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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF OMBITASVIR, PARITAPREVIR AND RITONAVIR IN TABLET DOSAGE FORMS

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ABSTRACT

A specific, stability indicating method was developed and validated for the simultaneous estimation of Ombitasvir, Paritaprevir and Ritonavir in the pharmaceutical dosage form using RP-HPLC. The separation was done using BDS (250mm x 4.6mm, 5µ particle size) column with a mobile phase consisting of ammonium acetate buffer and acetonitrile in the ratio 40:60%v/v on isocratic mode. The mobile phase is pumped into the system at a flow rate of 1.0ml/min. The column oven temperature is maintained at 30ºC. The samples were detected at the wavelength of 257nm. The method was validated as per ICH guidelines. The method was found to be specific, accurate, precise, rugged and robust. The method obeys Beer's law in the concentration range of 3.13µg/ml – 18.75µg/ml for Ombitasvir, 12.5µg/ml – 75µg/ml for Paritaprevir and 18.75µg/ml 112.5µg/ml for Ritonavir. The correlation coefficient was found to be 0.9996 for Ombitasvir, 0.9996 for Paritaprevir and 0.9997 for Ritonavir. Forced degradation studies were conducted for the standard drug solutions and found to be stable across various stressed conditions. The developed method can be used for the routine analysis of Ombitasvir, Paritaprevir and Ritonavir in pharmaceutical dosage form.

K**eywords:** Ombitasvir, Paritaprevir, Ritonavir, Stability indicating, Method development, Validation, RP-HPLC.

INTRODUCTION

Paritaprevir[1,2] (Figure 1A), chemically known as (2R,6S,12Z,13aS,14aR,16aS)-N-(cyclopropylsulfonyl)-6{[(5-methylpyrazin-2-yl)carbonyl]amino}-5,16-dioxo-2-(phenanthridin-6-yloxy)1,2,3,6,7,8,9,10,11,13a,14,15,16,16a-tetradecahydrocyclopropa[e]pyrrolo[1,2-a][1,4] diazacyclopentadecine-14a(5H)-carboxamide dehydrate. It is white or off-white powder, soluble in dimethyl sulfoxide or slightly soluble in ethanol belongs to antiviral category. It has a pKa value of 4.6. It is used for the treatment of Hepatitis C virus infection. Ritonavir[3] (Figure 1B), chemically known as [5S-(5R*,8R*,10R*,11R*)]10-Hydroxy-2-methyl-5-(1-methyethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-

3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12 tetraazatridecan-13-oic acid,5-thiazolylmethyl ester. It is white to off-white to light tan powder, practically insoluble in water and freely soluble in methanol and ethanol belongs to antiviral category. It has a pKa value of 2.8. It is used to treat and prevent the HIV/AIDS and also to treat Hepatitis C virus infection. Ombitasvir hydrate[4,5] (Figure 1C), chemically designated as Dimethyl ([(2S,5S)-1-(4-tert-butylphenyl) pyrrolidine-2,5diyl] bis{benzene-4,1-diylcarbamoyl(2S) pyrrolidine-2,1-diyl[(2S)-3-methyl-1-oxobutane-1,2diyl]}) biscarbamate hydrate. It is white to light yellow to light pink powder, practically insoluble in aqueous buffers but soluble in ethanol belongs to antiviral category. It has a pKa value of 2.5. It is used for the treatment of Hepatitis C virus infection.

Literature review reveals that there are very few methods such as RP-HPLC methods[6-8] for the simultaneous estimation of Ombitasvir, Paritaprevir and Ritonavir. The objective of the present study is to develop and validate a stability indicating method for the simultaneous estimation of Ombitasvir, Paritaprevir and Ritonavir in the pharmaceutical dosage form by RP-HPLC.

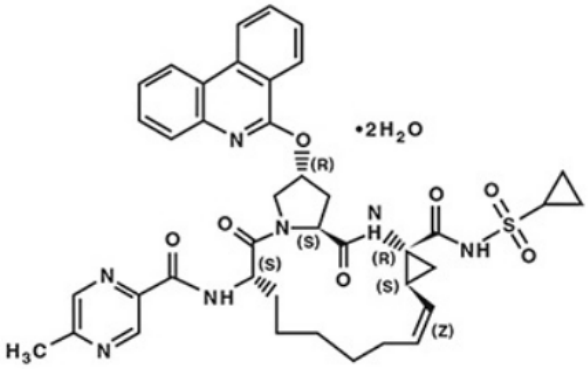

Fig.1A: Chemical structure of Paritaprevir

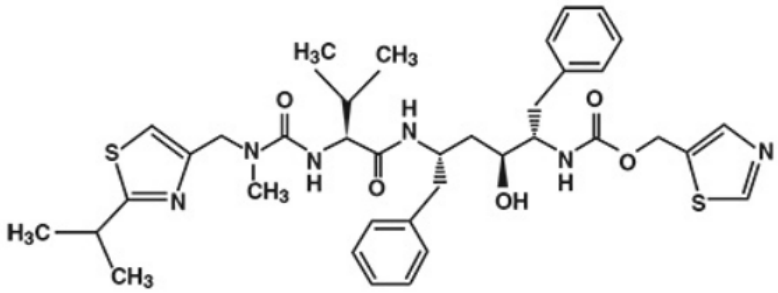

Fig.1B: Chemical structure of Ritonavir

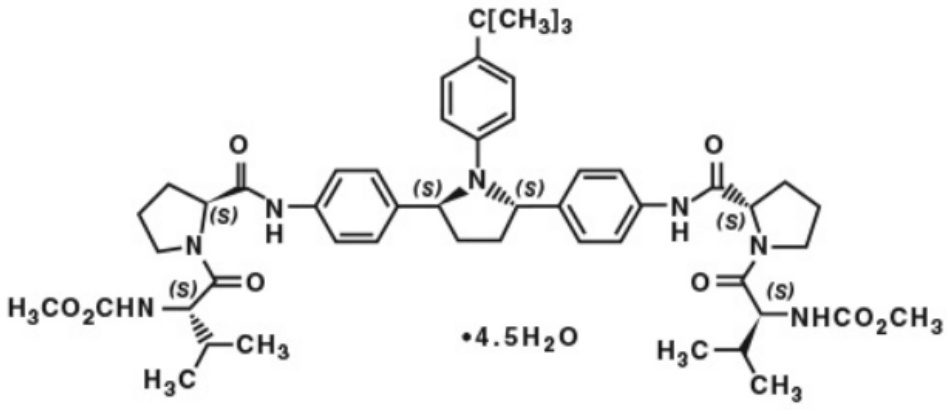

Fig.1C: Chemical structure of Ombitasvir hydrate

MATERIAL AND METHODS:

Chemicals and Reagents: The Ombitasvir standard, Paritaprevir standard and Ritonavir standard were supplied as gift sample from Spectrum labs, Hyderabad. The tablet dosage form (Viekira) was purchased from local pharmacy. All the chemicals used for development of method were of AR grade and all the solvents used were of HPLC grade.

Instrument and analytical conditions: Waters HPLC systems with binary solvent pump, autosampler and PDA detector running on empower 2 software was used to detect the drug samples. The HPLC system with BDS (250mm x 4.6 mm, 5µ) column was used to separate the drugs using mixture of ammonium acetate buffer and acetonitrile in 40:60%v/v ratio on isocratic mode at 1.0ml/min flow rate. The detection was done at 257nm. The other instruments used were pH meter (EI), Digital Balance (Infra Instruments), Ultrasonic Bath (Wadegati), Hot air oven (Cisco).

Preparation of mobile phase: 0.01N ammonium acetate was prepared and mixed with acetonitrile in the ratio 40:60%v/v, used as mobile phase.

Preparation of standard and sample solution: The standard solution was prepared by dissolving 7.5mg of Paritaprevir, 5mg of Ritonavir and 1.25mg of Ombitasvir in 10ml of diluent. From the above stock solution, pipette out 1ml of the solution and make the volume to 10ml with diluent.

The sample solution was prepared by weighing the 20 tablets (Viekira) and an amount equivalent to 7.5mg of Paritaprevir was dissolved in 10ml of diluent. The solution was sonicated for 30min with intermediate shaking. The solution was filtered using HPLC filters. Dilute 1ml of above solution to 10ml with diluent.

Method Validation: The developed method was validated in accordance with the ICH guidelines[9]. The analytical method validation parameters include following

System suitability: The system suitability parameters such as % Relative Standard Deviation (RSD), USP Plate count, USP tailing factor and USP resolution were evaluated by injecting standard solution into the HPLC system. The results were summarized in table 1.

Accuracy: The accuracy of the method was determined in the terms of % recovery. The solutions were prepared in three different concentration levels of 50%, 100% and 150%, injected into HPLC and % recoveries were calculated.

Precision: The %RSD of the method was determined by injecting standard solution six times in a day.

Specificity: Specificity of the method was determined by injecting the placebo solution into the system and then observing for the interference of placebo peaks with that of drug peaks.

Linearity: The linearity of the method was estimated by preparing serial dilutions of drug solutions in the concentration range of 12.5µg/ml - 75µg/ml, 18.75µg/ml – 112.50µg/ml and 3.13µg/ml – 18.75µg/ml for Paritaprevir, Ritonavir and Ombitasvir respectively and injecting into HPLC. A linearity graph was plotted between concentration and peak areas.

Limit of Detection (LOD) and Limit of Quantification (LOQ): For determining LOD and LOQ, initially standard deviations and slopes of calibration curves were calculated. Then by using these values as per formula maintained in ICH guidelines, these parameters were evaluated.

Ruggedness: Ruggedness of method was conducted using same system by same analyst on different days and analyzed under similar conditions as per developed method.

Robustness: Robustness of method was determined by analyzing the standard drug solution by varying the mobile phase composition (±10%), flow rate (±0.2ml/min) and column oven temperature (±5ºC).

Stability: The stability of the drugs in mobile phase was estimated by repeated analysis after storage of drug samples for 24hours under laboratory conditions.

Forced degradation studies: Stability indicating method was developed by subjecting the drug samples to various stress conditions such as acidic (2N Hydrochloric acid, 60 ºC for 30 mins), basic (2N sodium hydroxide, 60 ºC for 30 mins), oxidative (20% hydrogen peroxide, 60 ºC for 30 mins), neutral (refluxing the drugs in water for 6hrs at a temperature of 60ºC), thermal (drugs solution was placed in an oven at

105ºC for 6 hours) and photolytic (exposing the drugs solution to UV light by keeping the beaker in UV Chamber for 7 days or 200Watt hours/m^2 in photo stability chamber) conditions.

RESULTS AND DISCUSSION:

Initially many mobile phase compositions and columns were tried to separate the drugs, finally ammonium acetate buffer and acetonitrile (40:60%v/v) as mobile phase and BDS (250mm x 4.6 mm, 5µ) column were selected. The mobile phase was operated on an isocratic mode with a flow rate of 1.0ml/min. The column oven temperature was maintained at 30ºC and detection of drugs was done at 257nm as the drug shows maximum absorbance at this wavelength as shown in figure 2.

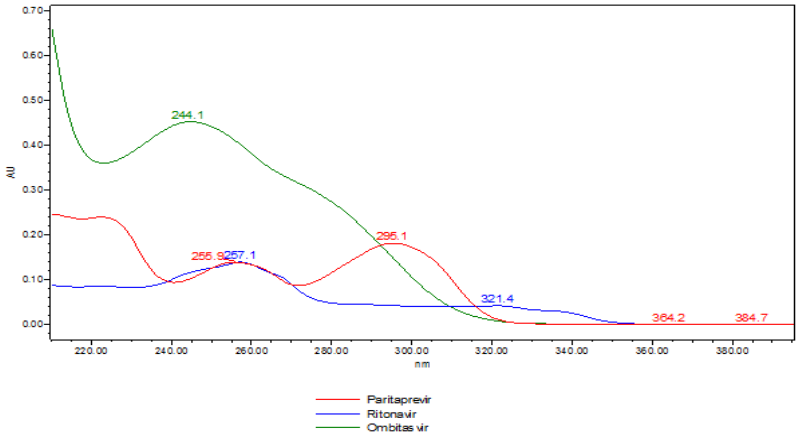

Fig.2: Overlay UV spectrum of Paritaprevir, Ritonavir and Ombitasvir

The standard, sample and blank solutions were prepared and their chromatograms were recorded as shown in figure 3A, 3Band 3C respectively. The system suitability parameters such as %Relative Standard Deviation (RSD), USP plate count, USP tailing factor and Resolution were measured from the standard solution.

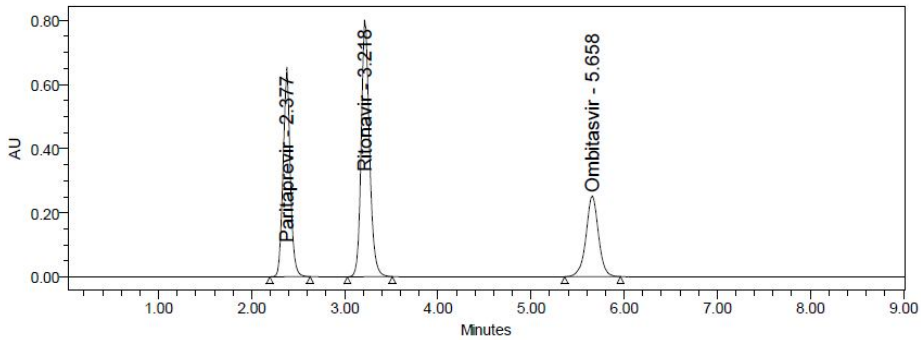

Fig.3A: Standard Chromatogram

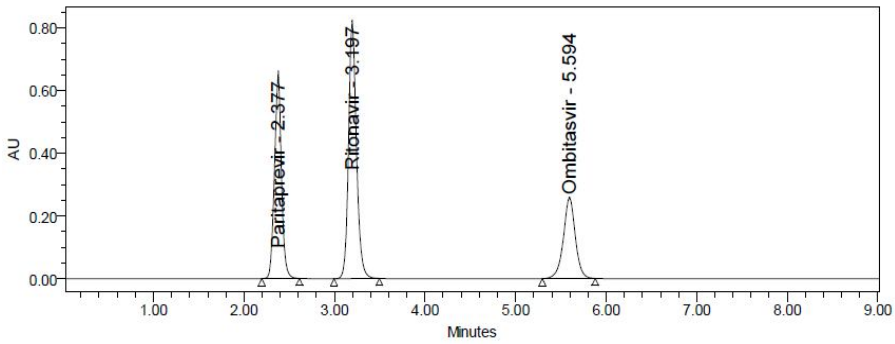

Fig.3B: Sample Chromatogram

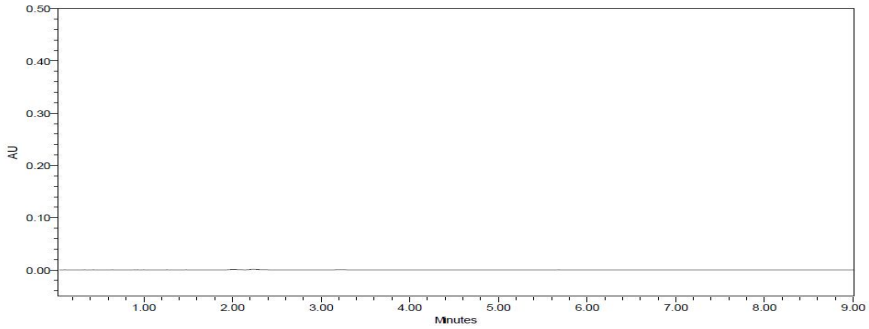

Fig.3C: Blank Chromatogram

Linearity of the method was determined by preparing serial dilutions of Paritaprevir, Ritonavir and Ombitasvir in the concentration range of 12.5µg/ml - 75µg/ml, 18.75µg/ml – 112.50µg/ml and 3.13µg/ml – 18.75µg/ml respectively. A good linear response was observed with correlation coefficient of 0.999 for the three drugs. The linearity plots were shown in figures 4A, 4B and 4C.

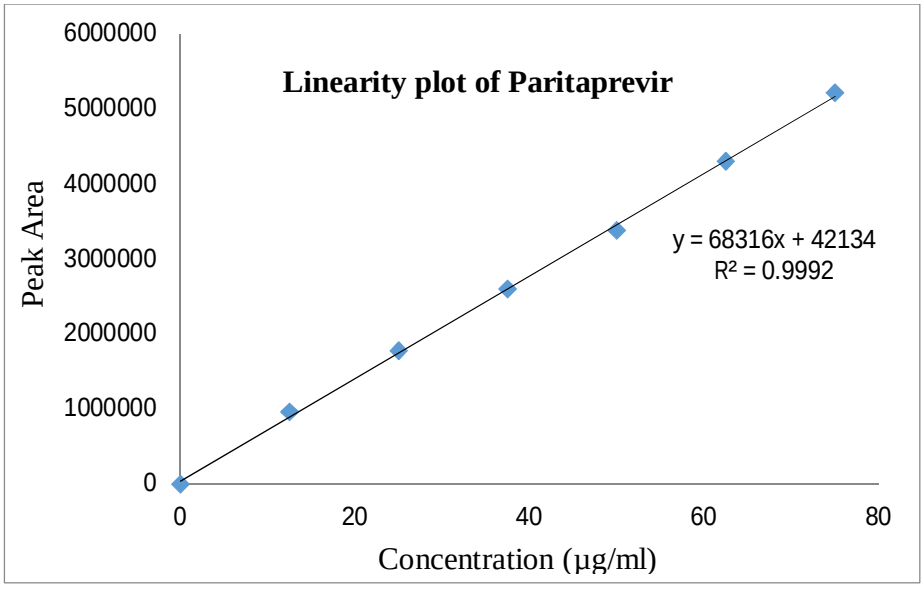

Fig.4A: Linearity plot of Paritaprevir

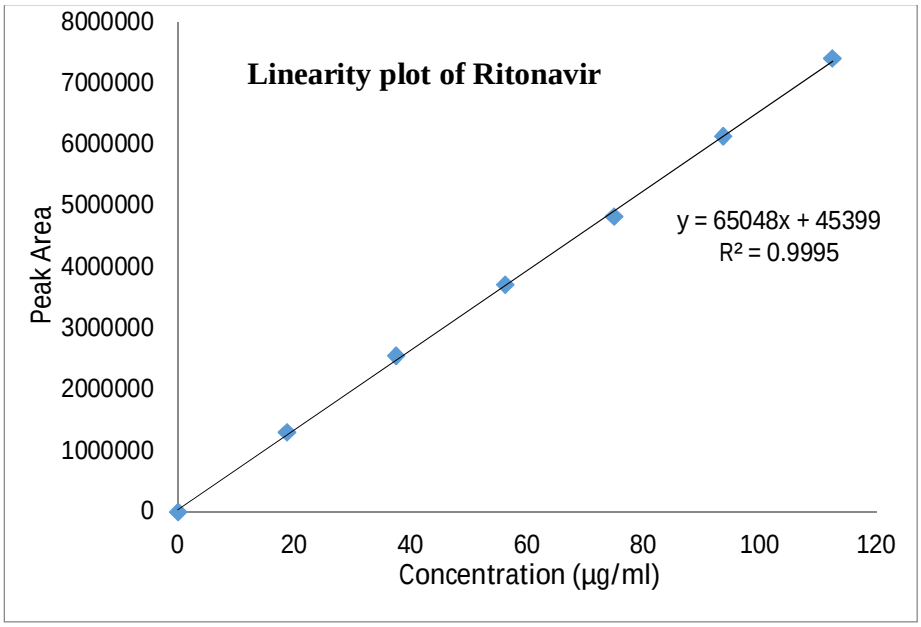

Fig.4B: Linearity plot of Ritonavir

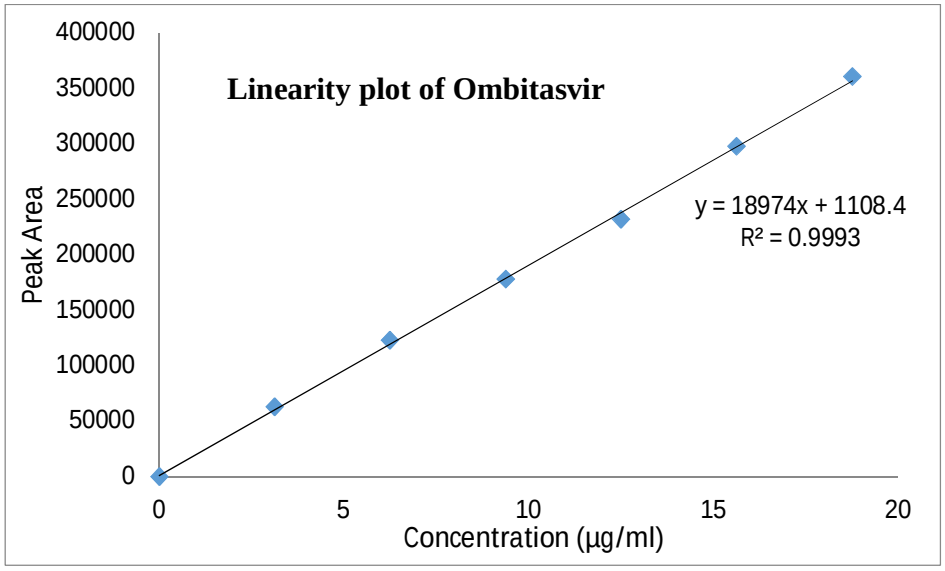

Fig.4C: Linearity plot of Ombitasvir

Specificity of the method was determined by comparing with placebo chromatogram. No interference of placebo peak was found with the drug peak, indicating the method to be specific. The placebo chromatogram was shown in figure 5.

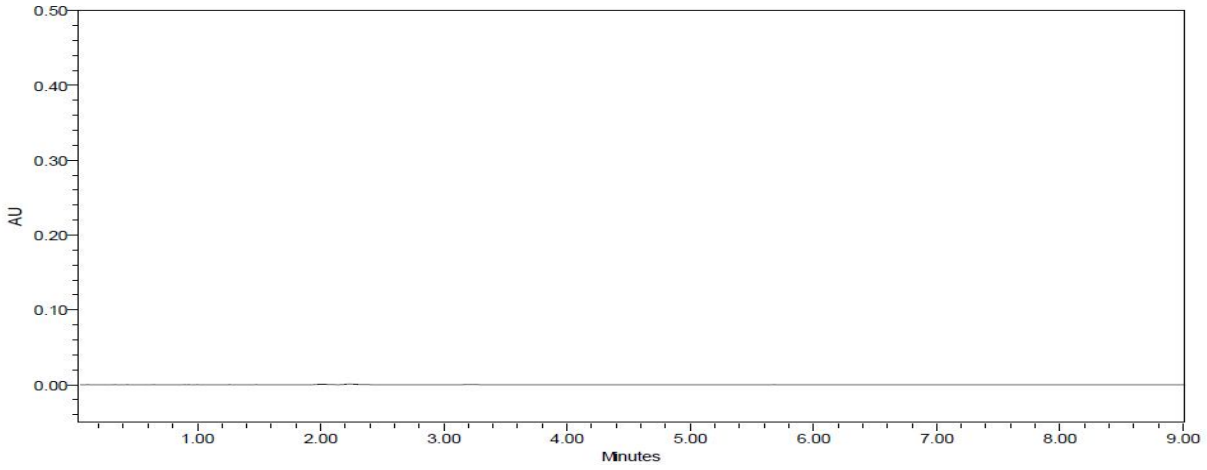

Fig.5: Placebo chromatogram

The method was found to be precise and accurate after determining the % RSD and % recoveries respectively. The % RSD was found to be 0.4 for Paritaprevir, 0.2 for Ritonavir and 0.3 for Ombitasvir. The % recovery was found to be 99.62% – 100.05% for Paritaprevir, 99.38% - 100.28% for Ritonavir and 98.88% - 99.80% for Ombitasvir. The method was also found to be rugged, robust and stable. All the validation parameters were summarized in table 1.

Table 1: SYSTEM SUITABILITY AND VALIDATION PARAMETER RESULTS

Parameter	**Paritaprevir**	**Ritonavir**	**Ombitasvir**
Specificity	Specific	Specific	Specific
Precision (%RSD)	0.4	0.2	0.3
Accuracy (% Recovery)	99.62%-100.05%	99.38%-100.28%	98.88%-99.80%
Linearity range (µg/ml)	12.5-75	18.75-112.5	3.13-18.75
Correlation coefficient, r	0.9996	0.9997	0.9996
Limit of Detection (µg/ml)	0.17	0.31	0.34
Limit of Quantitation (µg/ml)	0.52	0.93	1.04
Ruggedness (%RSD)	0.5	0.7	0.5
Robustness	Robust	Robust	Robust
Stability	Stable	Stable	Stable
USP Plate Count	4726	6741	9156
USP Tailing factor	1.05	1.16	0.98
USP Resolution		5.43	11.99

Forced degradation studies were conducted by exposing the standard drug solution to various stress conditions and net degradation was found to be within the limits. The forced degradation study results indicate that the method was stable in various stress conditions. The results were summarized in table 2 and chromatograms were shown in figure 6.

Table 2: FORCED DEGRADATION STUDIES RESULTS.

Drug	Parameters	Stress Condition					
		Acidic heat	Basic	Oxidative	Photolytic	Neutral	Dry
Paritaprevir	% Assay	95.35	95.51	97.33	98.81	99.36	98.69
	Purity Angle	0.160	0.338	0.342	0.174	0.161	0.202
Ritonavir	Purity Threshold	0.308	0.381	0.390	0.540	0.540	0.597
	% Assay	94.94	96.37	97.64	99.32	99.29	98.93
	Purity Angle	0.634	1.486	1.507	2.446	2.477	2.439
Ombitasvir	Purity Threshold	0.741	1.561	1.574	3.719	3.725	4.230
	% Assay	94.06	97.21	98.15	99.27	99.49	98.24
	Purity Angle	0.092	0.066	0.076	0.064	0.059	0.059
% Area of	Purity Threshold	0.305	0.283	0.296	0.290	0.291	0.290
	degradation Peak	1.07	0.82	0.87	-	-	-

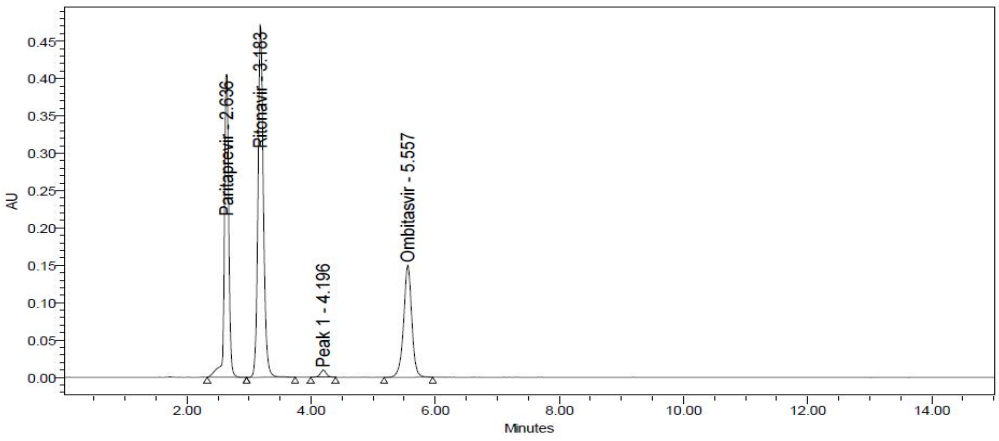

Fig.6A: Acid degradation chromatogram

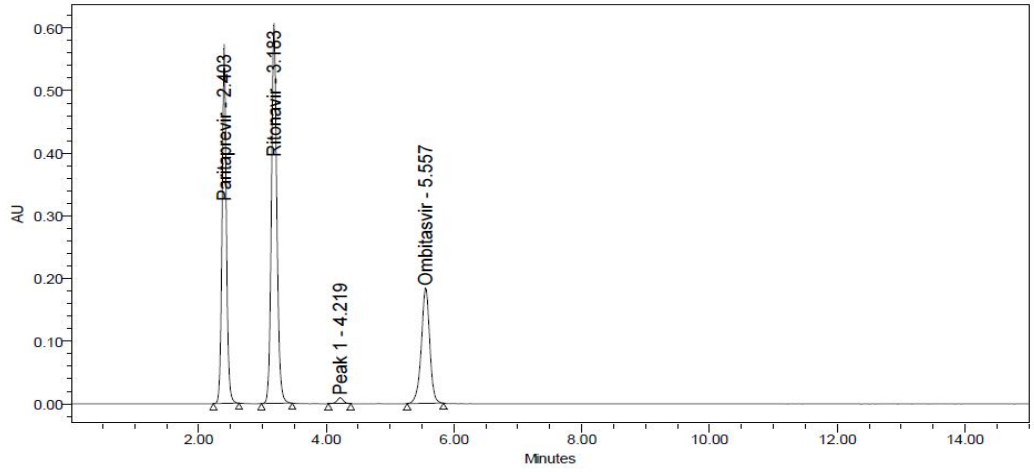

Fig.6B: Base degradation chromatogram

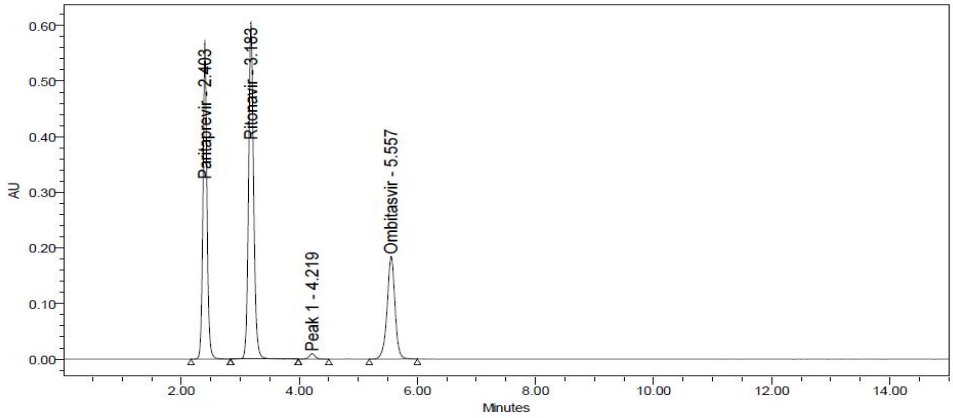

Fig.6C: Peroxide degradation chromatogram

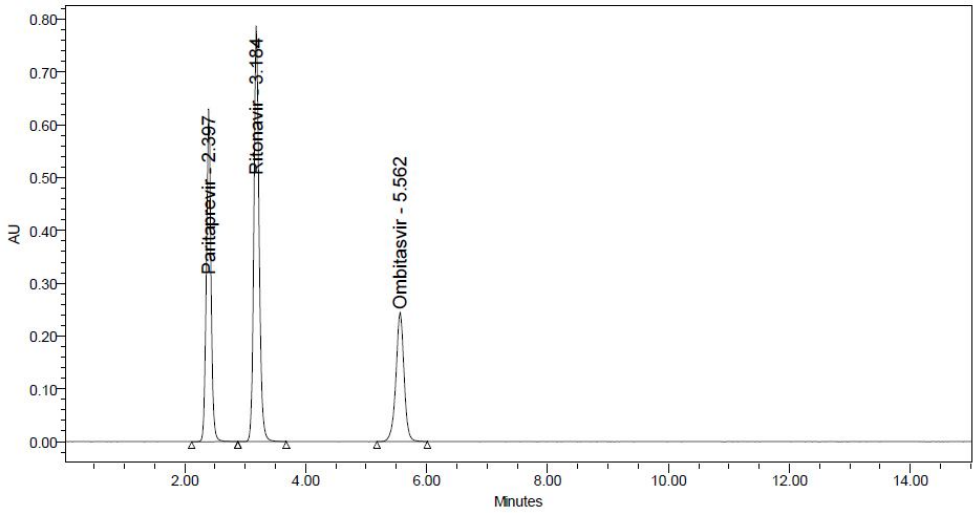

Fig.6D: Water stress study chromatogram

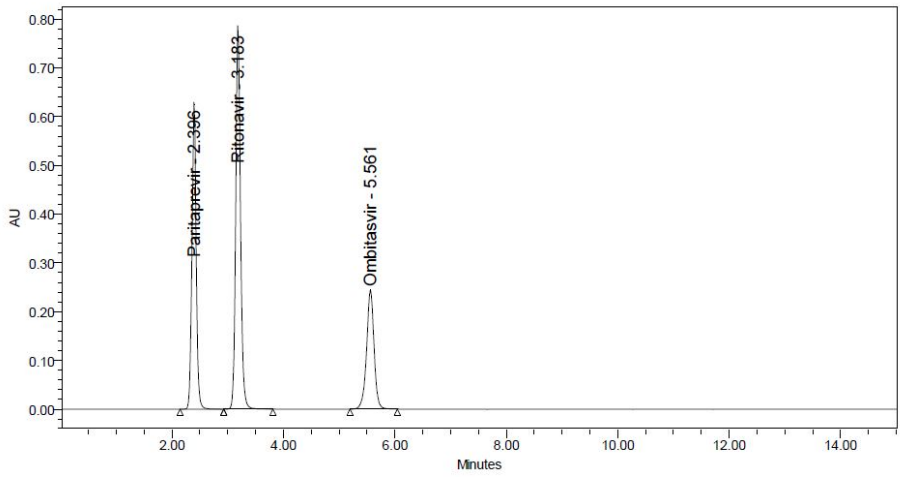

Fig.6E: Photo stability degradation chromatogram

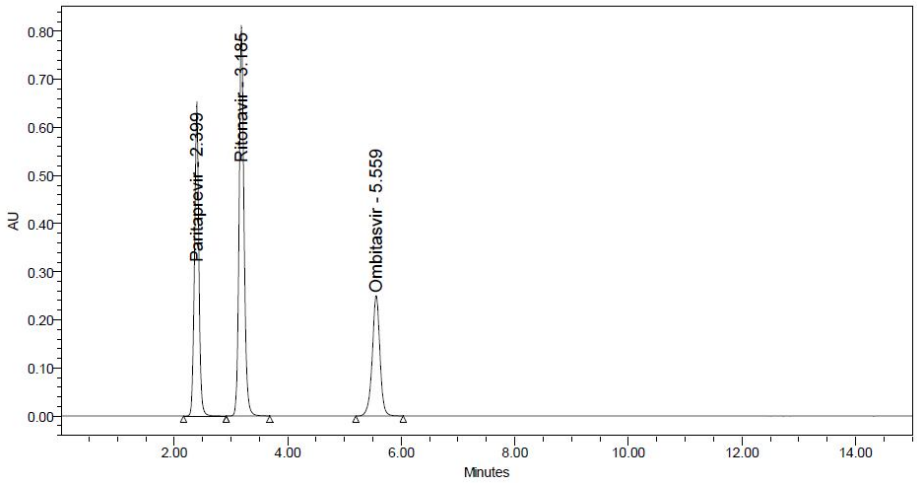

Fig.6F: Dry heat study chromatogram

CONCLUSION:

Stability indicating RP-HPLC method was developed for the simultaneous estimation of Paritaprevir, Ritonavir and Ombitasvir in pharmaceutical dosage form. The developed method was validated and found to be specific, accurate, precise, linear and robust. The drugs, Sofosbuvir and Velpatasvir were stable under different forced degradation conditions. The developed method can be used for the rapid quantification of Paritaprevir, Ritonavir and Ombitasvir in its pharmaceutical dosage form.

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