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RP-HPLC Method Development and Validation for Simultaneous Estimation of Sofosbuvir and Velpatasvir in Bulk and Dosage Forms

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ABSTRACT

This study focuses on the development and validation of a stability-indicating RP-HPLC method for the simultaneous estimation of Sofosbuvir and Velpatasvir in bulk and pharmaceutical dosage forms. Sofosbuvir and Velpatasvir are critical antiviral drugs used in the treatment of Hepatitis C, and their accurate quantification is essential for quality control in pharmaceutical manufacturing. The proposed RP-HPLC method offers a simple, rapid, and reliable approach to quantify both drugs with high sensitivity, specificity, and reproducibility. The method was optimized using a Waters Alliance 2695 HPLC system with a Hypersil BDS C8 column, and the chromatographic separation was achieved with a mobile phase consisting of 50% buffer and 50% acetonitrile. Validation of the method was carried out following ICH guidelines, confirming its accuracy, precision, and robustness. The method demonstrated excellent linearity with correlation coefficients of 0.999 for both drugs and showed low limits of detection (LOD) and quantification (LOQ), indicating high sensitivity. Forced degradation studies, including oxidation, acid, alkali, and thermal degradation, were conducted to assess the stability of Sofosbuvir and Velpatasvir under different conditions, and the method proved to be robust and suitable for routine quality control applications in the pharmaceutical industry. The assay results for both drugs were within acceptable limits, ensuring the method's reliability for routine use in pharmaceutical quality testing.

KEYWORDS: RP-HPLC, Sofosbuvir, Velpatasvir, Stability-Indicating Method, Pharmaceutical Quality Control..

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major global health concern that can lead to chronic liver disease, cirrhosis, and hepatocellular carcinoma. Combination therapy with direct-acting antiviral agents (DAAs) such as Sofosbuvir and Velpatasvir has emerged as an effective treatment strategy for HCV infection, offering high cure rates across multiple genotypes. Sofosbuvir, chemically known 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*, is a nucleotide analogue prodrug. It inhibits the HCV nonstructural protein 5B (NS5B), an RNA-dependent RNA polymerase responsible for viral RNA replication by catalyzing the polymerization of ribonucleoside triphosphates. By targeting NS5B, Sofosbuvir effectively blocks viral genome replication.

Velpatasvir, *Methyl{(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxy carbonyl) amino]-2-phenyl acetyl}-4-(methoxy methyl)-2-pyrrolidinyl]-1H-imidazol-4-yl}-1,11-dihydroisochrome no [4',3':6,7] naphtho[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate*, is a potent inhibitor of the HCV nonstructural protein 5A (NS5A), which plays a crucial role in viral replication and assembly. Additionally, Velpatasvir acts as a substrate for the transporter proteins P-glycoprotein (P-gp) and ATP-binding cassette superfamily G member 25.

Despite their therapeutic importance, limited analytical methods are available for the simultaneous quantification of Sofosbuvir and Velpatasvir, either individually or in combination with other antiviral drugs. Reported techniques include HPLC [6–12], HPTLC [13,14], LC–MS [15–17], and UV spectrophotometry [18,19]. However, most of these methods suffer from drawbacks such as longer run times, limited sensitivity, or lack of stability-indicating capacity. Therefore, the present study was undertaken to develop and validate a simple, rapid, and reliable stability-indicating RP-HPLC method for the simultaneous estimation of Sofosbuvir and Velpatasvir in bulk and pharmaceutical dosage forms. The developed method was optimized to achieve satisfactory sensitivity, selectivity, and reproducibility within a short chromatographic run, making it suitable for routine quality control applications.

MATERIALS AND METHODS

2.1 Materials

The RP-HPLC analysis was performed using a Waters Alliance 2695 HPLC system equipped with a 2998 Photodiode Array (PDA) detector, autosampler, and Empower 2 software for data acquisition and processing. Chromatographic separation was achieved on a Hypersil BDS C8 column (4.6 × 50 mm, 5 µm). Additional equipment included an ultrasonic bath sonicator

(Frontline FS 4, Mumbai, India), Denver electronic balance, and Whatman filter paper No. 41.

2.2 Reagents and Chemicals

Sofosbuvir and Velpatasvir were obtained as gift samples from Hetero Drugs Limited (Hyderabad, India). HPLC-grade acetonitrile was purchased from SD Fine Chemicals (India). Ortho-phosphoric acid and potassium dihydrogen phosphate (HPLC grade) were procured from Rankem Ltd. (India). Milli-Q water was used throughout the study.

2.3 Preparation of Buffer

Potassium dihydrogen phosphate (1.36 g) was accurately weighed and dissolved in 900 mL of Milli-Q water in a 1000 mL reagent bottle. The pH of the solution was adjusted to 3.5 with 0.1% ortho-phosphoric acid. The final volume was made up to 1000 mL with water, and the buffer was filtered through 0.45 µm membrane filter before use.

2.4 Preparation of Mobile Phase

The mobile phase consisted of buffer and acetonitrile in the ratio of 50:50 (v/v).

- **Diluent-1:** Acetonitrile:Water (50:50, v/v)
- **Diluent-2:** Buffer:Acetonitrile (50:50, v/v)

2.5 Preparation of Standard Stock Solutions

Sofosbuvir: Accurately weighed 40 mg of Sofosbuvir was transferred into a 50 mL volumetric flask. About 10 mL of Diluent-1 was added and the solution was sonicated for 10 min. The volume was then made up to the mark with Diluent-2 to obtain a concentration of 800 µg/mL. Velpatasvir: Accurately weighed 5 mg of Velpatasvir was transferred into a 50 mL volumetric flask. About 10 mL of Diluent-1 was added and the solution was sonicated for 10 min. The volume was then made up to the mark with Diluent-2 to obtain a concentration of 100 µg/mL.

2.6 Preparation of Standard Working Solutions

Aliquots of 1 mL each from Sofosbuvir and Velpatasvir stock solutions were transferred into a 10 mL volumetric flask and diluted with Diluent-2 to yield working standard solutions containing 80 µg/mL of Sofosbuvir and 10 µg/mL of Velpatasvir.

2.7 Preparation of Sample Stock Solution

Ten tablets were weighed and the average weight was calculated. A quantity equivalent to one tablet was finely powdered and transferred to a 50 mL volumetric flask. About 10 mL of Diluent-1 was added and the mixture was sonicated for 25 min. The volume was then made up with Diluent-1 and the solution filtered. The filtrate was centrifuged at 3000 rpm for 5 min and further diluted with Diluent-1 to obtain a concentration of 800 µg/mL of Sofosbuvir and 100 µg/mL of Velpatasvir.

2.8 Preparation of Sample Working Solutions

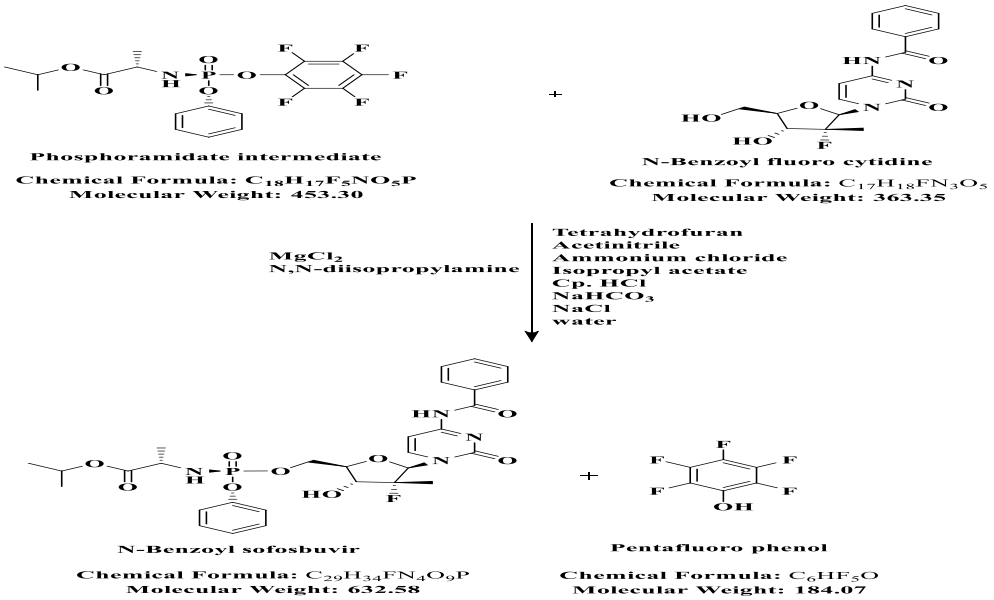
An aliquot of 1 mL of the prepared sample stock solution was transferred into a 10 mL volumetric flask and diluted with Diluent-2 to obtain a working solution containing 80 µg/mL of Sofosbuvir and 10 µg/mL of Velpatasvir.

2.9 Assay Procedure

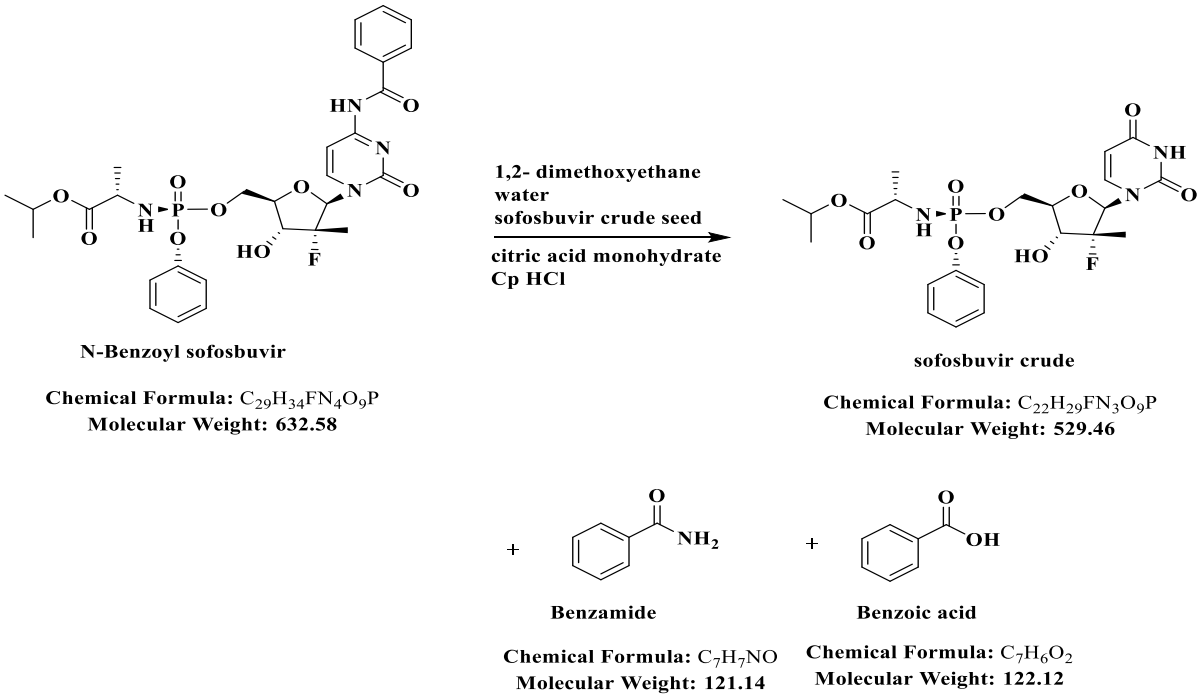
An aliquot of 10 µL of both standard and sample working solutions was injected six times into the chromatographic system under optimized conditions. Chromatograms were recorded and the peak areas of Sofosbuvir and Velpatasvir were compared to calculate the assay results

Sofusbuvir Preparation:

- Stage-1:



- Stage-II



N-Benzoyl sofosbuvir

Chemical Formula: $C_{29}H_{34}FN_4O_9P$
Molecular Weight: 632.58

sofosbuvir crude

Chemical Formula: $C_{22}H_{29}FN_3O_9P$
Molecular Weight: 529.46

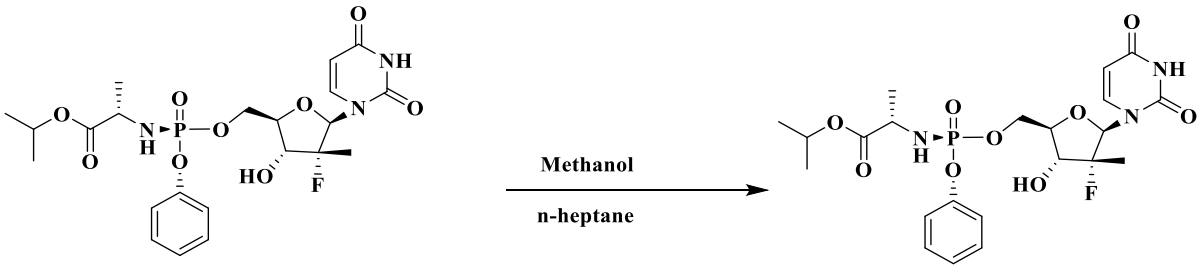
Benzamide

Chemical Formula: C_7H_7NO
Molecular Weight: 121.14

Benzoic acid

Chemical Formula: $C_7H_6O_2$
Molecular Weight: 122.12

- Stage-III



sofosbuvir crude

Chemical Formula: $C_{22}H_{29}FN_3O_9P$
Molecular Weight: 529.46

sofosbuvir

Chemical Formula: $C_{22}H_{29}FN_3O_9P$
Molecular Weight: 529.46

Sofosbuvir is 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 which was shown in figure.

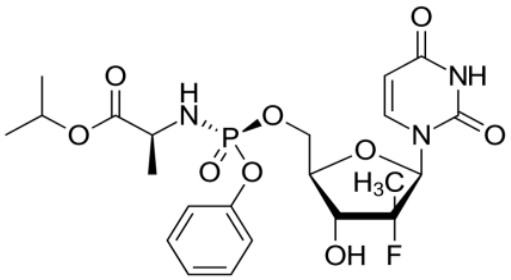
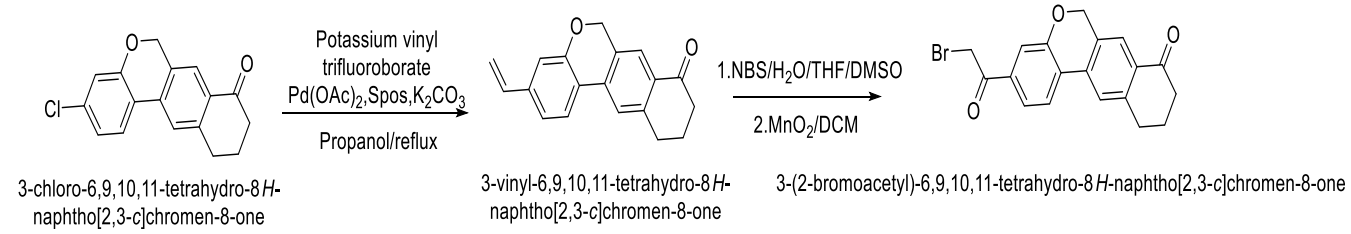


Fig. No.1 Structure of Sofosbuvir

Sofosbuvir inhibits the hepatitis C Nonstructural protein 5B (NS5B) is a viral protein found in the hepatitis C virus (HCV). It is an RNA-dependent RNA polymerase, having the key function of replicating HCV's viral RNA by using the viral positive RNA strand as a template to catalyze the polymerization of ribonucleoside triphosphates (rNTP) during RNA replication[1-4].

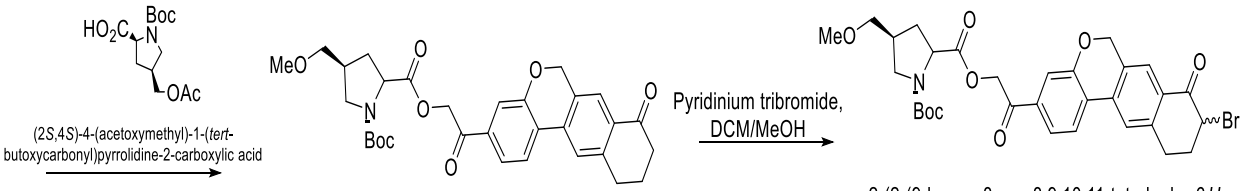
Velpatasvir Preparation:



3-chloro-6,9,10,11-tetrahydro-8*H*-naphtho[2,3-*c*]chromen-8-one

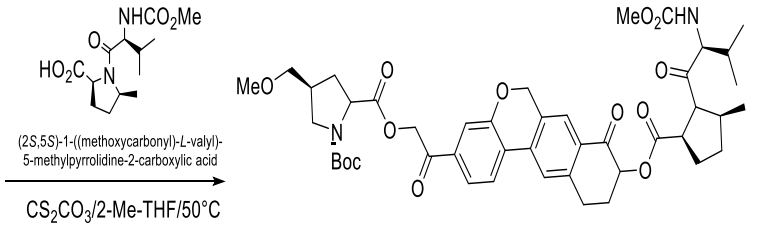
3-vinyl-6,9,10,11-tetrahydro-8*H*-naphtho[2,3-*c*]chromen-8-one

3-(2-bromoacetyl)-6,9,10,11-tetrahydro-8*H*-naphtho[2,3-*c*]chromen-8-one

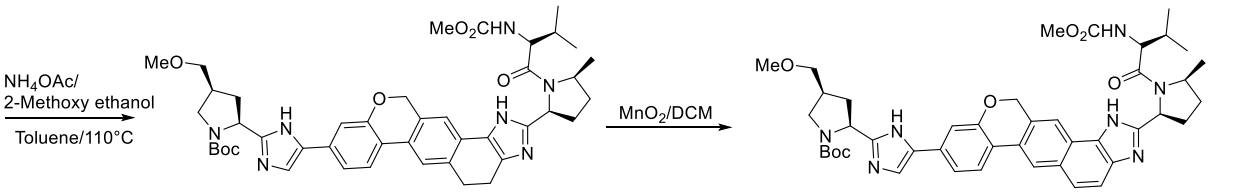


1-(*tert*-butyl) 2-(2-oxo-2-(8-oxo-8,9,10,11-tetrahydro-6*H*-naphtho[2,3-*c*]chromen-3-yl)ethyl) (4*S*)-4-(methoxymethyl)pyrrolidine-1,2-dicarboxylate

2-(2-(9-bromo-8-oxo-8,9,10,11-tetrahydro-6*H*-naphtho[2,3-*c*]chromen-3-yl)-2-oxoethyl) 1-(*tert*-butyl) (4*S*)-4-(methoxymethyl)pyrrolidine-1,2-dicarboxylate

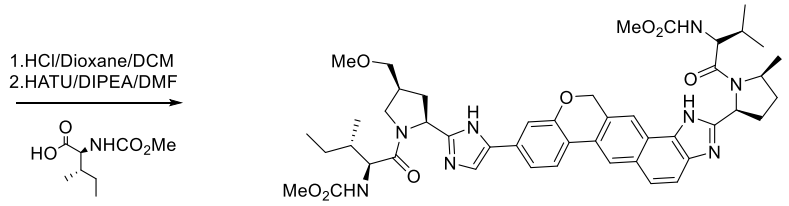


1-(*tert*-butyl) 2-(2-(9-(((1*R*,3*S*)-2-((methoxycarbonyl)-*L*-valyl)-3-methylcyclopentane-1-carbonyl)oxy)-8-oxo-8,9,10,11-tetrahydro-6*H*-naphtho[2,3-*c*]chromen-3-yl)-2-oxoethyl) (4*S*)-4-(methoxymethyl)pyrrolidine-1,2-dicarboxylate



tert-butyl (2*S*,4*S*)-2-(5-(2-((2*S*,5*S*)-1-((methoxycarbonyl)-*D*-valyl)-5-methylpyrrolidin-2-yl)-1,4,5,12-tetrahydrochromeno [4',3':6,7]naphtho[1,2-*d*]imidazol-9-yl)-1*H*-imidazol-2-yl)-4-(methoxymethyl)pyrrolidine-1-carboxylate

tert-butyl (2*S*,4*S*)-2-(5-(2-((2*S*,5*S*)-1-((methoxycarbonyl)-*D*-valyl)-5-methylpyrrolidin-2-yl)-1,12-dihydrochromeno [4',3':6,7]naphtho[1,2-*d*]imidazol-9-yl)-1*H*-imidazol-2-yl)-4-(methoxymethyl)pyrrolidine-1-carboxylate



Methyl ((*R*)-1-((2*S*,5*S*)-2-(9-(2-((2*S*,4*S*)-1-((methoxycarbonyl)-*L*-isoleucyl)-4-(methoxymethyl)pyrrolidin-2-yl)-1*H*-imidazol-5-yl)-1,12-dihydrochromeno[4',3':6,7]naphtho[1,2-*d*]imidazol-2-yl)-5-methylpyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate

➢ Velpatasvir is Methyl{(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxy carbonyl) amino]-2-phenyl acetyl}-4-(methoxy methyl)-2-pyrrolidinyl]-1H-imidazol-4-yl}-1,11-dihydroisochrome no [4',3':6,7] naphtho[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate which was shown in figure.

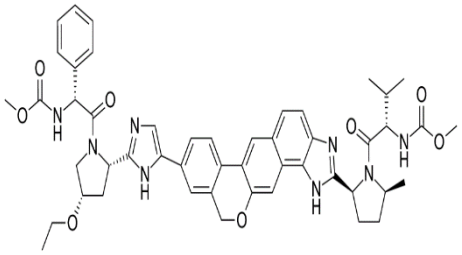


Fig. No.2 Structure of Velpatasvir

Velpatasvir is an inhibitor of Nonstructural protein 5A (NS5A) and a substrate of the transporter proteins P-glycoprotein (Pgp) and ATP-binding cassette super-family G member 2[5]. Literature review reveals that very few methods were reported for the quantification of Sofosbuvir and Velpatasvir individually and combined with other drugs. However, there is few work was reported either single or combination with other drugs for the quantification of Sofosbuvir and Velpatasvir by HPLC[6-12], HPTLC[13, 14], LC-MS[15-17] and UV Spectrophotometric[18, 19] method. Hence, in the present study an attempt has been made by the author to develop a rapid and reliable HPLC method for determination of Sofosbuvir and Velpatasvir. The HPLC technique was successfully employed to provide a satisfactory, sensitivity and selectivity in a desirable time of chromatographic run.

Standard and sample solution for assay studies

➢ An aliquot of 10µL of standard and sample solution contains 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir were injected six times into the chromatographic system and peaks eluted asper below figure-1 & 2

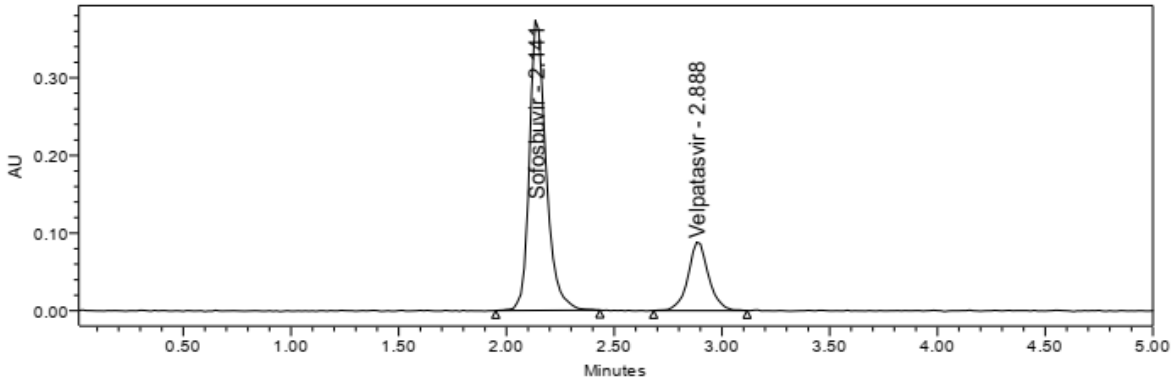


Fig-1 Chromatogram of working standard solution

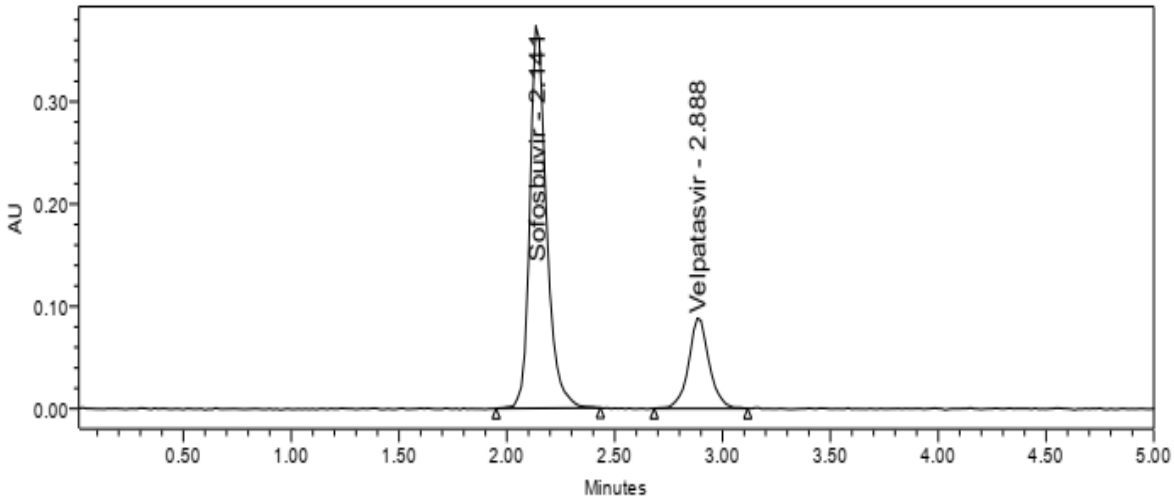


Fig-2 Chromatogram of working sample solution

➢ Area for Sofosbuvir and Velpatasvir were measured and assay % was calculated by comparing the peak area of standard and sample chromatogram was shown in Table-1.

➢ **Assay:** Assay was performed with the above solution. Average % Assay for Sofosbuvir and Velpatasvir obtained was 99.84% and 99.87% respectively.

Table-1: Assay Data of Sofosbuvir

S.no	Standard Area	Sample area	% Assay
1	2089767	2090768	100.26
2	2097171	2079433	99.72
3	2084873	2090725	100.26
4	2054911	2087749	100.12
5	2073048	2061219	98.84
6	2099592	2082340	99.86
Avg	2083227	2082039	99.84
SD	16811.5	11176.8	0.54
%RSD	0.8	0.5	0.54

Table-2: Assay Data of Velpatasvir

S.no	Standard Area	Sample area	% Assay
1	587753	590756	99.83
2	583205	591666	99.99
3	592039	594272	100.43
4	597173	589801	99.67
5	591419	588396	99.43
6	595330	590832	99.85
Avg	394069	590954	99.87
SD	5084.8	1971.9	0.33
%RSD	0.9	0.3	0.33

Table-3: Results for assay studies

Drug	Label claim (mg/tablet)	Amount found* (mg/tablet)	Label claim %	RSD %
Sofosbuvir	400	399.36	99.84	0.54
Velpatasvir	100	99.87	99.87	0.33

* Mean of six determinations

- **Method Validation:**

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Sofosbuvir and Velpatasvir (80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%. Results are tabulated and Chromatogram as shown below:

Table-4 System suitability parameters for Sofosbuvir and Velpatasvir.

Sl no	Sofosbuvir			Velpatasvir			
Inj	**RT(min)**	**TP**	**Tailing**	**RT(min)**	**TP**	**Tailing**	**Resolution**
1	2.136	3967	1.27	2.871	4916	1.05	4.7
2	2.138	3902	1.27	2.884	4927	1.11	4.9
3	2.141	4049	1.28	2.887	4989	1.04	4.8
4	2.141	4031	1.28	2.888	4844	1.05	4.7
5	2.152	4154	1.28	2.897	5099	1.06	4.9
6	2.156	4146	1.30	2.907	5166	1.07	4.8

TP: Theoretical plates

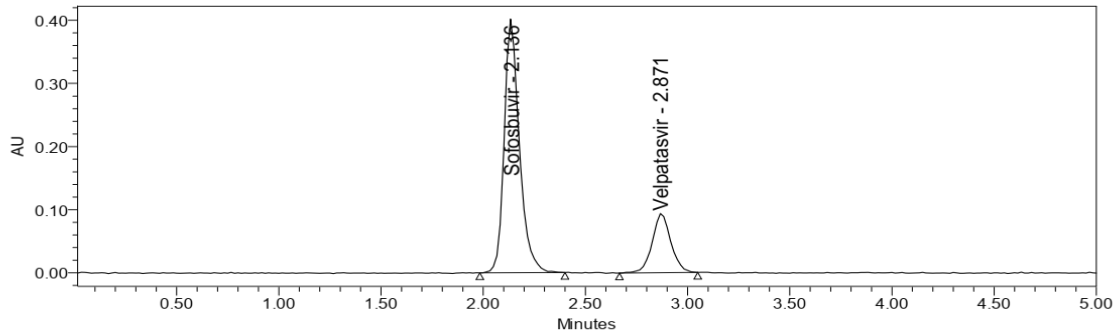


Fig-3 Optimized Chromatogram

Discussion: Plate count, tailing factor, resolution of Sofosbuvir and Velpatasvir wasaccording to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

2. Specificity:

Checking of the interference in the optimized method, it was found that no interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. Chromatograms are as shown below:

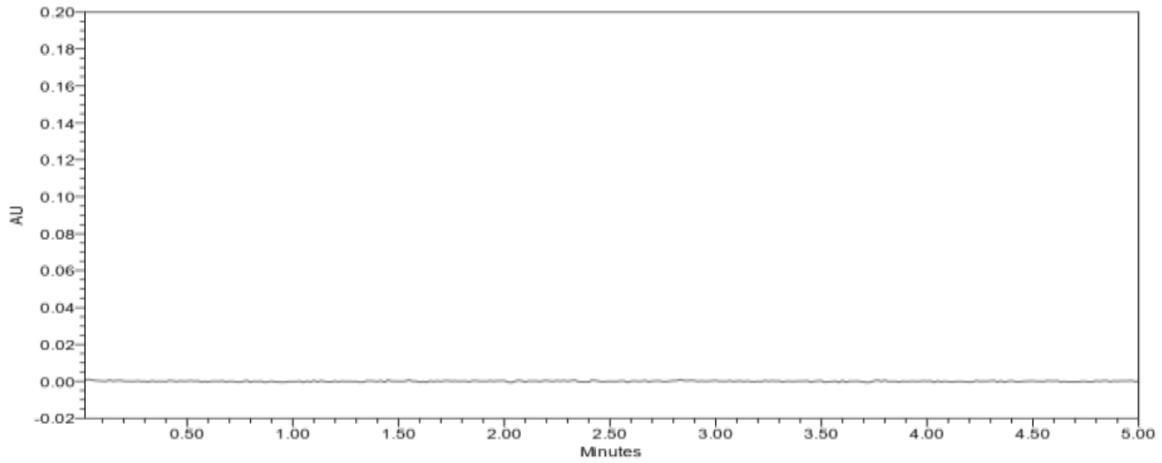


Fig-4 Blank chromatogram

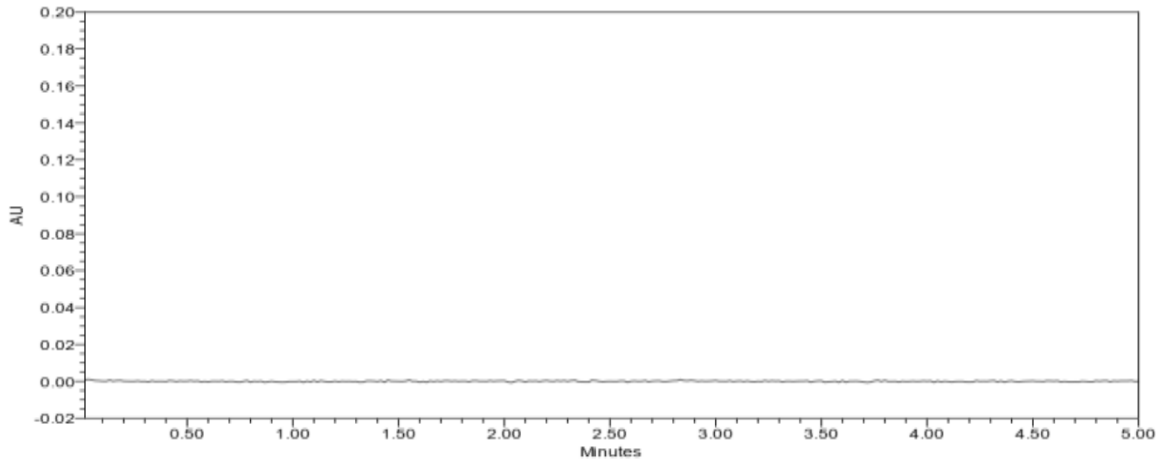


Fig-5 Placebo chromatogram

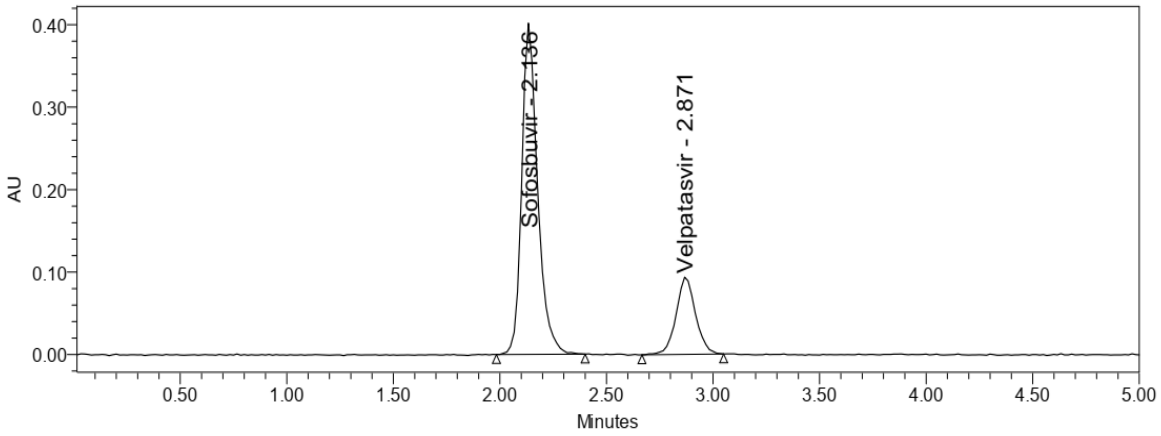


Fig-6 Optimized Chromatogram

Discussion: Retention times of Sofosbuvir and Velpatasvir were 2.136min and2.871min respectively. We did not found and interfering peaks in blank and placeboat retention times of these drugs in this method. So this method was said to be specific.

3. Precision:

Preparation of Sample stock solutions: 10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was taken and finely powdered with motor and pestle and transferred into a 50 mL volumetric flask, 10mL of diluent-1 added and sonicated for 25 min, further the volume made up with diluent-2 and filtered and the resulting solution was centrifuged at 3000 rpm for 5 min and after suitable dilution the sample solution was then filtered using 0.45-µm nylon filter to get the concentration 800µg/ml of Sofosbuvir and 100µg/ml Velpatasvir.

Preparation of Sample working solutions: From the filtered solution 1 ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent-2 to get the concentration 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir. The precision was determined by preparing sample solutions of 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir and the solutions were injected six times, the % RSD for the area of six standard injections results should not be more than 2%.

Optimized method:

Chromatographic conditions:

- **Mobile phase** : **50**% 0.1N KH2PO4 buffer: 50% Acetonitrile
- **Flow rate** : **1.0** mL/min
- **Column** : BDS C8 (4.6 x 50mm, 5µm)
- **Detector wave length** : 288.0nm
- **Column temperature** : 30°C
- **Injection volume** : 10□L
- **Run time** : 9 min
- **Results** : Both peaks have good resolution, tailing theoretical plate count and resolution.

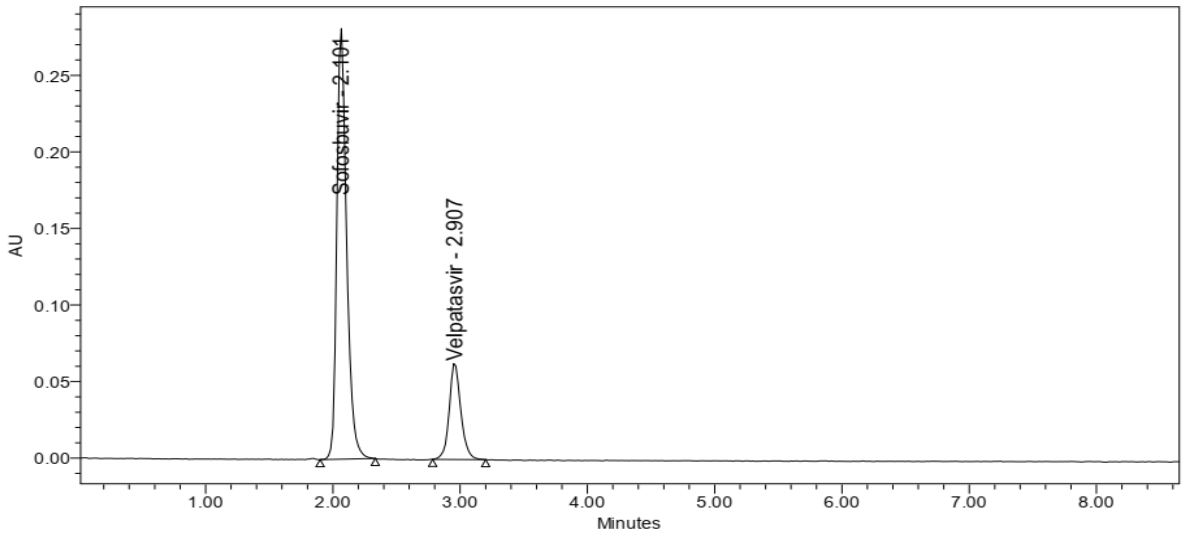


Fig-7 Optimized Chromatogram

Observation: Sofosbuvir and Velpatasvir were eluted at 2.101min and 2.907min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and proceeded for validation

Method Precision:**Table-5: Repeatability table of Sofosbuvir and Velpatasvir.**

S. No	Concentration (µg/ml)	Area of Sofosbuvir	Concentration (µg/ml)	Area of Velpatasvir
1.	80	2090768	10	590756
2.	80	2079433	10	591666
3.	80	2090725	10	594272
4.	80	2087749	10	589801
5.	80	2061219	10	588396
6.	80	2082340	10	590832
Mean		2082039	Mean	590954
S.D		11176.8	S.D	1971.9
%RSD		0.5	%RSD	0.3

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each work sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.3% respectively for Sofosbuvir and Velpatasvir. As the limit of Precision was less than "2" the system precision was passed in this method.

4. Linearity:

Preparation of Standard stock solutions: Accurately weighed 40mg of Sofosbuvir and 5mg of Velpatasvir transferred to 50ml volumetric flask. 10ml of diluent-1 was added to flask and sonicated for 10mins. Flask was made up with the diluent-2 to get the concentration 800µg/ml of Sofosbuvir and 100µg/ml Velpatasvir.

- 25% Standard solution: 0.25ml each from three standard stock solutions was pipette out and made up to 10ml to get the concentration 20µg/ml of Sofosbuvir and 2.5µg/ml of Velpatasvir.
- 50% Standard solution: 0.5ml each from three standard stock solutions was pipette out and made up to 10ml to get the concentration 40µg/ml of Sofosbuvir and 5µg/ml of Velpatasvir.
- 75% Standard solution: 0.75ml each from three standard stock solutions was pipette out and made up to 10ml to get the concentration 60µg/ml of Sofosbuvir and 7.5µg/ml of Velpatasvir.
- 100% Standard solution: 1.0ml each from three standard stock solutions was pipette out and made up to 10ml to get the concentration 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir.
- 125% Standard solution: 1.25ml each from three standard stock solutions was pipette out and made up to 10ml to get the concentration 100µg/ml of Sofosbuvir and 12.5µg/ml of Velpatasvir.
- 150% Standard solution: 1.5ml each from three standard stock solutions was pipette out and made up to 10ml to get the concentration 120µg/ml of Sofosbuvir and 15µg/ml of Velpatasvir.
- Above mentioned 25%,50%75%,100%,125% and 150% solutions are injected into the HPLC system and results are tabulated below:

Table-6: Linearity table for Sofosbuvir and Velpatasvir.

Sofosbuvir		**Velpatasvir**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
20	518858	2.5	151547
40	1047581	5	297597
60	1502795	7.5	447465
80	2085606	10	592112
100	2542313	12.5	751020
120	3078920	15	892625

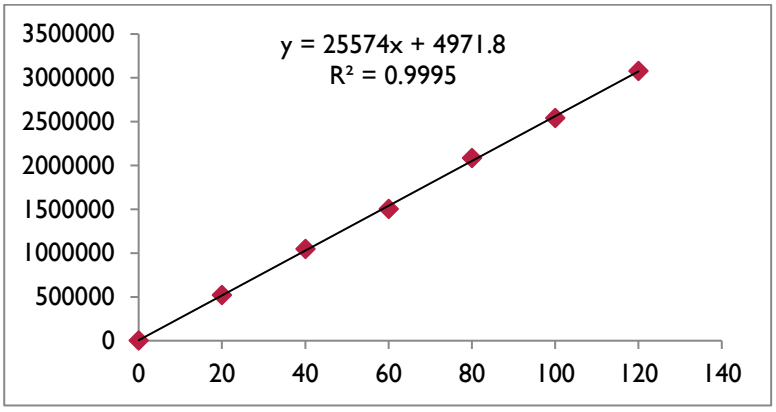


Fig-8 Calibration curve of Sofosbuvir

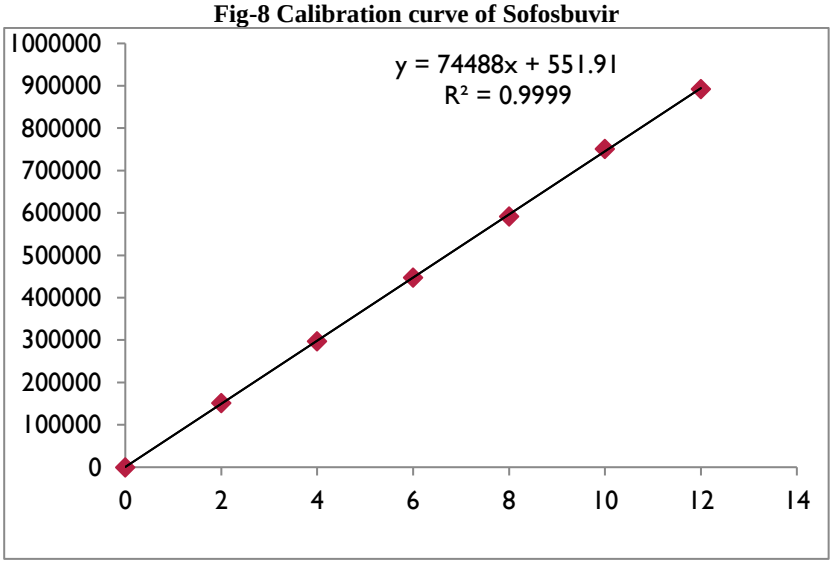


Fig-9 Calibration curve of Velpatasvir

Discussion: Six linear concentrations of Sofosbuvir (20-120µg/ml) and Velpatasvir (2.5-15µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Sofosbuvir was y = 25574x + 4971 and Velpatasvir was y = 74488x + 551.9. Correlation coefficient obtained was 0.999 for the two drugs.

5. Accuracy:

Preparation of Standard stock solutions: Accurately weighed 40mg of Sofosbuvir and 5mg of Velpatasvir transferred to 50ml volumetric flask. 10ml of Diluent-1 was added to flask and sonicated for 10mins. Flask was make up with the diluent-2 to get the concentration 800µg/ml of Sofosbuvir and 100µg/ml Velpatasvir.

Preparation of Sample stock solutions: 10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was taken and finely powdered with motor and pestle and transferred into a 50 mL volumetric flask, 10mL of diluent-1 added and sonicated for 25 min, further the volume made up with diluent-2 and filtered and the resulting solution was centrifuged at 3000 rpm for 5 min and after suitable dilution the sample solution was then filtered using 0.45-µm nylon filter to get the concentration 800µg/ml of Sofosbuvir and 100µg/ml Velpatasvir.

- **Preparation of 50% Spiked Solution**: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent-2.
- **Preparation of 100% Spiked Solution**: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent-2.
- **Preparation of 150% Spiked Solution**: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent-2.
- **Above 50%,100% and 150% Spiked solutions are injected into HPLC system and results are summarized below**:

Table-7: Accuracy table of Sofosbuvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	40	40.13	100.31	100.11%
	40	39.83	99.56	
	40	39.82	99.56	
100%	80	80.44	100.55	
	80	79.76	99.70	
	80	79.99	99.99	
150%	120	122.77	102.30	
	120	119.29	99.40	
	120	119.51	99.59	

Table-8: Accuracy table of Velpatasvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	4.97	99.37	99.31%
	5	4.93	98.56	
	5	4.97	99.50	
100%	10	9.94	99.39	
	10	9.86	98.60	
	10	9.96	99.64	
150%	15	14.89	99.27	
	15	14.98	99.88	
	15	14.94	99.58	

Discussion: Three levels of Accuracy samples were prepared by standard addition method.Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 100.11% and 99.31% for Sofosbuvir and Velpatasvir respectively.

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.

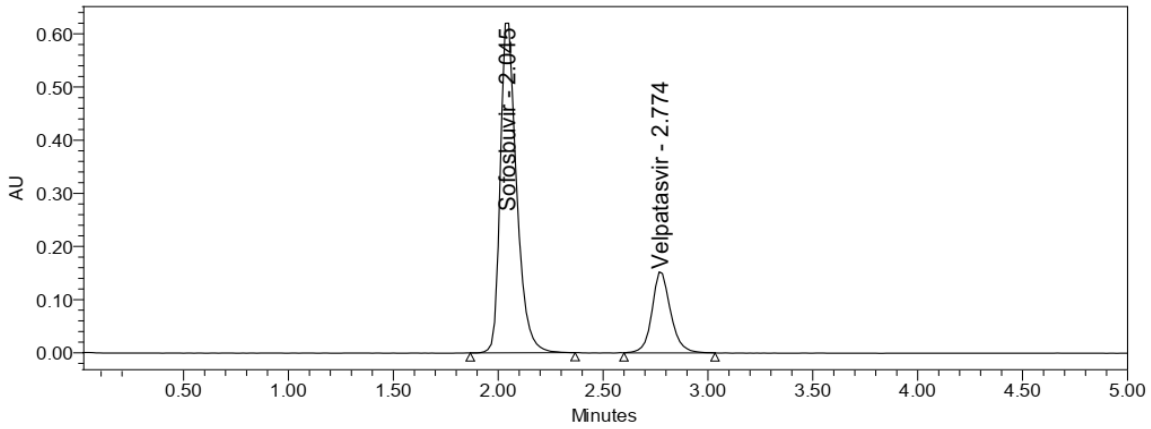


Fig -10 Accuracy 50% Chromatogram of Sofosbuvir and Velpatasvir

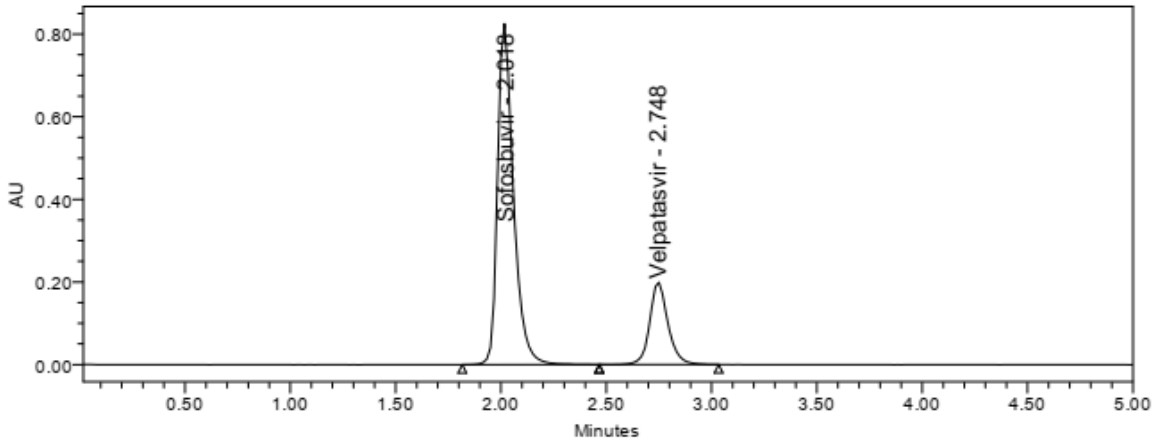


Fig 11 Accuracy 100% Chromatogram of Sofosbuvir and Velpatasvir

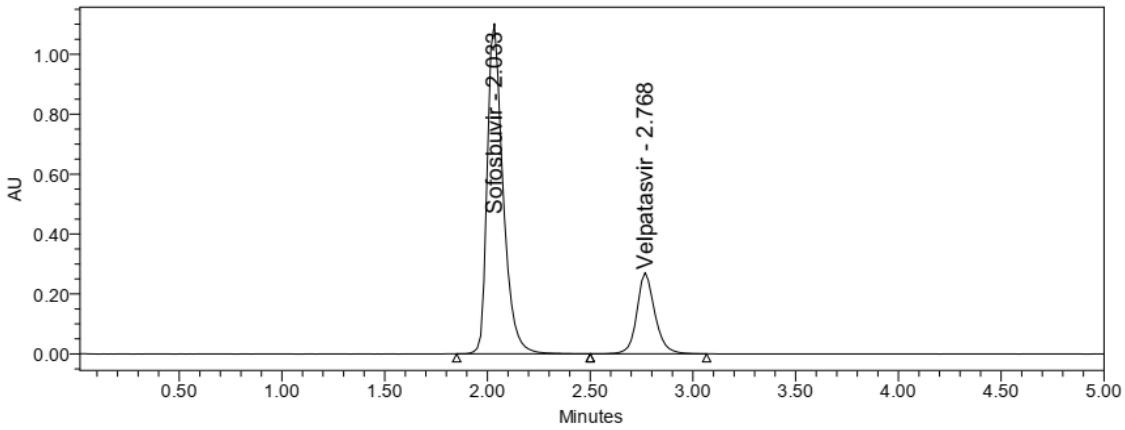


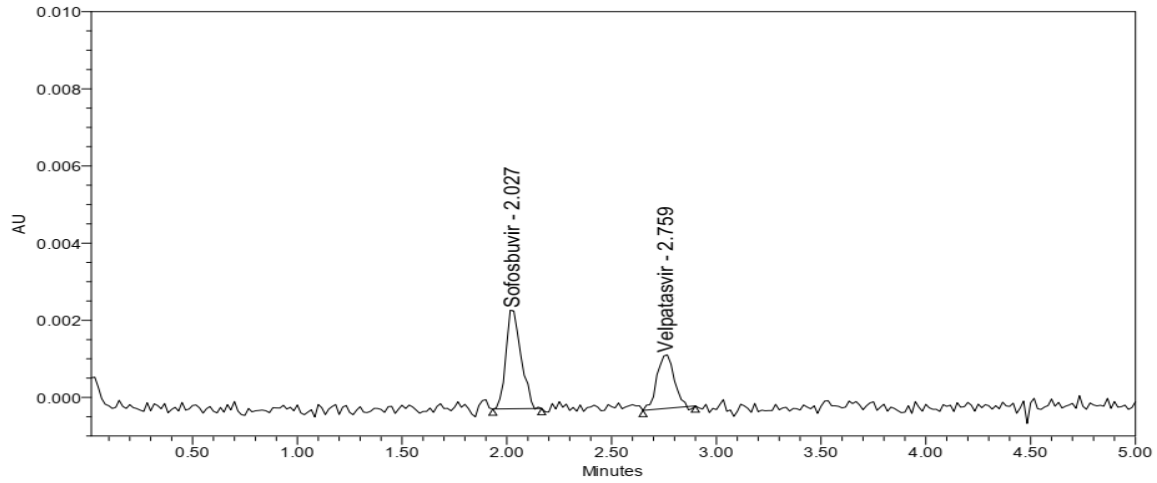
Fig 12 Accuracy 150% Chromatogram of Sofosbuvir and Velpatasvir

6. Sensitivity:

LOD sample Preparation: 0.25ml of standard stock solutions was pipette out and transferred to 10ml volumetric flask and made up with diluent-2 from the above solutions 0.1ml of Sofosbuvir and Velpatasvir solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25ml of standard stock solutions was pipette out and transferred to 10ml volumetric flask and made up with diluent-2 from the above solutions 0.1ml of Sofosbuvir and Velpatasvir solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

Fig 13 LOD Chromatogram of Standard



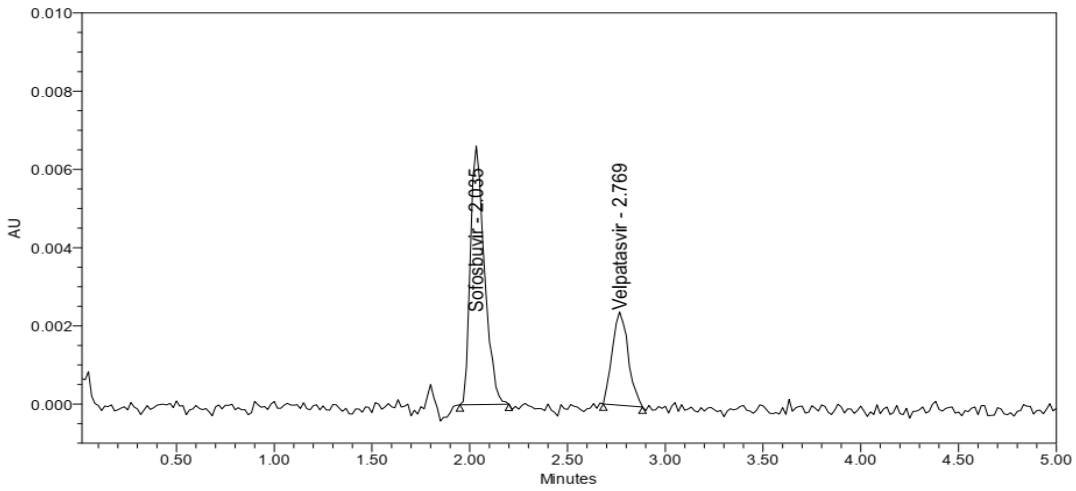


Fig 14 LOQ Chromatogram of Standard

Table-9: Sensitivity table of Sofosbuvir and Velpatasvir.

Drugs	LOD(µg/ml)	LOQ(µg/ml)
Sofosbuvir	0.13	0.40
Velpatasvir	0.01	0.04

7. Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guide lines. **Robustness conditions**: Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°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.

Table-10: Robustness data for Sofosbuvir and Velpatasvir

S.no	Condition	%RSD of Sofosbuvir	%RSD of Velpatasvir
1	Flow rate (-) 0.9ml/min	0.4	0.2
2	Flow rate (+) 1.1ml/min	0.4	0.6
3	Mobile phase (-) 35B:65A	0.6	0.1
4	Mobile phase (+) 45B:55A	0.8	0.4
5	Temperature (-) 25°C	0.4	0.1
6	Temperature (+) 35°C	0.3	0.5

Discussion: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (35B:65A), mobile phase plus (45B:55A), temperature minus (25°C) and temperature plus (35°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.

Robustness conditions chromatograms:

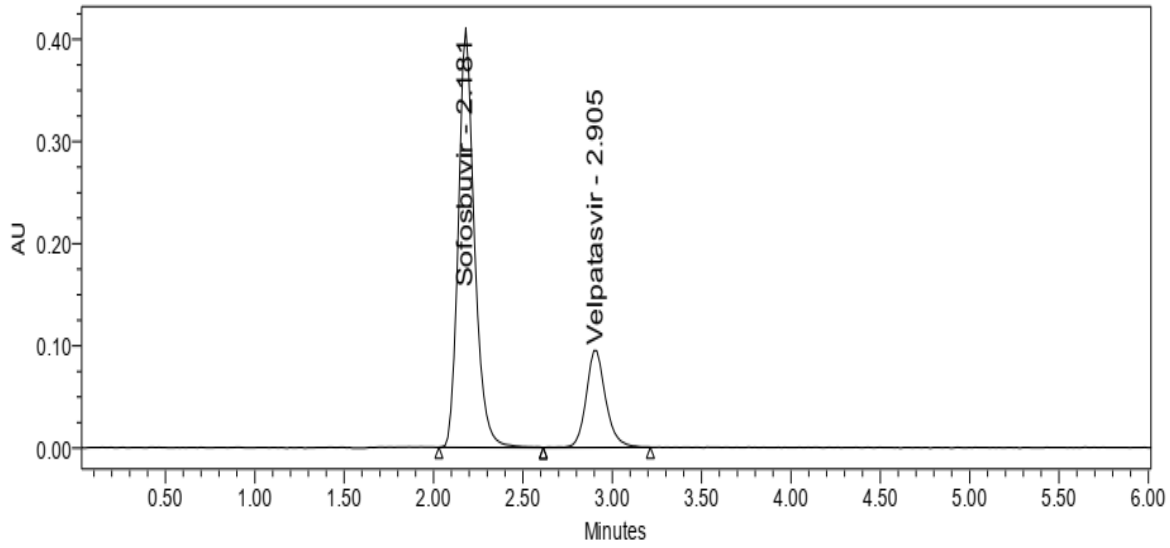


Fig 15 Flow minus Chromatogram of Sofosbuvir and Velpatasvir.

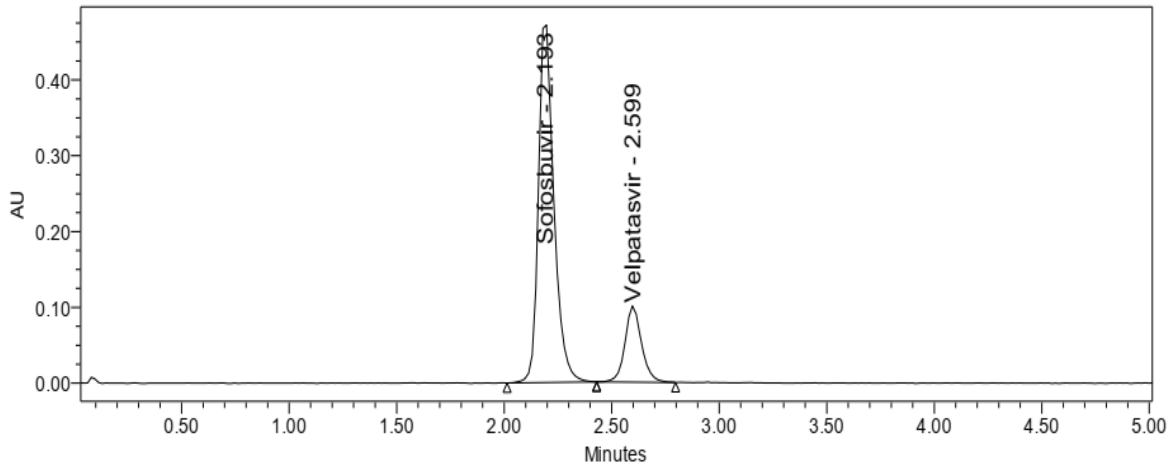


Fig 16 Flow plus Chromatogram of Sofosbuvir and Velpatasvir.

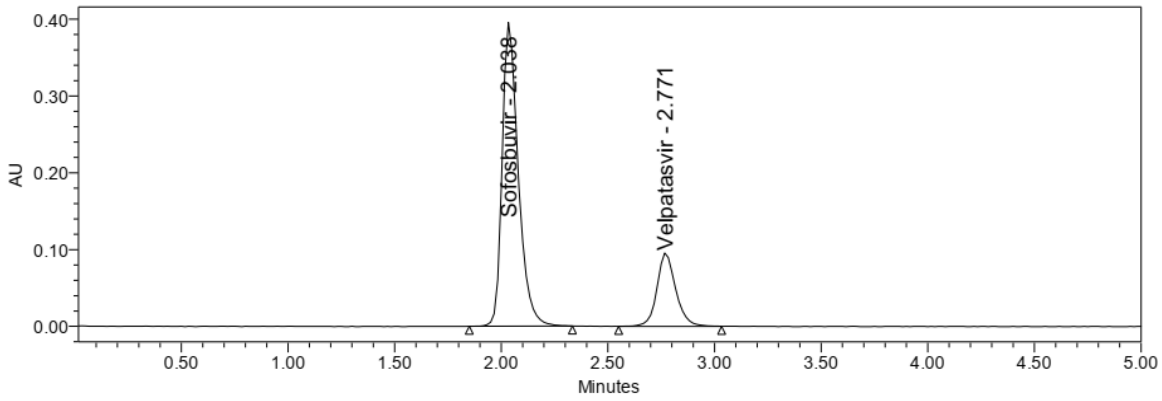


Fig 17 Mobile phase minus Chromatogram of Sofosbuvir and Velpatasvir

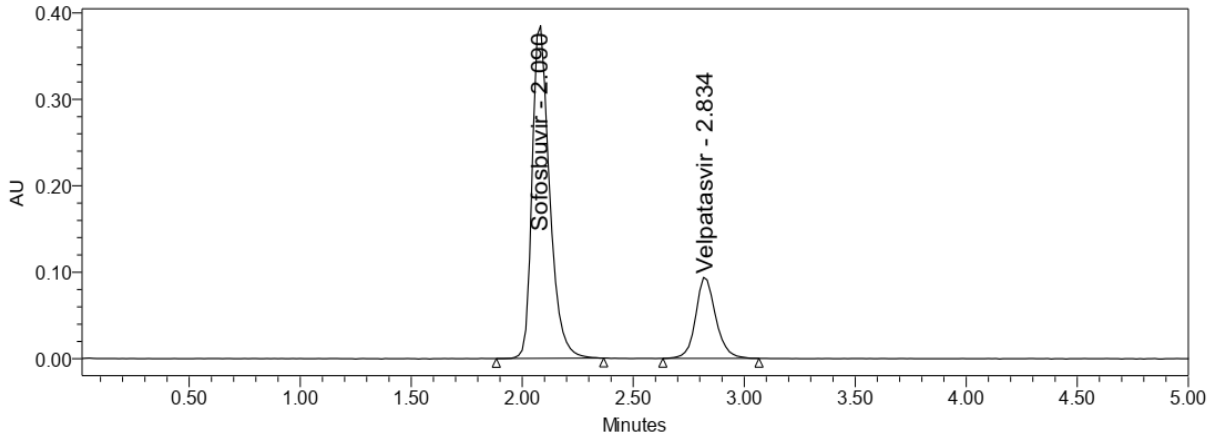


Fig 18 Mobile phase Plus Chromatogram of Sofosbuvir and Velpatasvir.

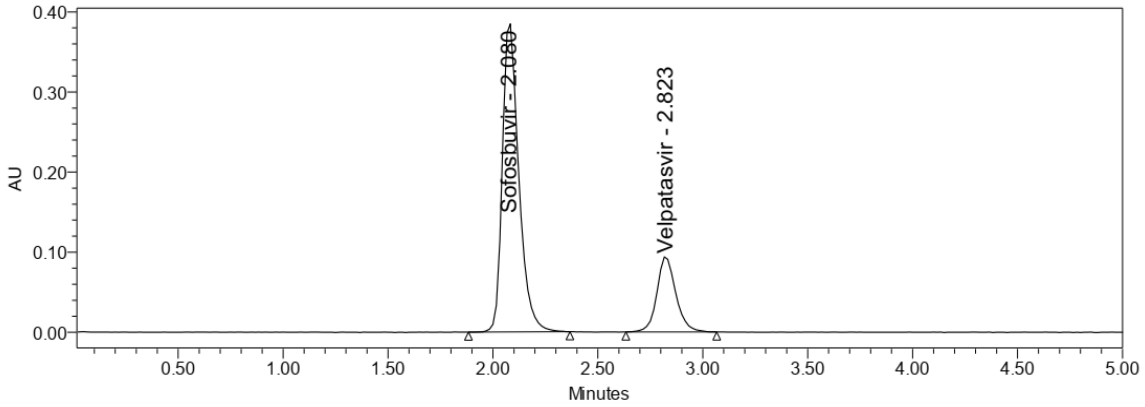


Fig 19 Temperature minus Chromatogram of Sofosbuvir and Velpatasvir.

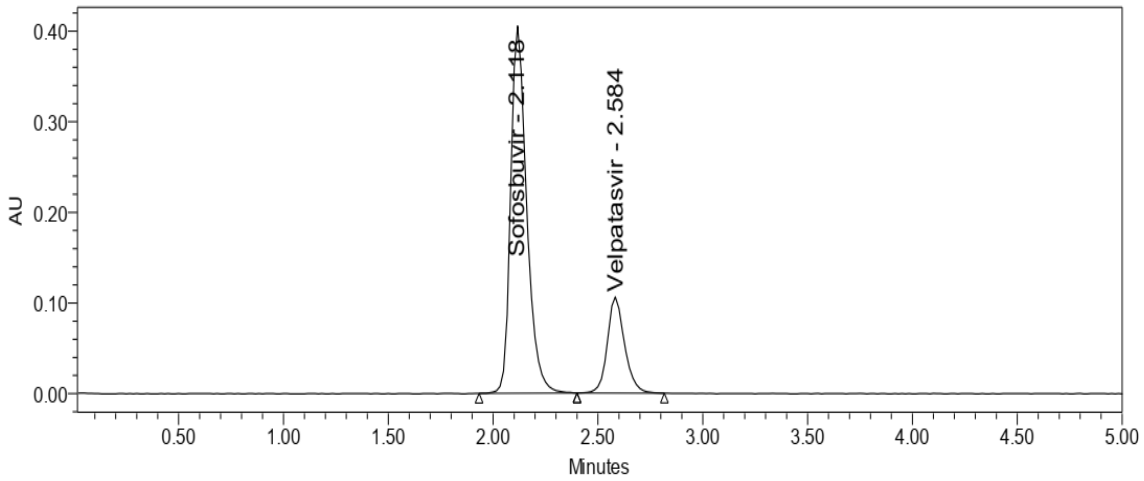


Fig 20 Temperature plus Chromatogram of Sofosbuvir and Velpatasvir

8. Forced Degradation Study:

- **Oxidation:**

To 1 ml of stock solutions of Sofosbuvir and Velpatasvir, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

- **Acid Degradation Studies**:

To 1 ml of stock ssolution Sofosbuvir and Velpatasvir, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at

60^0C.The resultant solution was diluted to obtain 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir and10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

- **Alkali Degradation Studies**:

To 1 ml of stock solution Sofosbuvir and Velpatasvir, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

- **Thermal DegradationStudies**:

The standard drug solution of Sofosbuvir and Velpatasvir was placed in oven at 105^0C for1h to study dry heat degradation. For HPLC study, the resultant solution was diluted obtain 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

- **Photo Stability studies**:

The photochemical stability of the drug was also studied by exposing the Sofosbuvir and Velpatasvir with concentration 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

- **Neutral Degradation Studies**:

Stress testing under neutral conditions was studied by refluxing the drug inwater for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to obtain 80µg/ml of Sofosbuvir and 10µg/ml of Velpatasvir and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

➢ **Degradation Studies:** 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.

Table-12: Degradation Data of Sofosbuvir

S.No	Degradation Condition	% Drug UnDegraded	% Drug Degraded
1	Acid	92.72	7.28
2	Alkali	95.54	4.46
3	Oxidation	96.66	3.34
4	Thermal	97.19	2.81
5	UV	98.38	1.62
6	Water	98.38	1.62

Table-13: Degradation Data of Velpatasvir

S.No	Degradation Condition	% Drug UnDegraded	% Drug Degraded
1	Acid	94.74	5.26
2	Alkali	95.00	5
3	Oxidation	96.29	3.71
4	Thermal	97.30	2.7
5	UV	98.43	1.57
6	Water	99.57	0.43

9. Intermediate precision:

Table-11 Intermediate precision table of Sofosbuvir and Velpatasvir.

S. No	Concentration (µg/ml)	Area of Sofosbuvir	Concentration (µg/ml)	Area of Velpatasvir
1.	80	2040768	10	584756
2.	80	2079433	10	581666
3.	80	2051725	10	584272
4.	80	2037749	10	583633
5.	80	2061219	10	588396
6.	80	2079307	10	590832
Mean		2058367	Mean	585593
S.D		18272.0	S.D	3376.8
%RSD		0.9	%RSD	0.6

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained area were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.9% and 0.6% respectively for Sofosbuvir and Velpatasvir. As the limit of Precision was less than "2" the system precision was passed in this method.

➢ **Degradation Studies:** 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

Table-12: Degradation Data of Sofosbuvir

S.No	Degradation Condition	% Drug UnDegraded	% Drug Degraded
1	Acid	92.72	7.28
2	Alkali	95.54	4.46
3	Oxidation	96.66	3.34
4	Thermal	97.19	2.81
5	UV	98.38	1.62
6	Water	98.38	1.62

Table Degradation Data of Velpatasvir

S.NO	Degradation Condition	% Drug UnDegraded	% Drug Degraded
1	Acid	94.74	5.26
2	Alkali	95.00	5
3	Oxidation	96.29	3.71
4	Thermal	97.30	2.7
5	UV	98.43	1.57
6	Water	99.57	0.43

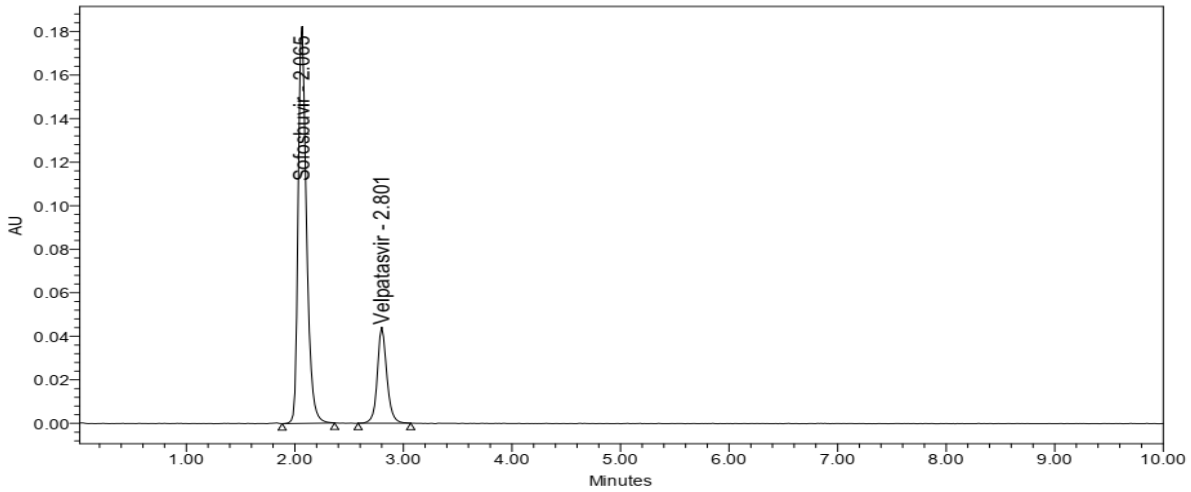


Fig 21 Acid degradation chromatogram of Sofosbuvir and Velpatasvir

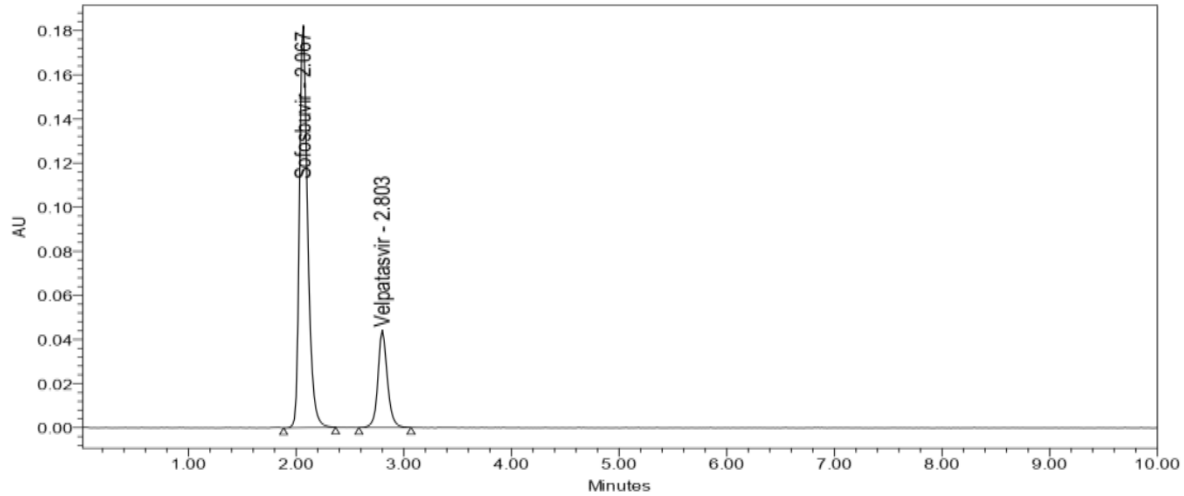


Fig 22 Base degradation chromatogram of Sofosbuvir and Velpatasvir

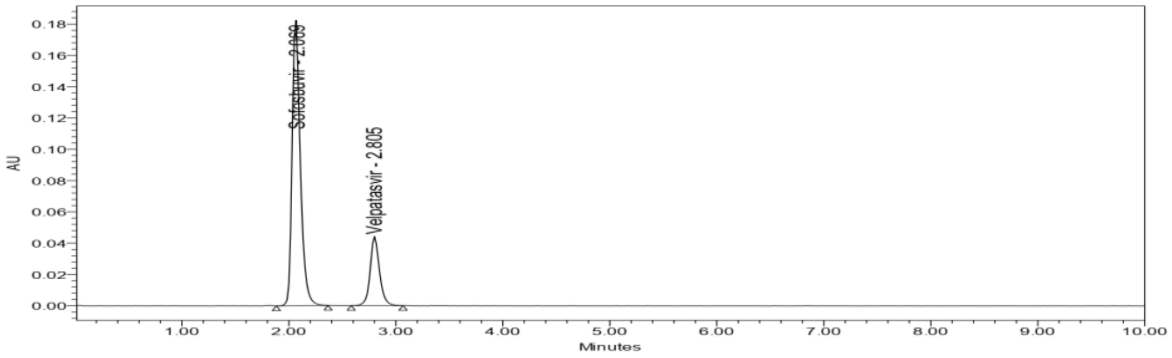


Fig 23 Peroxide degradation chromatogram of Sofosbuvir and Velpatasvir

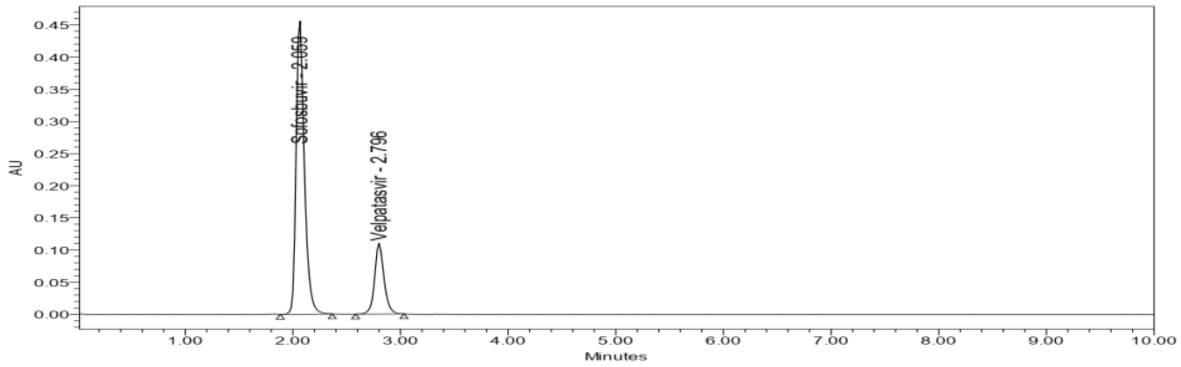


Fig 24 Thermal degradation chromatogram of Sofosbuvir and Velpatasvir

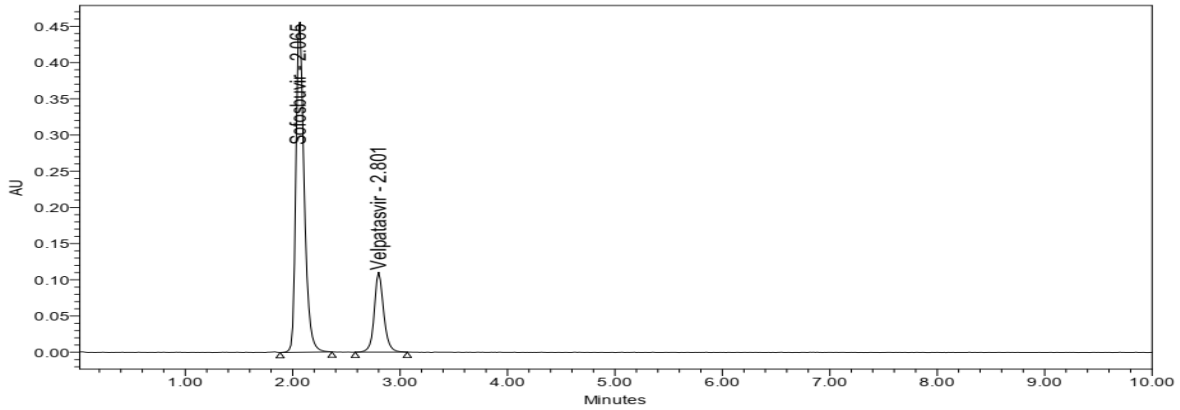


Fig 25 Photo degradation chromatogram of Sofosbuvir and Velpatasvir

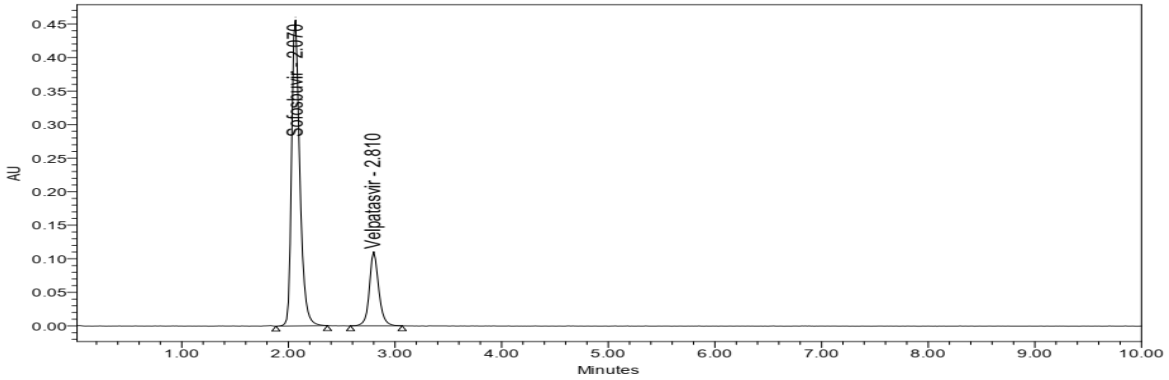


Fig 26 Water degradation chromatogram of Sofosbuvir and Velpatasvir

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Sofosbuvir and Velpatasvir in tablet dosage form. Retention time of Sofosbuvir and Velpatasvir were found to be 2.136 min and 2.871 min. %RSD of system precision for Sofosbuvir and Velpatasvir were and found to be 0.8 and 0.9 respectively. %RSD of method precision for Sofosbuvir and Velpatasvir were and found to be 0.5 and 0.3 respectively % recovery was obtained as 100.11% and 99.31% for Sofosbuvir and Velpatasvir respectively.LOD values are obtained from regression equations of Sofosbuvir and Velpatasvir were 0.13 µg/ml and 0.01 µg/ml and LOQ values are obtained from regression equations of Sofosbuvir and Velpatasvir were 0.40 µg/ml and 0.04

µg/ml respectively. Regression equation of Sofosbuvir was y = 25574x + 4971 and Velpatasvir was y = 74488x + 551.9. Retention times are decreased so the method developed was simple and economical that can be adopted in regular quality control test in Industries.

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