entrance of the column, and injection volume was 10µL. Separations and simultaneous determination of Ritonavir,

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Ombitasvir and Paritaprevir were performed using the mixture of 0.1% OPA and Acetinitrile (60:40) as a mobile phase. Mobile phase was filtered through a 0.45 µm Millipore filter. The flow rate was 1.0 mL min−1 and the UV detection was performed at 260 nm**.**

Analytical Procedure

Preparation of Standard stock solutions:

Precisely measured 25mg of Ritonavir, 6.25mg of Ombitasvir and 37.5mg of Paritaprevir and transferred to three 50ml volumetric flasks independently. 10ml of methanol was added and sonicated for 15mins. Volume were made up with water and acetonitrile (50:50) and marked as Standard stock arrangement1,2 and 3.

From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluents. (50ppm&12.5ppm&75ppm)

Assay

The label claim per unit formulation 25mg of Ritonavir, 6.25mg of Ombitasvir and 37.5mg of Paritaprevir Assay was performed with the above formulation. Average % Assay forRitonavir, Ombitasvir and Paritaprevir. Obtained was 100.07%, 99.67% and100.12% respectively.

Validation:

Chromatographic separation was optimized in the aim to obtain a resolution above 1.5 between all components, with the respect of stationary and mobile phase compositions, flow rate, sample volume, detection wavelength and temperature.

The method was validated for linearity, precision (repeatability and intermediated precision), specificity, limit of quantitation, limit of detection and robustness.

Linearity

Standard calibration curves were prepared with six calibrators over a concentration range of 12.5 to 75 µg/ml of Ritonavier , 3.125-18.75 µg/ml of Ombitasvir and 18.75-112.5µg/ml of Paritaprevir. The data of peak area versus drug concentration were treated by linear least square regression analysis. Thestandard curves were evaluated for linearity

Precision:

The precision of the assay was studied with respect to both repeatability and intermediated precision. Repeatability was calculated from six replicate injections of freshly prepared solution in the same equipment on the same day. The % of the relative standard deviation (R.S.D.) for Ritonavir, Ombitasvir and Paritepravir was realized with a 1.3 , 1 and 0.4.

Specificity: Checking of the interference in the optimized method. We should not found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Limis of detection and quantitation

Limits of detection (LOD) and limits of quantitation (LOQ) were provided and calculation was made with the following equations:

$$LOD=3.3\ \sigma /S$$

$$LOQ=10\ \sigma /S$$

When σ was the standard deviation of the response (estimated from the standard deviation of y-intercepts or regression lines) and S was the slope of the standard curve.

Sensitivity

The sensitivity (6x) of an analytical method is defined by the minimum variation that requires to be applied to the magnitude measured in order to obtain a significant variation in the signal measured.

Robustness

Robustness of method was investigated by varying the chromatographic conditions such as change of flow rate(±20%), organic content in mobile phase (± 2%). Robustness of the developed method was indicated by the overall %RSD between the data at each variable condition.

III. RESULTS AND DISCUSSION

Firstly, HPLC conditions were optimized to obtain a desired peak with high purity and resolution. Therefore, the various parameters affecting the peak shape, retention time and resolution were investigated in detail. The separation efficiency of Waters BDS C18 150 x 4.6 mm, 5um was compared to the Phenylhexyl 250x4.6 mm,5µ for the determination under the same conditions, and the proposed column was chosen for the further optimization of parameters. During our preliminary experiments, the series of aqueous mobile phases containing buffer solutions with the different pH values in combination with different organic modifiers including0.1% OPA and Acetonitrile taken in the Ratio (50:50) thewere tested for obtaining the optimum separation conditions. Acetonitrileand Ortho-Phosphoric acid were selected as the eluents. The chromatographic analysis time was shortened with high organic solvent content, and also, the buffer solutions in the

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mobile phase ensured stable chromatographic retention times preventing broad peaks. The effect of the mobile phase pH on the retention time and peak shape of the analyte was studied especially in the acidic region. The best retention time and peak shape were achieved with OPA buffer. The best separation was achieved with the mobile phase consisting of 0.1% ortho phosphoric acid:acetonitrile (60:40 v/v). The calibration curves analysis were constructed by plotting the peak area aganist the concentration of the drugs

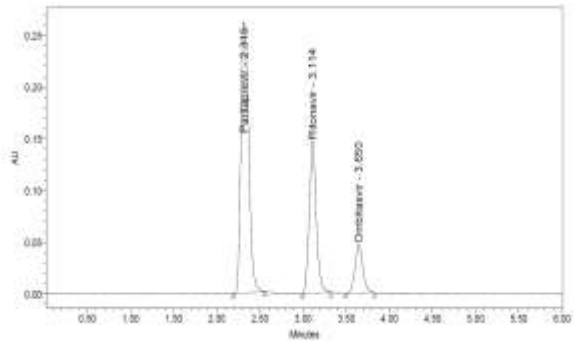

Fig.4Typical Chromatogram

METHODVALIDATION

The method was validated for linearity, precision, accuracy, robustness, rugudness, forced degradation and stability.

Linearity:

Six linear concentrations of Ritonavir.(12.5-75µg/ml), Ombitasvir(3.125-18.75µg/ml) and Paritaprevir(18.75-112.5µg/ml) were injected in a triplicate maner. Average areas were mentioned above and linearity equations obtained for Ritonavir. was y = 16094x + 1378, Ombitasvire was y = 19972x + 1651and of Paritaprevir was y = 20711x + 19981.

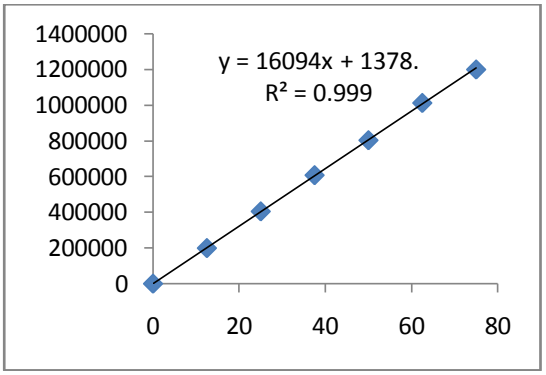

Fig.5: Linearity plot for Ritonavir

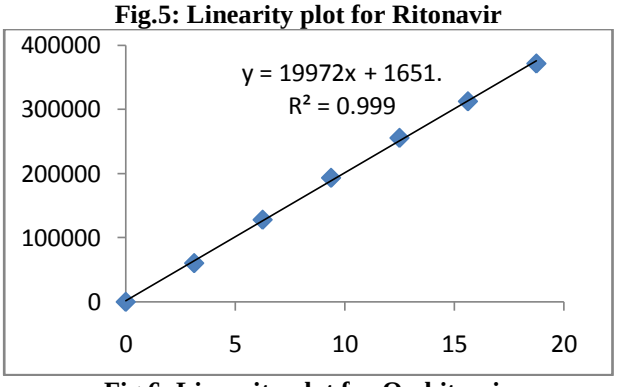

Fig.6: Linearity plot for Ombitasvir

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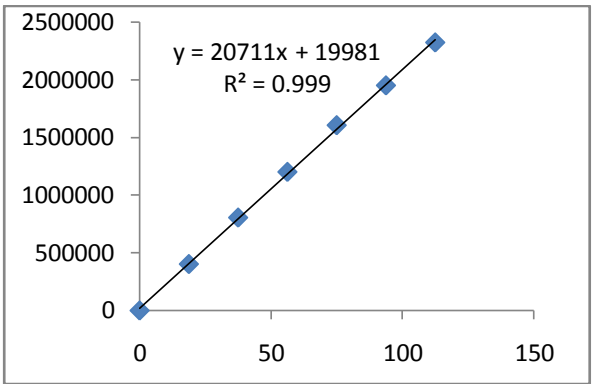

Fig.7: Linearity plot for Paritaprevir

Precision of this method was studied in inter day and intra day variation. The precision of intraday studies of six different concentration of the drug was repeated thrice in a day and in the inter day variation studies of six different concentration of the drug was repeated on three consecutive days. The developed method was found to be precise as the percentage of RSD values for inter-day and intra-day precision studies were found to be less than 2%.

Table1:Recoveryof Ritonavir drug

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	25	25.191	100.76	100.07%
	25	25.304	101.22	
	25	25.261	101.04	
100%	50	49.659	99.32	
	50	49.511	99.02	
	50	50.377	100.75	
150%	75	73.982	98.64	
	75	75.052	100.07	
	75	74.864	99.82	

Table2:Recovery of Ombitasvir drug

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	6.25	6.236	99.77	99.67%
	6.25	6.290	100.64	
	6.25	6.258	100.13	
100%	12.5	12.364	98.91	
	12.5	12.531	100.25	
	12.5	12.524	100.19	
150%	18.75	18.569	99.03	
	18.75	18.561	98.99	
	18.75	18.590	99.15	

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Table3:Recovery of Paritaprevir drug

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	37.5	37.35	99.60	100.38%
	37.5	37.07	98.86	
	37.5	38.09	101.57	
100%	75	74.71	99.62	
	75	76.05	101.40	
	75	75.62	100.83	
150%	112.5	113.17	100.60	
	112.5	112.82	100.29	
	112.5	113.21	100.63	

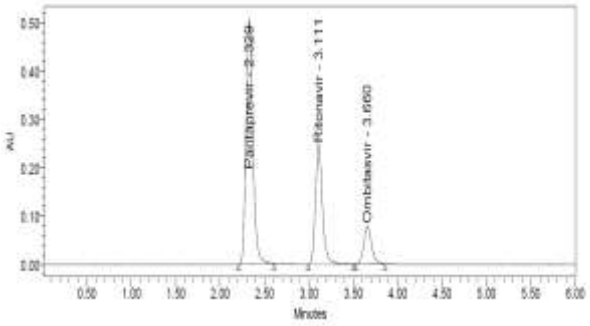

Fig.8:Chromatogram for Accuracy50%

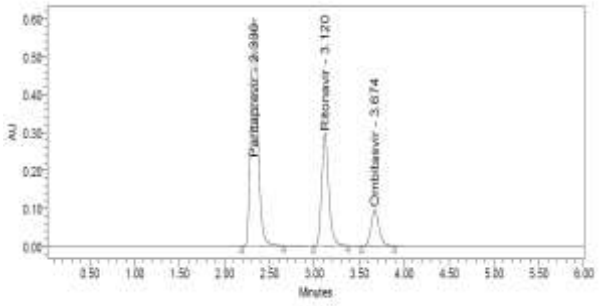

Fig.9:Chromatogram for Accuracy100%

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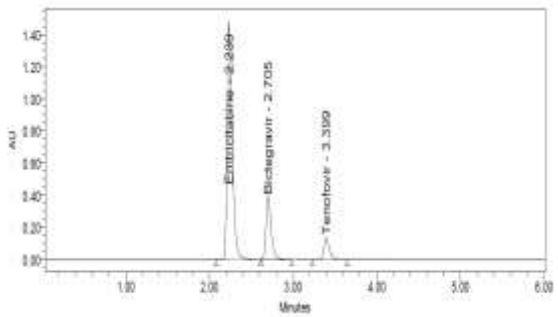

Fig.10:Chromatogram for Accuracy150%

Molecule	LOD(µg/ml)	LOQ(µg/ml)
Ritonavir	0.12 µg/ml	0.38 µg/ml
Ombitasvir	0.19 µg/ml	0.57µg/ml
Paritaprevir	0.44 µg/ml	1.32 µg/ml

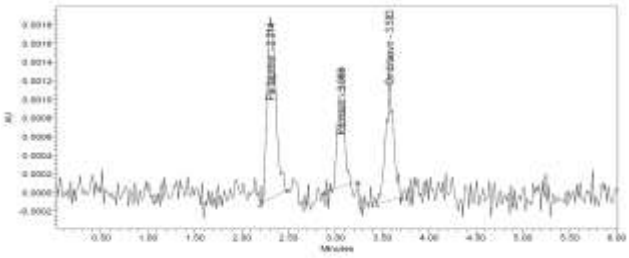

Fig.9:Chromatogram for LOD

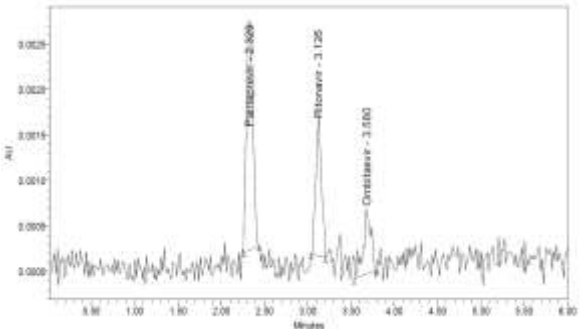

Fig.9:Chromatogram for LOQ

Forced degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

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Table4: Degradation data of Ritonavir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.09	0.407	0.438
2	Alkali	3.86	0.220	0.42
3	Oxidation	2.94	0.213	0.409
4	Thermal	2.41	0.224	0.429
5	UV	1.88	0.234	0.431
6	Water	0.52	0.224	0.435

Table5: Degradation data of Ombitasvir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.41	0.389	0.607
2	Alkali	5.03	0.383	0.587
3	Oxidation	3.35	0.367	0.566
4	Thermal	1.22	0.400	0.623
5	UV	4.19	0.387	0.622
6	Water	0.08	0.415	0.648

Table6: Degradation data of Paritepravir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	5.40	0.151	0.305
2	Alkali	4.16	0.293	0.303
3	Oxidation	4.65	0.243	0.303
4	Thermal	2.99	0.126	0.304
5	UV	1.77	0.123	0.305
6	Water	0.38	0.306	0.309

Fig.11:Chromatogramfor Acid Degradation of Ritonavir, Ombitasvir and Paritaprevir

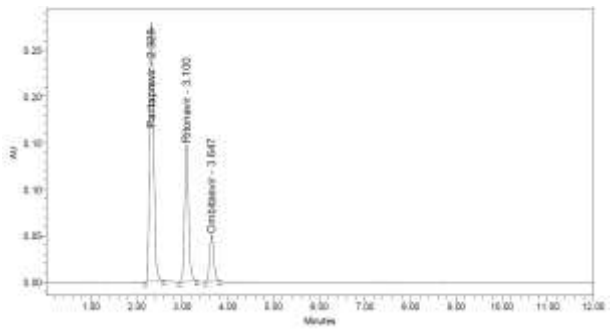

Fig.12:Chromatogramfor Base Degradation of Ritonavir, Ombitasvir and Paritaprevir

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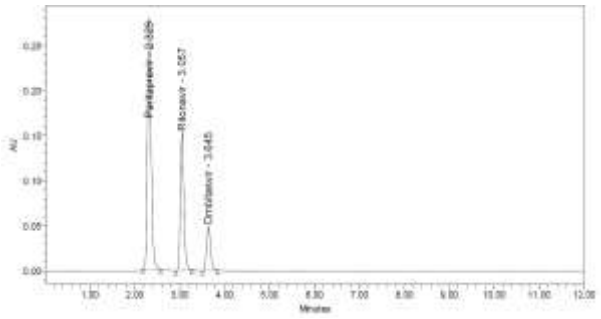

Fig.13:Chromatogram for Peroxide Degradation Ritonavir, Ombitasvir and Paritaprevir

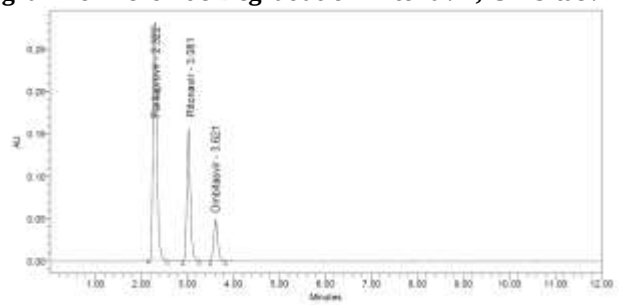

Fig. 14: Chromatogram for Thermal Degradation of Ritonavir, Ombitasvir and Paritaprevir

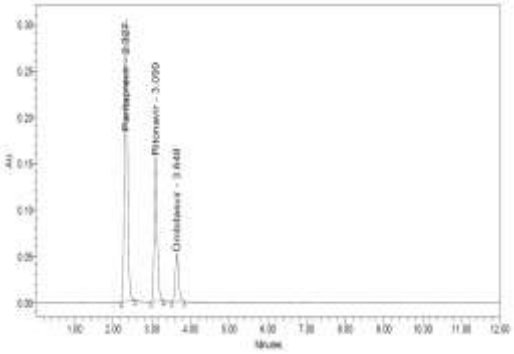

Fig. 15: chromatogram for UV Degradation of Ritonavir, Ombitasvir and Paritaprevir

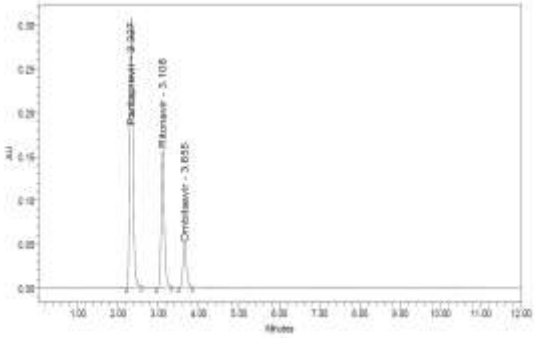

Fig. 16: Chromatogram for water degradation of Ritonavir, Ombitasvir and Paritaprevir

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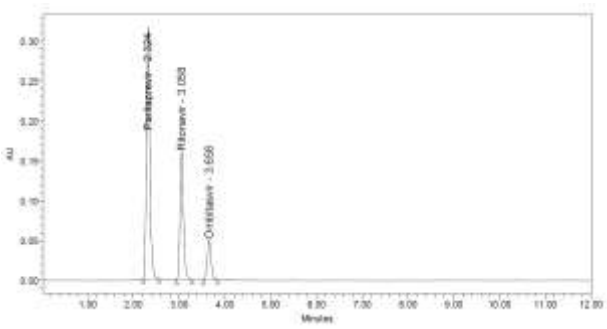

Robustness

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (45B:55A), temperature minus (23°C) and temperature plus(27°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit

Table7: Robustness data for Ritonavir, Ombitasvir and Paritaprevir

S.no	Condition	%RSD of Paritaprevir	%RSD of Ritonavir	%RSD of Ombitasvir
1	Flow rate (-) 0.9ml/min	0.6	0.4	1.5
2	Flow rate (+) 1.1 ml/min	1.0	0.4	0.5
3	Mobile phase (-) 55B:45A	0.9	1.0	0.8
4	Mobile phase (+) 45B:55A	0.6	0.7	0.7
5	Temperature (-) 25°C	0.4	0.3	0.6
6	Temperature (+) 35°C	0.8	0.9	0.8

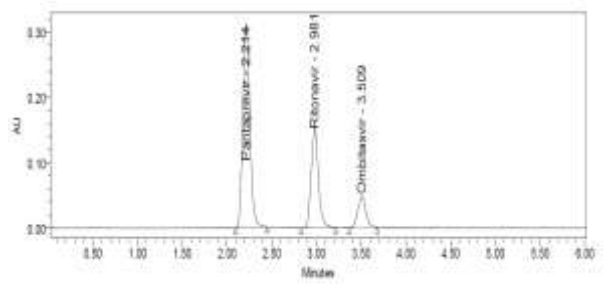

Fig.17 Chromotagram for flow plus

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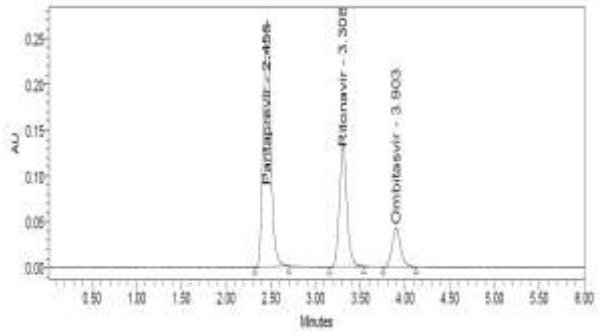

Fig. 18 Chromotagram for flow minus

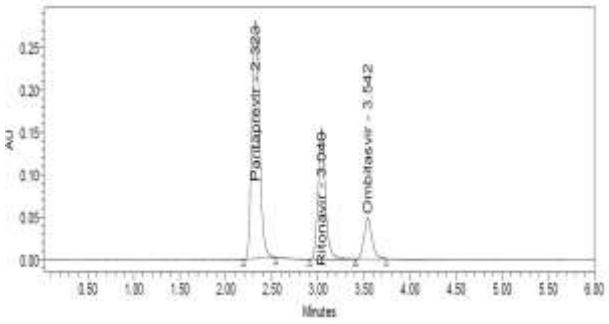

Fig.19Chromotagram for Mobile Phase minus

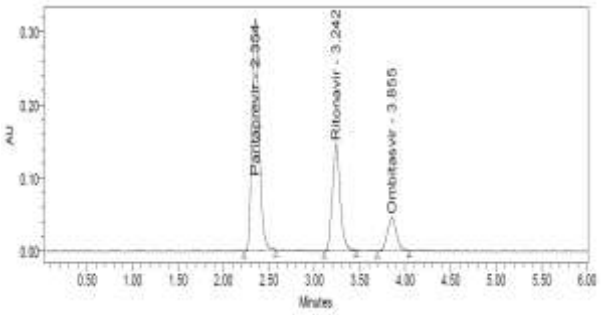

Fig.20 Chromotagram for Mobile Phase plus

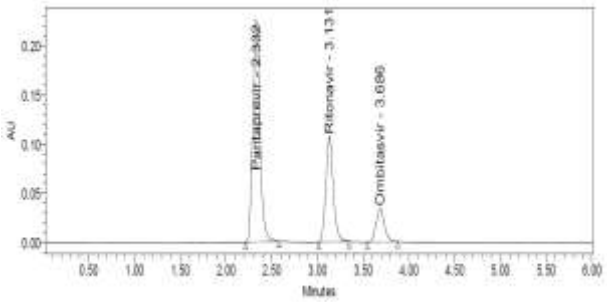

Fig:21 Temperature minus chromatogram injection

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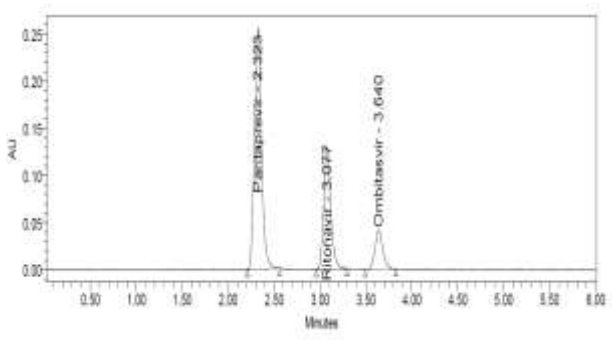

Fig:21 Temperature plus chromatogram injection

IV. CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ritonavir, Ombitasvir and Paritaprevir in Tablet dosage form. Retention time of Ritonavir, Ombitasvir and Paritaprevir Were found to be 2.315 min; 3.114 min and 3.650 min %RSD of system precision for Paritaprevir , Ritonavir and Ombitasvir were and found to be 0.4, 1.3 and 1.0 respectively. %RSD of method precision for Paritaprevir , Ritonavir and Ombitasvir were and found to be 0.2, 0.4 and 0.9 respectively. % recovery was obtained as 100.30%, 100.19% and 100.15%for Paritaprevir , Ritonavir and Ombitasvir respectively. LOD, LOQ values are obtained from regression equations of Paritaprevir , Ritonavir and Ombitasvir were 0.44ppm, 0.12ppm, 0.19ppm, 1.32ppm and 0.38ppm, 0.57ppm respectively

REFERENCES

[1]. https://en.wikipedia.org/wiki/Ritonavir#/media/File:Ritonavir_structure.
[2]. https://pubchem.ncbi.nlm.nih.gov/compound/Ombitasvir.
[3]. https://en.wikipedia.org/wiki/Paritaprevir
[4]. Medisetti Pravallika*, Dr. Devanaboyina Narendra and Gadi Vijaya Lakshmi method development and validation for simultaneous estimation of ritonavir, ombitasvir & paritaprevir by rp-hplc method. European journal of pharmaceutical and medical research. 2020,7(7), 849-855
[5]. Syed ibrahimbaje, b. Jyothi, n. Madhavi. Rp-hplc method for simultaneous estimation of ritonavir, ombitasvir and paritaprevir in tablet dosage forms and their stress degradation studies. International journal of applied pharmaceutics. Vol 11, issue 2 (mar-apr), 2019.