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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN TABLET

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ABSTRACT

A simple, specific, precise, accurate, rapid and robust stability indicating RP-HPLC method has been developed and subsequently validated for the simultaneous estimation of Sofosbuvir and Velpatasvir in tablet. In Reverse Phase High Performance Liquid Chromatography Method the chromatographic system was equipped with Inertsil ODS C_{18} column (150mm x 4.6mm, 5µm), as stationary phase and DAD detector set at 270 nm, in conjunction with a mobile phase of 0.05M Potassium Dihydrogen Phosphate buffer (pH- 3.5, adjusted with 1% Orthophosphoric acid) and Acetonitrile in the ratio of 60:40 % v/v at a flow rate of 1.0 ml/min. The described method was linear over a concentration range of 20 - 60 µg/ml and 5 – 15 µg/ml for Sofosbuvir and Velpatasvir respectively. The retention time of Sofosbuvir and Velpatasvir were 2.720 min and 4.430 min respectively. The % recoveries of Sofosbuvir and Velpatasvir were found to be 99.605% - 101.034% and 100.519% - 100.651% respectively. Method was statistically validated for accuracy, precision, specificity, LOD, LOQ and robustness according to ICH guidelines. Sofosbuvir and Velpatasvir were subjected to stress condition to check the degradation behaviour of them. Sofosbuvir was found stable in Acid and Oxidative condition. Velpatasvir was found stable in Base and Oxidative condition. Both the drug degraded in Thermal condition. The proposed method enables rapid quantification and simultaneous analysis of both drugs from commercial formulations without any interference of excipients. So, the method can be used for routine analysis of Sofosbuvir and Velpatasvir in tablet dosage form.

KEYWORDS: Sofosbuvir, Velpatasvir, RP-HPLC, Stability indicating, Method Validation.

INTRODUCTION

Sofosbuvir, is chemically designated as Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate and has the molecular wt. 529.453 g/mol (figure-1).[1] Sofosbuvir is a nucleotide hepatitis C virus (HCV) Nonstructural protein (NS) 5B polymerase inhibitors. Various analytical methods have been reported for the estimation of Sofosbuvir as alone. They include RP-HPLC,[2-7] Stability indicating RP-HPLC,[8] UV spectrophotometric methods.[9]

Velpatasvir, is chemically designated Methyl{(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxycarbonyl)amino]-2-phenylacetyl}-4-(methoxymethyl)-2-pyrrolidinyl]-1H-midazol-4-yl}-1,11-dihydroiso chromeno[4',3':6,7] naphtha[1, 2-d] imidazol-2-yl) -5 -methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl}carbamate and has the molecular wt. 883.02 g/mol (figure-2).[10] Velpatasvir is Velpatasvir is HCV NS5A replication complex inhibitors. There is not individual methods have been reported for the estimation of Velpatasvir as alone. Literature review reveals that there are various reported analytical methods are available for estimation of Sofosbuvir and Velpatasvir alone and with other combination.[11-16]

Literature survey does not reveals stability indicating RP-HPLC method for simultaneous estimation of Sofosbuvir and Velpatasvir in combined dosage form.

MATERIALS AND METHOD

Reagents and Chemicals

Sofosbuvir and Velpatasvir were obtained as gift samples from Advance Analytical Research Institute, Ahmedabad. The branded tablet formulation Velasof Tablet (sofosbuvir 400 mg and velpatasvir 100 mg) was purchased from the local market. HPLC grade Acetonitrile, Water and Ortho-phosphoric acid, and Potassium Dihydrogen Phosphate of analytical grade were obtained from Finar Chemicals Ltd.

Instruments and Chromatographic Conditions

The analysis was performed on Young lin (YL 9100) software. The separation was achieved on Inertsil ODS C_{18} column (150 x 4.6mm, 5µm) column. The column was maintained at

room temperature and the eluent was monitored at 270 nm using DAD detector. The mixture of 0.05M Phosphate Buffer pH 3.5 and Acetonitrile in proportion of 60:40%v/v at a flow rate of 1.0 ml/min was used as mobile phase. The injection volume is 10µl.

Preparation of Mobile Phase

Preparation of Diluted Ortho phosphoric acid

Diluted 1 mL of ortho phosphoric acid to 100 mL with HPLC grade water and mixed.

Preparation of 0.05 M phosphate buffer (pH 3.5)

Weigh accurately and transferred 3.4 gm of KH_2PO_4 into 500 mL of volumetric flask. Added 250 mL of HPLC grade water & shaken to dissolved. Diluted up to the mark with HPLC grade water and mixed. Adjusted pH 3.5 with diluted ortho phosphoric acid.

Preparation of mobile phase (0.05M phosphate buffer (KH_2PO_4) Acetonitrile, 60:40 % v/v)

Accurately measured 400 mL of Acetonitrile and 600 mL of above prepared 0.05M phosphate buffer (KH_2PO_4**),** Mix thoroughly and degassed by sonication.

Preparation of Diluents

Mixed water & Acetonitrile in the ratio (50:50 % v/v).

Preparation of stock solutions

Standard stock solution of Sofosbuvir (400 µg/ml)

A 40 mg of Sofosbuvir weighed accurately and transferred in to 100 ml volumetric flask and dissolved with few ml of diluent, then volume was made up to the mark with diluent and mixed thoroughly.

Standard stock solution of Velpatasvir (100 µg/ml)

A 10 mg of Velpatasvir weighed accurately and transferred in to 100 ml volumetric flask and dissolved with few ml of diluent, then volume was made up to the mark with diluent and mixed thoroughly.

Working standard solution of SOF and VEL (40: 10 µg/ml)

A 5 ml of standard stock solution of Sofosbuvir (400 µg/ml) and 5 ml of standard stock solution of Velpatasvir (100 µg/ml) were transferred in to 50 ml volumetric flask, volume was made up to the mark with diluent and mixed thoroughly.

Preparation of Sample solution

An average weight of 20 tablets was determined and tablets were crushed in to powder form. Accurately weighed amount of powder equivalent to 400 mg of Sofosbuvir and 100 mg of Velpatasvir was transferred in to 100 ml volumetric flask. About 40 ml of diluent was added and solution was sonicated for 30 min. to ensure complete solubilisation of drugs. Then solution was filtered through whatman filter paper and then volume was made up to the mark with diluent. 1ml of this solution was diluted to 10 ml with diluent. (400 µg/ml of Sofosbuvir and 100 µg/ml of Velpatasvir), Again 1ml of this solution was diluted to 10 ml with diluent. (Resulting solution containing 40 µg/ml of Sofosbuvir and 10 µg/ml of Velpatasvir).

Peak ID solution of Sofosbuvir (40 µg/ml)

A 2 ml of standard stock solution of Sofosbuvir (10 µg/ml) was transferred in to 20 ml volumetric flask, volume was made up to the mark with diluent and mixed thoroughly.

Peak ID solution of Velpatasvir (10 µg/ml)

A 2 ml of standard stock solution of Velpatasvir (10 µg/ml) was transferred in to 20 ml volumetric flask, volume was made up to the mark with diluent and mixed thoroughly.

Forced degradation study

Forced Degradation Studies of the drugs, in combination, were performed under different stress conditions as mentioned in ICH guideline Q1A (R2).[17-19] The standard solution containing 400µg/ml Sofosbuvir and 100µg/ml Velpatasvir was subjected to acidic, alkaline, oxidative, thermal condition. Acidic and alkaline degradation were performed up to 2N strength of acid/base at different temperature. Oxidative stress studies were carried out for using 3-10% H_2O_2.The sample solution containing Sofosbuvir was found stable in Acid and Oxidative condition. Velpatasvir was found stable in Base and Oxidative condition. Both the drug degraded in Thermal condition.

Acid degradation

Acid decomposition studies were performed by Transferring One ml of stock solution in to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and put for 8 hrs, Reflux. After this 2ml 0.1N NaOH solution was added to neutralize the reaction. After time period the volume was adjusted with diluent to get 40µg/ml for Sofosbuvir and 10µg/ml for Velpatasvir and filtered through 0.45 µm membrane filter paper and injected in

to HPLC system. Similarly solutions were prepared for respective conditions mentioned in Table.2, while Fig: 5 represent the graph for acidic degradation optimization.

Base degradation

Basic decomposition studies were performed by Transferring One ml of stock solution in to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 8 hrs, Reflux. After this 2ml 0.1N HCl solution was added to neutralize the reaction. After time period the volume was adjusted with diluent to get 40µg/ml for Sofosbuvir and 10µg/ml for Velpatasvir and filtered through 0.45 µm membrane filter paper and injected in to HPLC system. Similarly solutions were prepared for respective conditions mentioned in Table.2, while Fig: 6 represent the graph for basic degradation optimization.

Oxidative degradation

Oxidative decomposition studies were performed by Transferring One ml of stock solution in to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and put for 6 hrs, at RT. After time period the volume was adjusted with diluent to get 40µg/ml for Sofosbuvir and 10µg/ml for Velpatasvir and filtered through 0.45 µm membrane filter paper and injected in to HPLC system. Similarly solutions were prepared for respective conditions mentioned in Table.2, while Fig: 7 represent the graph for oxidative degradation optimization.

Thermal degradation

Thermal decomposition studies were performed by transferring 400mg Sofosbuvir and 100mg Velpatasvir drug powder in Petri dish. Petri dish was stored in oven at 80°C for 3hrs thermal hydrolysis. Powder equivalent to 40 mg Sofosbuvir and 10 mg Velpatasvir was weighed and transferred into 100 ml volumetric flask then volume was made up to the mark with diluent.(400µg/ml Sofosbuvir and 100µg/ml Velpatasvir) One ml of stock solution was transferred in to 10 ml of volumetric flask. The volume was adjusted with diluent to get 40µg/ml for Sofosbuvir and 10µg/ml for Velpatasvir and filtered through 0.45 µm membrane filter paper and injected in to HPLC system. Similarly solutions were prepared for respective conditions mentioned in Table.2, while Fig: 8 represent the graph for thermal degradation optimization.

METHOD VALIDATION

1) Specificity

Specificity was checked by comparison of chromatogram of diluents, chromatogram of Sofosbuvir (40 µg/ml), chromatogram of Velpatasvir (10µg/ml) and chromatogram of Standard Sofosbuvir and Velpatasvir (40:10 µg/ml) in combination.Fig.9-11.

2) Linearity and Range

The Linearity of peak area responses versus concentration was demonstrated by linear least square regression analysis. Good linearity (correlation coefficient >0.9977) was observed for Sofosbuvir and (correlation coefficient > 0.9974) was observed for Velpatasvir over the Concentration range of 20 to 60 µg/ml and 5-15 µg/ml, respectively. An aliquot of 10µl of each solution was injected 3 times for each standard solutions and peak area was observed. Plot of average peak area versus the concentration is plotted and from this the correlation coefficient and regression equation were generated. The calibration data of Sofosbuvir and Velpatasvir is given in Table 4-5, while Figure 12-13 represents linearity graphs of Sofosbuvir and Velpatasvir.

3). Precision

1. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Six repeated injections of standard solutions of 40 µg/ml of Sofosbuvir and 10 µg/ml of Velpatasvir were made and the response factor of drug peaks and % RSD were calculated. Repeatability data for Sofosbuvir and Velpatasvir are given in Table no-6.

2. Intraday precision

Combined solution containing the mixture of Sofosbuvir (20, 40, 60 µg/mL) and Velpatasvir (5, 10, 15 µg/mL) were analyzed for 3 times on the same day, peak areas were determined and %RSD was calculated. The result given in Table no-6.

3. Interday precision

Combined solution containing the mixture of Sofosbuvir (20, 40, 60 µg/mL) and Velpatasvir (5, 10, 15 µg/mL) were analyzed for 3 different days, peak areas were determined and %RSD was calculated. The result given in Table no-6.

4). Accuracy

The accuracy of the method was determined at 50%, 100%, and 150% level by calculating recoveries of Sofosbuvir and Velpatasvir by the standard addition method. Known amount of standard solutions of Sofosbuvir (10, 20, 30µg/ml) and Velpatasvir (2.5, 5, 7.5µg/ml) were added to a pre-quantified sample solution of Sofosbuvir (20 µg/ml) and Velpatasvir (5µg/ml). Each solution was injected in triplicate and the percentage recovery was calculated by measuring the peak areas and fitting these values into the regression equation of the respective calibration curves. The result given in Table no-7-8.

5). LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = (3.3× σ)/ S

LOQ = (10 × σ)/ S

Where, σ = standard deviation of the response, S = slope of the calibration curve.

The LOD and LOQ data of Sofosbuvir and Velpatasvir is given in Table-09.

6). Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation. The Robustness data of Sofosbuvir and Velpatasvir is given in Table-10.

1. Flow rate of mobile phase was changed (± 0.02 ml/min) 0.98 ml/min and 1.02 ml/min.
2. Ratio of Mobile phase was changed (±2) Buffer: Acetonitrile (58:42) and Buffer: Acetonitrile (62:38).
3. pH of buffer was changed (±0.2) pH 3.3 and pH 3.7.

7). Application to Tablet Dosage form

Applicability of proposed method was tested by analyzing tablet dosage form. The result is shown in Table-11.

RESULT AND DISCUSSION

System Suitability Study

The detection was carried out in the DAD detector set at 270 nm region. The different composition of mobile phase was testing and the composition giving retention time of 2.720

min for Sofosbuvir and 4.430 min for Velpatasvir with good resolution and theoretical plates and optimized Mobile phase was 0.05M Potassium Dihydrogen Phosphate buffer (pH- 3.5, adjusted with 1% Orthophosphoric acid) and Acetonitrile in the ratio of 60:40% v/v. A Chromatogram of the Mixture in optimized conditions is shown Figure and the system suitability parameters are shown in Table 1.

Table 1: Result for System Suitability Parameters.

Parameters	Sofosbuvir (mean ± SD) n=3	Velpatasvir (mean ± SD) n=3
Retention time (min)	2.720 ± 0.04	4.430 ± 0.05
Theoretical plate	4099 ± 73.45	7148± 27
Tailing factor	1.667 ± 0.11	1.345 ± 0.06
Resolution	9.011 ± 0.18	

Table 2: Results for Force Degradation.

Degradation method	Optimized Condition	% Degradation	
		Sofosbuvir	Velpatasvir
Acid	2N HCl for 24 hrs, Reflux.	3.384	18.764
Base	2N NaOH for 24 hrs, Reflux.	20.412	4.014
Oxidative	10% H_2O_2 for 24 hrs, at RT.	1.923	3.032
Thermal	80 °C for 6 hrs.	18.866	23.054

Table 3: Preparation of calibration curve.

Linearity Solution No.	Linearity Solutions		Volume standard stock solution of Sofosbuvir (400µg/ml)(ml)	Volume Standardstock solution of Velpatasvir (100µg/ml) (ml)	Diluted up to Mark with diluent (ml)
	Sofosbuvir (µg/ml)	Velpatasvir (µg/ml)			
1	20	5	0.5	0.5	10
2	30	7.5	0.75	0.75	10
3	40	10	1	1	10
4	50	12.5	1.25	1.25	10
5	60	15	1.5	1.5	10

Table 4. Data for linearity and range of Sofosbuvir.

Sofosbuvir							
Sr.No.	Concentration (µg/ml)	Area-1	Area-2	Area-3	Average Area	SD	%RSD
1	20	1240.252	1250.161	1269.400	1253.271	14.821	1.183
2	30	1987.946	1994.420	2012.780	1998.382	12.882	0.645
3	40	2543.489	2546.295	2525.942	2538.575	11.030	0.435
4	50	3268.535	3296.397	3240.049	3268.327	28.175	0.862
5	60	3849.441	3849.441	3844.566	3847.816	2.815	0.073

Table 5. Data for linearity and range of Velpatasvir.

Velpatasvir							
Sr. No	**Concentration (µg/ml)**	**Area-1**	**Area-2**	**Area-3**	**Average Area**	**SD**	**%RSD**
1	5	684.401	689.856	697.325	690.527	6.488	0.940
2	7.5	1096.425	1099.995	1110.105	1102.175	7.096	0.644
3	10	1402.949	1404.443	1387.019	1398.137	9.657	0.691
4	12.5	1814.894	1818.390	1787.219	1806.834	17.077	0.945
5	15	2123.887	2123.887	2108.908	2118.894	8.648	0.408

Table 6: Precision Study For Sofosbuvir and Velpatasvir.

Parameters	**Concentration (µg/ml)**		**%RSD**	
	Sofosbuvir	**Velpatasvir**	**Sofosbuvir**	**Velpatasvir**
Repeatability	40	10	0.8520.852	0.977
Intraday	20	5	0.295	0.534
	40	10	0.791	0.402
	60	15	0.551	0.433
Interday	20	5	0.724	1.119
	40	10	1.477	1.094
	60	15	0.938	1.009

Table 7: Recovery data for Sofosbuvir.

SR. NO.	**Conc. Level (%)**	**Sample amount (µg/ml)**	**Amount Added (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± S.D**
1	50 %	20	10	10.088	100.883	101.034 ± 1.547
2		20	10	10.265	102.650	
3		20	10	9.957	99.568	
4	100 %	20	20	19.924	99.619	99.605 ± 1.198
5		20	20	20.159	100.796	
6		20	20	19.680	98.400	
7	150 %	20	30	30.059	100.197	100.800 ± 0.578
8		20	30	30.405	101.351	
9		20	30	30.255	100.851	

Table 8: Recovery data for Velpatasvir.

SR. NO.	**Conc. Level (%)**	**Sample Amount**	**Amount Added**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± S.D**
1	50 %	5	2.5	2.470	98.804	100.519 ± 1.531
2		5	2.5	2.525	101.005	
3		5	2.5	2.544	101.747	
4	100 %	5	5	5.013	100.260	100.651 ± 0.683
5		5	5	5.072	101.440	
6		5	5	5.013	100.252	
7	150 %	5	7.5	7.547	100.621	100.606± 1.174
8		5	7.5	7.633	101.772	
9		5	7.5	7.457	99.425	

Table 9: LOD and LOQ For Sofosbuvir and Velpatasvir.

Sr.no	Drug	LOD	LOQ
1	Sofosbuvir	2.865 µg/ml	8.682 µg/ml
2	Velpatasvir	0.769 µg/ml	2.330 µg/ml

Table 10: Robustness study for Sofosbuvir and Velpatasvir.

Parameters		Area (n=3)	
		Sofosbuvir	Velpatasvir
pH (±0.2)	3.3	2490.107	1370.903
	3.5	2530.143	1394.221
	3.7	2508.315	1365.127
	Mean ± SD	2509.522 ± 20.04526	1376.75 ±15.4032
	% RSD	0.79	1.11
Flow Rate (±0.02 ml/min)	0.98 ml/min	2503.391	1367.246
	1.0 ml/min	2530.143	1394.221
	1.02 ml/min	2487.693	1356.166
	Mean ± SD	2507.076 ± 21.4635	1372.544 ±19.57294
	% RSD	0.85	1.42
Mobile PhaseComposition Buffer: ACN (± 2 mL)	58:42	2499.361	1360.335
	60:40	2530.143	1394.221
	62:38	2480.364	1380.332
	Mean ± SD	2503.289 ± 25.12093	1378.296 ± 17.0345
	% RSD	1.00	1.23

Table 11: Analysis of Formulation of Sofosbuvir and Velpatasvir by Proposed Method.

Sofosbuvir			Velpatasvir		
Labelled amount (mg)	**Amount found (mg)**	**% Assay**	**Labelled amount (mg)**	**Amount found (mg)**	**% Assay**
400 mg	395.834	98.958	**100 mg**	102.937	102.937
	394.428	98.607		102.576	102.576
	398.812	99.703		103.416	103.416
Mean ± SD	396.358 ±2.238	99.089±0.560	**Mean ± SD**	102.976 ±0.421	102.976 ±0.421
%RSD	0.565	0.565	**%RSD**	0.409	0.409

Figures

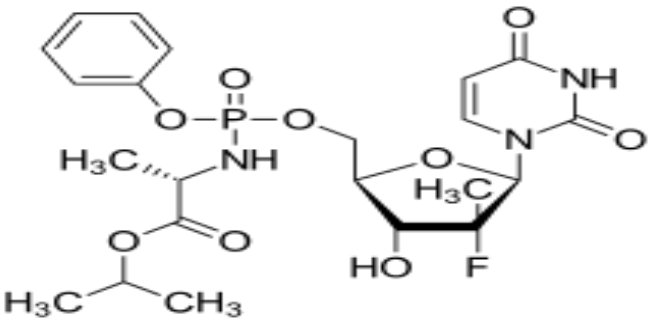

Fig. 1: Chemical Structure of Sofosbuvir.

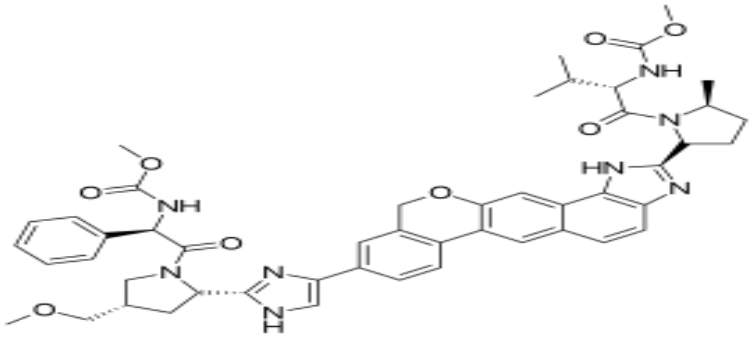

Fig. 2: Chemical Structure of Velpatasvir.

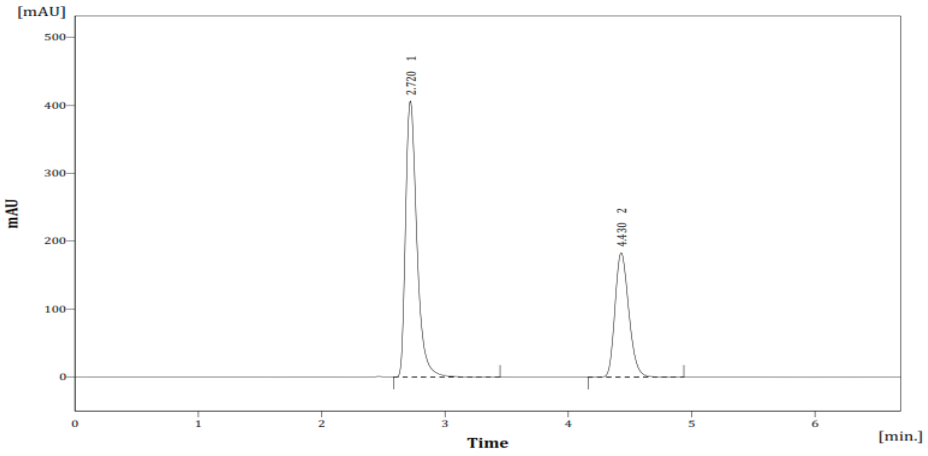

Fig 3: Chromatogram of Sofosbuvir (40µg/ml) and Velpatasvir (10µg/ml) in 0.05M KH_2PO_4 Buffer (pH 3.5): Acetonitrile (60:40%v/v).

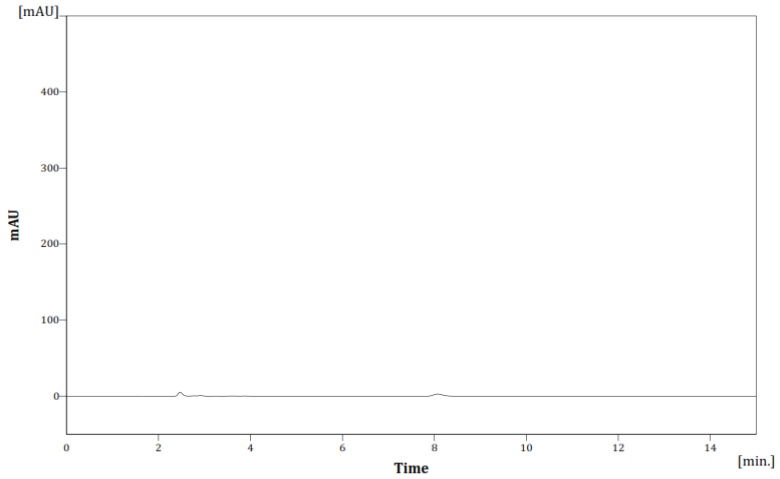

Fig.4: Chromatogram of Blank For Sofosbuvir and Velpatasvir.

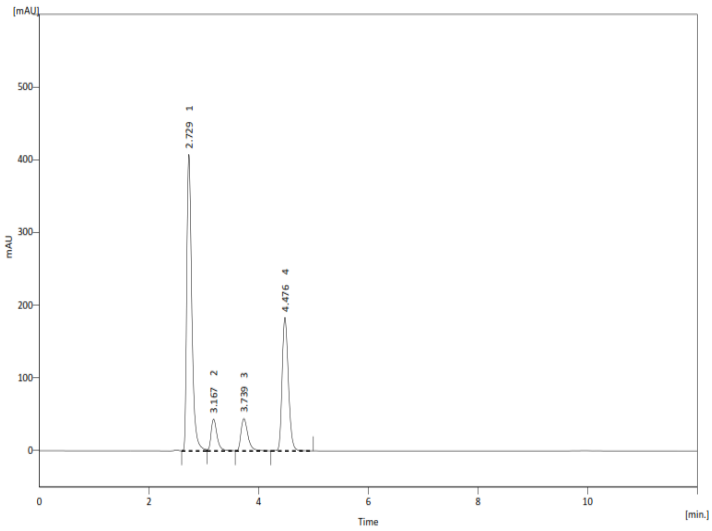

Fig. 5: Chromatogram of Optimized condition of Acid degradation For Sofosbuvir and Velpatasvir.

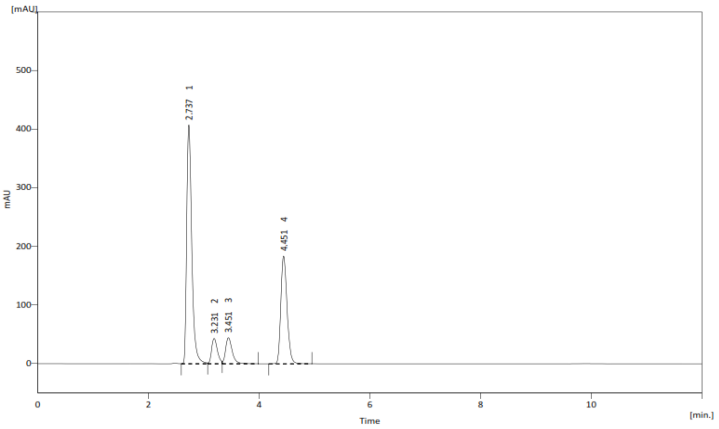

Fig. 6: Chromatogram of Optimized condition of Base degradation For Sofosbuvir and Velpatasvir.

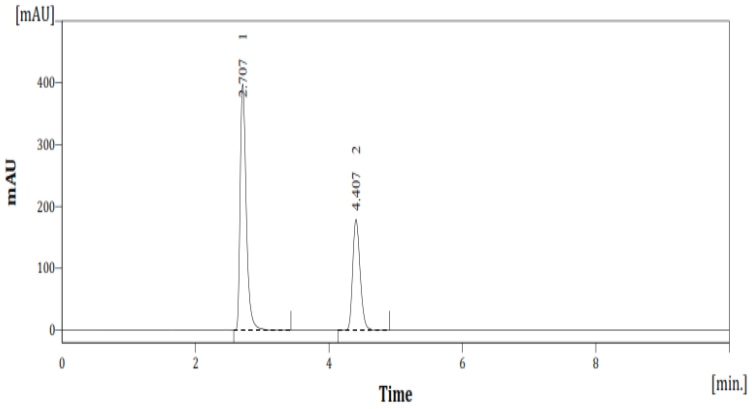

Fig. 7: Chromatogram of Optimized condition of Oxidative degradation For Sofosbuvir and Velpatasvir.

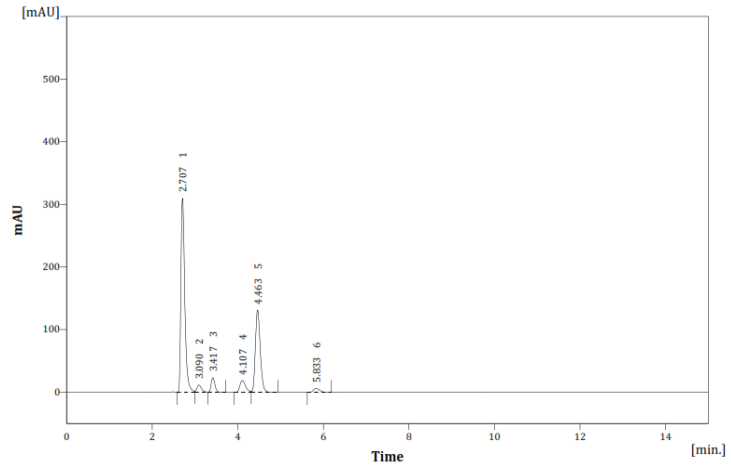

Fig. 8: Chromatogram of Optimized condition of Thermal degradation For Sofosbuvir and Velpatasvir.

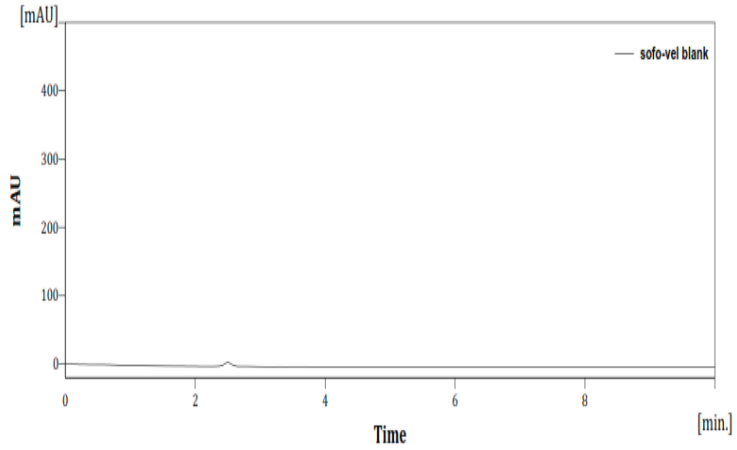

Fig. 9: Specificity chromatogram of Blank.

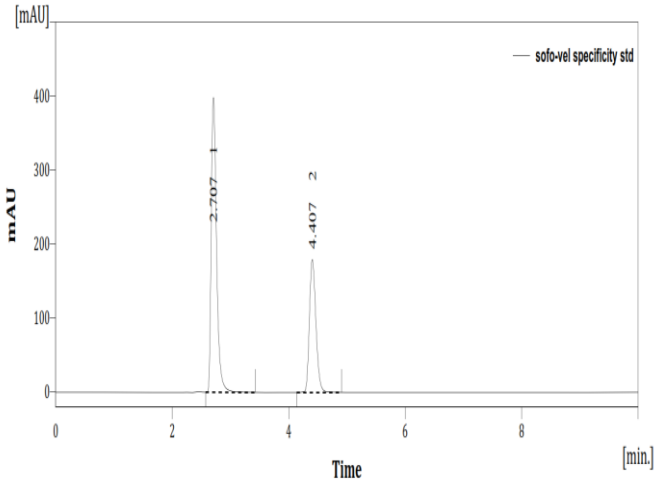

Fig. 10: Specificity chromatogram of standard Sofosbuvir and Velpatasvir (40:10µg/ml).

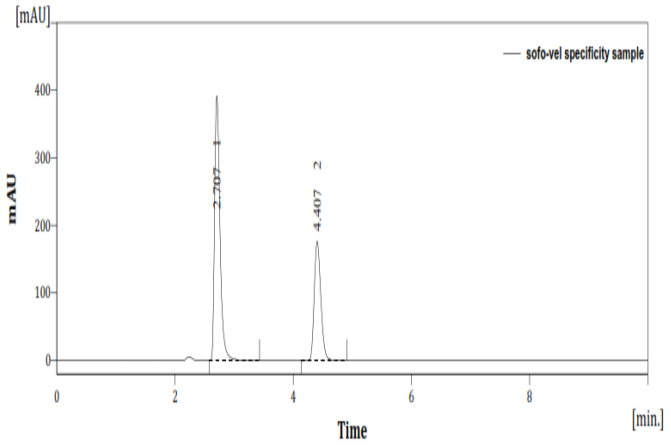

Fig. 11: Specificity chromatogram of sample Sofosbuvir and Velpatasvir (40:10µg/ml).

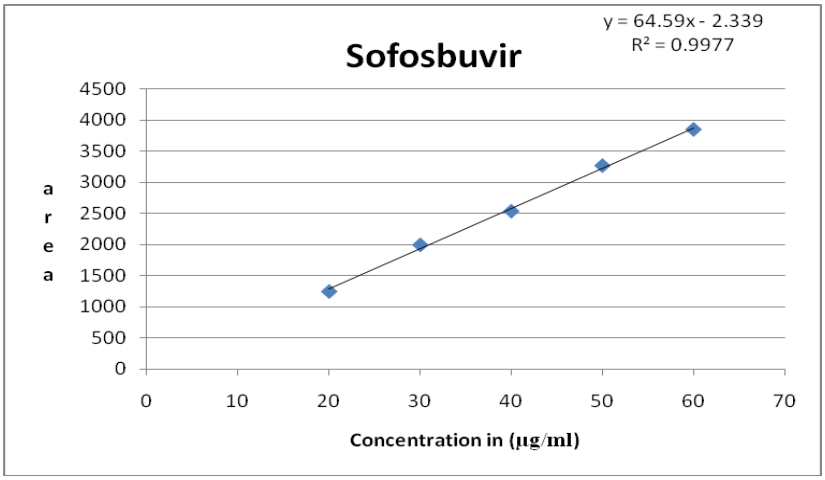

Fig. 12: Linearity Graph for Sofosbuvir.

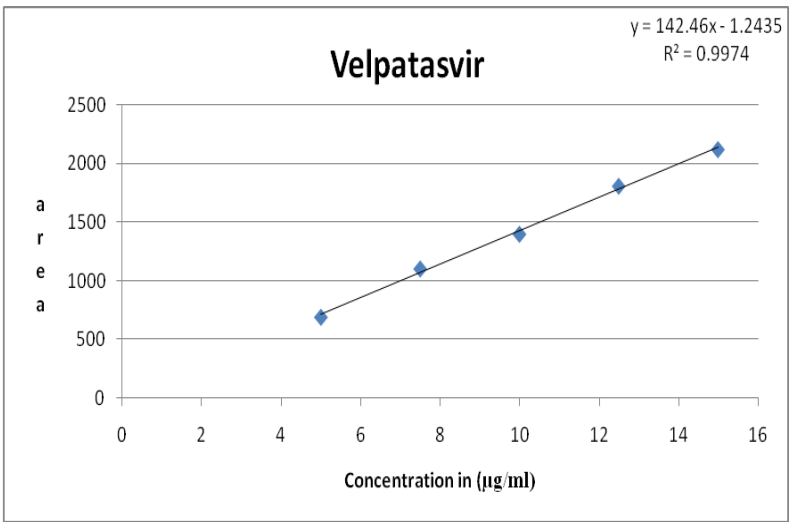

Fig. 13: Linearity Graph for Velpatasvir.

CONCLUSION

RP-HPLC method was developed and Forced degradation study was carried out. Sofosbuvir was found stable in Acid and Oxidative condition. Velpatasvir was found stable in Base and Oxidative condition. Both the drug degraded in Thermal condition. The developed method was validated and found to be simple, specific, precise, accurate, rapid and robust, as it separates components with good chromatographic criteria. All results were found satisfactory so, the stability indicating develop and validated method can be applied to the tablet dosage form.

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