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Original Article

Stability Indicating HPLC Method for the Simultaneous Determination of Sofosbuvir and Velpatasvir

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ABSTRACT

Aim: Effective pharmaceutical techniques are necessary to guarantee the quality, safety, and effectiveness of antiviral medication combinations employed for managing *Hepatitis* C, a dangerous viral disease that affects millions of people worldwide. This work's objective was to develop and validate a straightforward quick accurate these stability-indicating reverse phase high-performance liquid chromatographic technique for the combined measurement by the drug velpatasvir with sofosbuvir. **Materials and Methods:** Luna phenyl hexyl column 150×46 mm 35 mm and an isocratic solvent system made up of acetonitrile and 01 trifluoroacetic acid in a 30:70 v/v ratio chromatographic separation was accomplished the flow rate was kept constant at 10 mL/min using monitoring using a 10 µg/mL injection volume also detection of wavelength of 261 nm ICH recommendations for system compatibility linearity, consistency, correctness, specificity robustness detection limit LOD and quantification of limit LOD were followed in the validation of developed method forced degradation experiments in acidic alkaline oxidation-induced, heat-induced light-induced and neutral conditions also used to assess stability-indicating capabilities with **Results and Conclusion:** Retention time roughly 277 min sofosbuvir and 512 min for velpatasvir the data showed satisfactory chromatographic separation within the concentration intervals of 1060 µg/mL for sofosbuvir and 530 µg/mL for velpatasvir the technique exhibited excellent linearity with correlation coefficients near unity accuracy studies produced percentage recovery whereas precision studies revealed RSD values below 2 values ranging from 99 to 101 for routine quality evaluation of sofosbuvir and velpatasvir over bulk substance forms the established RP-HPLC technology is hence dependable stability-assessing and suitable

Keywords: HPLC, Method Validation, Pharmaceutical Analysis, Sofosbuvir, Velpatasvir.

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INTRODUCTION

Hepatitis C is a severe viral disease impacts millions of individuals globally and can lead to long term liver disorders including cirrhosis also liver cell carcinoma.[1,2] In recent years the development of direct-acting antiviral agent has greatly enhanced the therapeutic results for HCV infection.[3] the drug sofosbuvir and the medication velpatasvir are commonly utilized combined therapy because they act on different stages of the viral replication cycle and offers high recovery rates across different strains from the *Hepatitis* c pathogens.[4] sofosbuvir is a nucleotide analogue blocker of the ns5b RNA-dependent RNA polymerase enzyme that is essential to viral replication.[5] where as velpatasvir is an inhibitor of the ns5a protein that is important for viral RNA synthesis and formation.[6] the combined two drugs is widespread application in the treatment of long term *Hepatitis* is infection caused by the *Hepatitis C virus* chronic.[4] Due to their extensive use in pharmaceutical formulations it is important to develop consistent analytical methods for the real-time estimation of these drugs to ensure quality, "safety and efficacy of the dosage forms.[7] Several analytical methods have been stated to these determination of sofosbuvir and velpatasvir treatment individually either in combination.[8] However, many of the reported methods are complex time-consuming or lack comprehensive stability-indicating capability hence there is a need to grow a simple rapid accurate and stability-indicating analytical method for their simultaneous estimation therefore the present study aims to develop and validate a stability-indicating RP-HPLC method for the concurrent determination of sofosbuvir and velpatasvir in bulk drugs and pharmaceutical dosage forms in accordance with ICH guidelines.[9]

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MATERIALS AND METHODS

Analytical standards for the medicament Sofosbuvir and Velpatasvir were sourced from an accredited chemical vendor. The Commercial dosage form comprising Sofosbuvir and Velpatasvir was bought from a authorized drug store. HPLC grade ACN, (TFA), and water were bought from standard analytical chemical suppliers. All reagents and chemical compounds employed in the study were of analytical or HPLC grade.[10,11]

Methods

Experimental setup

HPLC chromatographic analysis performed on a RP-HPLC system equipped with a UV detector, an auto-sampler and a data acquisition software. The elution was performed on the Luna Phenyl Hexyl Stationary phase (3.5 µm, 150 mm×4.6 mm particle size). Isocratic conditions were used for the operation of the system.

Chromatographic Conditions

Acetonitrile and 0.1% trifluoroacetic acid in a 30:70 (v/v) ratio made up the mobile phase. Before being utilized, mobile phase was sonicated and cleared using a 0.45 µm membrane filter. The following chromatographic criteria were observed:

- Flow rate=1.0 mL/min.
- Detection wavelength=261 nm.
- Injection volume=10 µL.
- Column temperature: The surrounding air temperature.
- It takes about 8 min to run.

Standard Solution preparation

To create stock solutions, precisely weighed amounts of Velpatasvir and Sofosbuvir reference standards were put into a volumetric flask and dissolved in the mobile phase. To create working standard solutions in concentration range of 10-60 µg/mL for Sofosbuvir and 5-30 µg/mL for Velpatasvir, further dilutions were conducted using mobile phase.

Sample Solution preparation

Weighed 20 tabs and crushed them to a fine powder. A volumetric flask containing the mobile phase was charged with a weighed amount of the powder corresponding to that for the labelled amount of Sofosbuvir and Velpatasvir. The mixture was sonicated for 15 min to ensure complete release of the drug, and then filtered through a 0.45 µm membrane filter. The mobile phase was diluted as necessary to obtain the necessary attention for HPLC analysis.

Validation of Methods

The parameters were used to validate the developed RP-HPLC method with ICH guidelines. A forced deterioration study was utilized to demonstrate the method's ability to demonstrate stability. A variety of stressors were applied to the drug samples. Samples were then degraded followed by addition of appropriate amount of mobile phase and when necessary, neutralized and evaluated by the developed HPLC method for the partitioning of the degradation product peak from the main drug peak.

Analysis of statistics

To estimate the accuracy & dependability of the created approach, all experimental data from validation experiments were statistically analyzed using standard metrics, including mean, standard deviation (SD), & %relative standard deviation (%RSD).

Comparative Evaluation and Method Novelty

To demonstrate the analytical superiority, efficiency, and novelty of the proposed stability-indicating RP-HPLC method, its operational and validation parameters were systematically compared against previously published literature for the concurrent estimation of Sofosbuvir and Velpatasvir. A detailed performance overview covering stationary phases, mobile phases, runtime, sensitivity metrics, and retention behaviours is structured in Table 1.

Discussion of Practical Advantages and Novelty

As detailed in Table 8, the principal scientific advantage and structural novelty of the proposed method over existing literature (e.g., Kumar KK *et al*., 2025 or Seetharamaiah P., 2023) stems from the deliberate transition away from conventional, purely aliphatic octadecylsilane (C18) columns to a selective Luna Phenyl-Hexyl stationary phase. Traditional C18 matrices separate compounds strictly based on hydrophobic interactions, which frequently leads to co-elution or severe peak tailing for complex aromatic rings like those within Velpatasvir and Sofosbuvir.

The Phenyl-Hexyl matrix introduces dense, supplementary electron cloud interactions between the column's phenyl functional molecules and the conjugated aromatic configurations of both direct-acting antivirals. This unique retention dynamic generates exceptional spatial baseline resolution () and near-perfect peak symmetries (Sofosbuvir tailing = 1.05, Velpatasvir tailing = 0.91). Furthermore, this enhanced selectivity permits a significantly optimized isocratic runtime of just 8.0 minutes with an ultra-low detection sensitivity (LOD of 0.0801 for Sofosbuvir). This provides a highly economical, green-chemistry-aligned, and high-throughput alternative perfectly suited for routine commercial quality control and pharmaceutical formulation auditing (Tables 2-8).

METHODS VALIDATION

System suitability parameters

Using optimal chromatographic conditions, five duplicate injections of 10 µL of standard solutions of Sofosbuvir and

Velpatasvir were inoculated into the chromatographic system to conduct the system suitability test. The tailing factor, %RSD for retention duration, peak regions, resolution, and theoretical plates were assessed as system appropriateness characteristics.[12,13]

Linearity

Six concentration levels (25-150%) of the stock solution containing 800 µg/mL Sofosbuvir and 200 µg/mL Velpatasvir were prepared in order to assess linearity. To achieve final concentrations of 10-80 µg/mL for Sofosbuvir and 2-30 µg/mL for Velpatasvir, the solutions were diluted with diluent. The 10 µL injection of each solution was used to determine peak areas. Calibration curves were plotted, and it was observed that there was a linear relationship between peak area and concentration over the range studied. Then, intercept, slope and correlation coefficient were computed.

Precision

System, method, and intermediate precision were utilized to assess the method's precision. The standard solution was inoculated into the chromatographic system 6 times and the chromatograms were recorded to assess the precision of the chromatographic system. Method precision was determined by vaccination of 10 µL of sample solution six times and intermediate precision was determined by vaccination of 10 µL of reference solution six times on different days. Peak area of Sofosbuvir and Velpatasvir were calculated and the results presented as %RSD.[14,15]

The method was tested for accuracy by making three concentrations, 50%, 100% and 150%, and calculating the percentage recovery of the method over the range. Each of the concentrations was injected into the chromatographic apparatus in triplicate following the sample preparation to which a known quantity of standard was added (standard addition) and chromatograms were obtained. The recoveries of Sofosbuvir and Velpatasvir from each level were calculated as percentage.

Robustness

The robustness of the proposed approach was evaluated by deliberate change of chromatographic conditions including the composition of mobile phases, flow rates, wavelength and column temperature. The test method-prepared standard solutions were injected into the chromatograph in triplicate at different flow rate (0.1 mL±0.1 mL/min), mobile organic phase composition (10%±10%), wavelength (5 nm±5 nm) and column temperature (50°C±50°C). The chromatograms so obtained were utilized to measure system appropriateness characteristics.

Table 1: Comparative Performance Profiles of the Proposed Method vs. Reported Literature.

Analytical Parameters	Proposed Method	Kumar KK *et al.*, 2025	Bhavyasri K *et al.*, 2023	Seetharamaiah P., 2023
Stationary Phase (Column)	Luna Phenyl-Hexyl	Conventional Aliphatic C18 Column	Aliphatic Octadecylsilane (C18) Column	Aliphatic Octadecylsilane (C18) Column
MobilePhase Chemistry	Acetonitrile: 0.1% Trifluoroacetic Acid (30:70 v/v)	Methanol/Acetonitrile with Phosphate Buffer	Organic mixtures with high aqueous buffer ratios	Methanol and Phosphate Buffer combinations
Total Chromatographic Run Time	8.0minutes (Rapid elution)	Extended run cycles (>12 min)	Moderate to long run cycles	Long equilibration cycles
Retention Times (, min)	Sofosbuvir: 2.770 Velpatasvir: 5.124	Delayed retention windows	Delayed retention profiles	Broadened elution bands
Linearity Range	Sofosbuvir: 10–60 Velpatasvir: 5–30	10–80 2–30	Varied dynamic ranges	Restricted calibration constraints
Sensitivity (LOD / LOQ)	LOD: 0.0801 / 0.020 LOQ: 0.80 / 0.20	High limits (Lower sensitivity)	Moderate sensitivity boundaries	Standard lower detection limitations
USP Peak Tailing Factor	Sofosbuvir: 1.05 Velpatasvir: 0.91 (Highly symmetric)	Visible tailing effects (>1.5)	Pronounced peak asymmetries	Peak asymmetry factors near boundary parameters
Main Advantage / Novelty	- electron interactions; enhanced resolution with reduced mobile phase volume.	High organic solvent waste profiles	Complex multi-stage buffer preparation workflows	Prone to silanol interactions and peak broadening

Table 2: Optimization of RP-HPLC method.

Trial No.	Column	Mobile phase composition	Injection volume(µl)	λ_{max} (nm)	Flowrate (mL/min)	Observation
1	Waters X-BridgeC_{18} 150X4.6 mm, 3.5 µ	Acetonitrile and 0.1% OPA 60:40	10	261	1.0	Less resolution is observed.
2	Luna Phenyl Hexyl 150X4.6 mm, 3.5 µ	Acetonitrile and 0.1% TFA 60:40	10	261	1.0	Plate count is not within the limit
3	Waters Symmetry C_{18} 150X4.6 mm, 3.5 µ	Acetonitrile and 0.1% OPA 30:70	10	261	1.0	Second peak tailing is not within the limit
4	Luna Phenyl Hexyl 150X4.6 mm, 3.5 µ	Acetonitrile and 0.1% TFA 60:40	10	261	1.0	Peaks are not separated
5	Acetonitrile and 0.1% TFA 50:50 Luna Phenyl Hexyl 150X4.6 mm, 3.5 µ	Acetonitrile and 0.1% TFA 50:50	10	261	1.0	Peak height is not within the limit
6	Luna Phenyl Hexyl 150X4.6 mm, 3.5 µ	Acetonitrile and 0.1%TFA 30:70	10	261	1.0	Good peak and high resolution

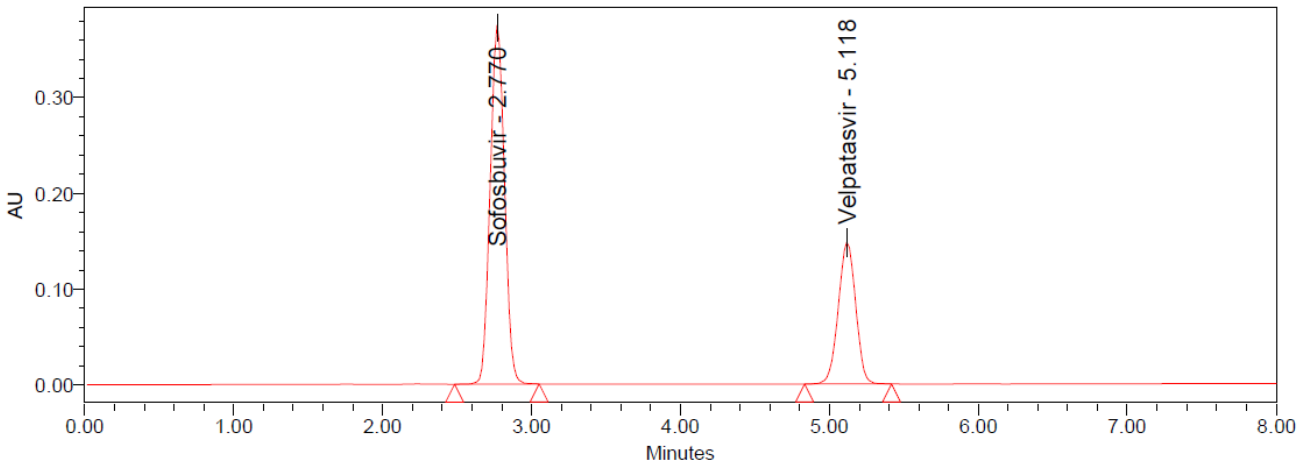

Figure 1: Optimized HPLC chromatogram.

Specificity

Blank and placebo solution were vaccinated in triplicate along with standard and sample solution to test the specificity of the technique for interference of blank and placebo. Specificity of the proposed RP-HPLC procedure was further evaluated by comparing the chromatograms of blank, placebo, standard and sample solutions.

Limit of Detection (LOD) and Quantification (LOQ)

A study was carried out to determine the LOD and LOQ for velpatasvir and sofosbuvir. As per the test procedure, a few different strengths of LOD and LOQ solutions were prepared and then injected into HPLC apparatus in triplicates. Chromatograms were captured. Signal-to-noise ratio (S/N) was used to calculate the LOD and LOQ. LOQ was determined by the concentration at which the s/n ratio is approximately 10 while LOD was determined by the concentration at which the s/n ratio is approximately.[15,16]

Forced degradation studies

Under the following stress circumstances, stress research was carried out to show how degradations could be successfully separated from the sample's primary analyte peaks. Using optimal chromatographic conditions, all stressed samples were appropriately diluted to the necessary concentration with diluent and inoculated twice into the HPLC system. The chromatograms were then recorded and peak purity determined. The degradation percentages of Sofosbuvir and Velpatasvir were calculated.

Solution stability

An initial, 12-hr, 24-hr, and 48-hr benchtop investigation was carried out to determine the stability of standard and test solutions. This was accomplished by making test and standard preparations in accordance with the test procedure, injecting them twice into the chromatographic system under ideal chromatographic

conditions, and recording the chromatograms. For velpatasvir and sofosbuvir, the difference in assay percentage was computed.

RESULTS AND DISCUSSION

Methods Development and Optimization: To achieve optimum simultaneous chromatographic resolution, peak shape symmetry, and controlled run times for the highly polar Sofosbuvir and comparatively lipophilic Velpatasvir, systematically structured mobile phase and stationary phase optimization trials were conducted. Initial scouting trials utilized conventional C18 chemistries (Trial 1: Waters X-Bridge C18; Trial 3: Waters Symmetry C18) with Acetonitrile and aqueous buffers. However, these stationary phases yielded insufficient spatial resolution between the active components and displayed unacceptable peak tailing for the later-eluting Velpatasvir peak (Tailing > 2.0).

To enhance chromatographic performance, a Luna Phenyl-Hexyl column (150 × 4.6 mm, 3.5 µm) was selected. The Phenyl-Hexyl phase provides unique interactions via its aromatic rings alongside moderate hydrophobic interactions, which significantly enhance the selectivity for the aromatic networks found in both Sofosbuvir and Velpatasvir. Early Phenyl-Hexyl configurations using an organic-rich profile (60:40 v/v Acetonitrile to 0.1% TFA) resulted in co-elution or inadequate column efficiency due to rapid elution (Trials 2 and 4). Lowering the organic modifier concentration slightly to 50:50 v/v (Trial 5) enhanced retention but compromised peak symmetry and baseline peak heights.

Optimal separation parameters were conclusively achieved in Trial 6 utilizing a mobile phase composition of Acetonitrile and 0.1% Trifluoroacetic Acid (TFA) in an optimized isocratic ratio of 30:70 v/v at a constant flow rate of 1.0 mL/min. The incorporation of 0.1% TFA acted as an effective ion-pairing and pH-adjusting agent, repressing silanol ionization, reducing peak asymmetry, and sharpening peak shapes. This system yielded an exceptional baseline resolution (Rs = 11.66), symmetrical peaks (Tailing factors of 1.05 and 0.91), and high column efficiency (Theoretical plates of 3,762 and 9,227 for Sofosbuvir and Velpatasvir, respectively) within an accelerated run profile (Figures 1-4).

Methods Validation

System suitability studies

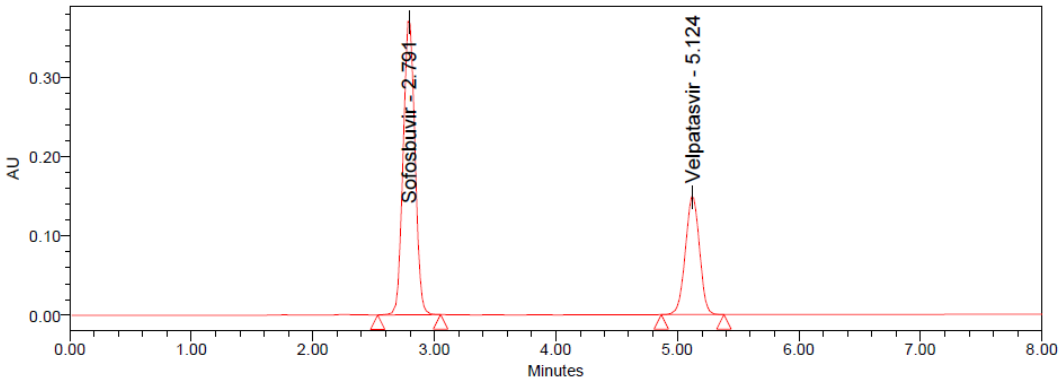

Figure 2: Displays the results of the system suitability chromatography.

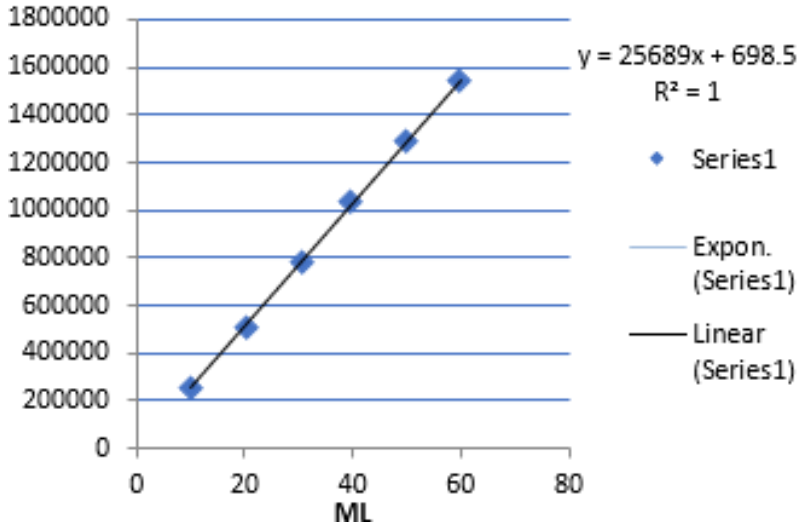

Figure 3: Standard Calibration graph of Sofosbuvir.

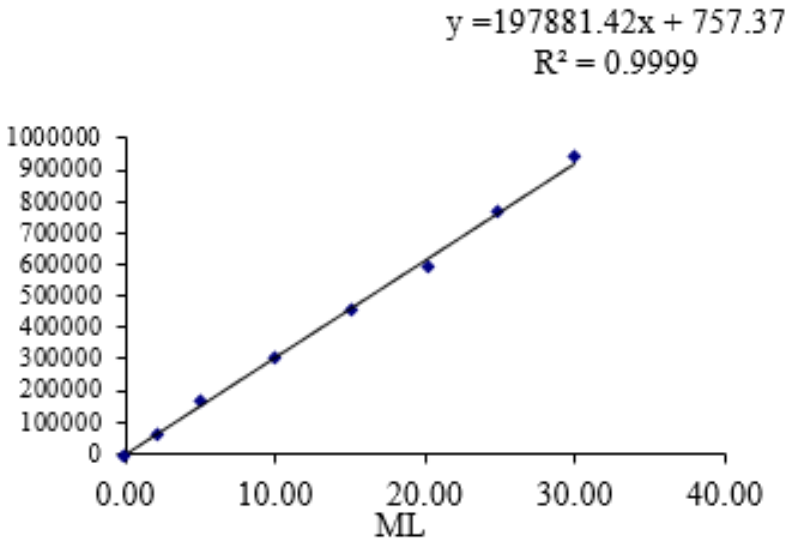

Figure 4: Standard Calibration graph of Velpatasvir.

Table 3: System suitability test parameter.

Sl. No.	Name of the drug	Rt(min)	USP Resolution	USP tailing	USP plate count	%RSD
1.	Sofosbuvir	2.791	-	1.05	3762	0.18
2.	Velpatasvir	5.124	11.66	0.91	9227	0.26

Table 4: Linearity studies of the proposed method.

% Level of concentration	Sofosbuvir		Velpatasvir	
	Conc. (µg/mL)	Peak Area	Conc. (µg/mL)	Peak Area
25	10	256890	5	62716
50	20	513780	10	171818
75	30	770670	15	308822
100	40	1027560	20	453374
125	50	1284450	25	595546
150	60	1541340	30	760828

DISCUSSION

ICH guidelines state that the resolution must be greater than two, the tailing factor must be less than two, and the plate count must be greater than 2000. Every appropriate parameter for the system was passed and was inside the bounds. It was discovered that the percentage RSD for the peak areas of reactions and retention times of five replicate inoculations of standard solutions of velpatasvir and sofosbuvir was less than 2%. For any combination, there were more than 2000 theoretical plates. It was discovered that the resolution between neighbouring peaks was greater than 5.0 and the tailing factor was less than 2.0.

Linearity

Discussion

Six concentrations of Sofosbuvir (10-80 µg/mL) and Velpatasvir (2-30 µg/mL) were inoculated in a duplicate manner. Average areas were mentioned above, and the linearity equations found for Sofosbuvir were y=25689x +698.5, and for velpatasvir were y=19788.1+757.37. The correlation coefficient was found for the two drugs, 1 and 0.9999.

It was discovered that the correlation coefficient values fell within the bounds.

Precision

System precision (Intra-day precision)

The %RSD of the peak areas for Sofosbuvir and Velpatasvir is shown below

Result of Repeatability studies (Intra-day precision)

Methods precision: The %RSD of the peak areas for sofosbuvir and velpatasvir is shown in Table 6

Discussion

Six injections were made from a single volumetric flask of working standard solution, and the areas that were obtained are listed above. For two medications, the average area, standard deviation, and percentage RSD were computed. The percentage RSD for velpatasvir and sofosbuvir was 0.75% and 0.31%, respectively. In this manner, the system precision was passed since the precision limit was less than "2."

Inter-day precision

RSD for retention time and peak regions of velpatasvir and sofosbuvir. The retention time and peak regions of velpatasvir and sofosbuvir acquired from six replicate inoculations in the precision studies are consistent, as shown by the %RSD values (below 2%). Therefore, it can be determined that the suggested RP-HPLC process was optimized.

Discussion

After several samples were taken from a sample stock solution, six working sample solutions with identical concentrations were made. On the day following sample preparation, each inoculation from each working sample solution was administered; the obtained areas are listed in the above Table. For two medications, the average area, SD, and %RSD were determined to be 0.18,0.256,0.4,0.21 respectively, for Sofosbuvir and Velpatasvir.

Table 5: Regression characteristics of proposed method.

Parameter	Results	
	Sofosbuvir	Velpatasvir
Linearity Range (µg/mL)	10-60	5-30
Regression equation(y=mx+b)	25689x + 698.5	197881.42x + 757.37
Slope(m)	28859	197881.42
Intercept(b)	698.5	757.37
Correlation Coeefficient(r2)	1	0.999

Table 6: Result of Repeatability Studies (Intra-day precision).

No. of Injection	Retention time (mins)		Peak Area	
	Sofosbuvir	Velpatasvir	Sofosbuvir	Velpatasvir
1	2.770	5.118	2020286	595540
2	2.791	5.135	2013277	592520
3	2.796	5.134	2020624	593847
4	2.796	5.132	2018056	596328
5	2.793	5.128	2011947	593809
6	2.791	5.124	2018119	592728
Statistical Parameters	Mean		2017052	594129
	SD		3624.254	1520.535
	%RSD		0.18	0.26

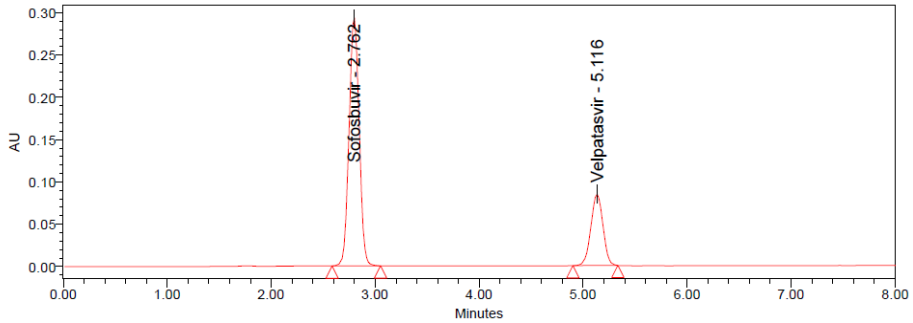

Figure 5: HPLC Chromatogram of Acid Degraded Sample.

Table 7: Results of Method Precision Studies.

No. of Injection	Retention time (mins)		Peak Area	
	Sofosbuvir	Velpatasvir	Sofosbuvir	Velpatasvir
1	2.768	5.122	2013125	595379
2	2.794	5.141	2015871	595559
3	2.787	5.129	2027154	594358
4	2.793	5.134	2016245	594652
5	2.799	5.136	2016871	594768
6	2.786	5.147	2027652	595883
Statistical Parameters	Mean		2019486.3	593433.1
	SD		6366.65	4470.63
	%RSD		0.31	0.75

Table 8: Results of Intermediate (Inter-Day) precision studies.

No. of Injection	Peak Area (Day-1)		Peak Area (Day-2)	
	Sofosbuvir	Velpatasvir	Sofosbuvir	Velpatasvir
1	2020286	595540	2001286	595977
2	2013277	592520	2020871	597574
3	2020624	593847	2012783	597354
4	2018056	596328	2006874	594856
5	2011947	593809	2017136	595669
6	2018119	592728	2021784	597983
Mean	2017052	594129	2013455	596568
SD	3624.25	1520.53	8121.4	1242.6
%RSD	0.18	0.256	0.4	0.21

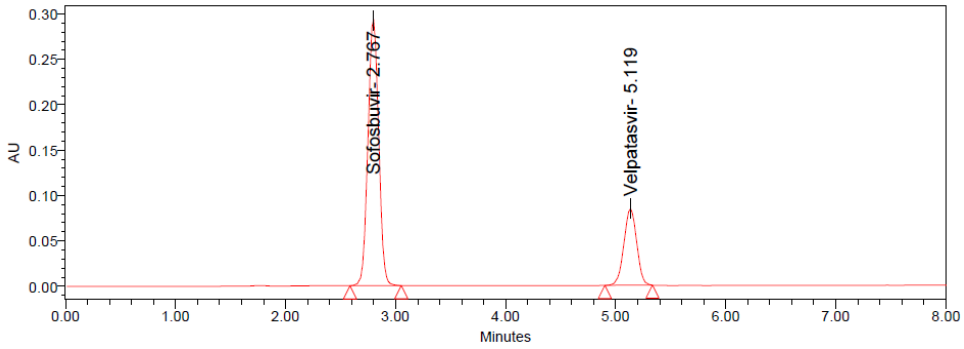

Figure 6: HPLC Chromatogram of Alkali Degraded Sample.

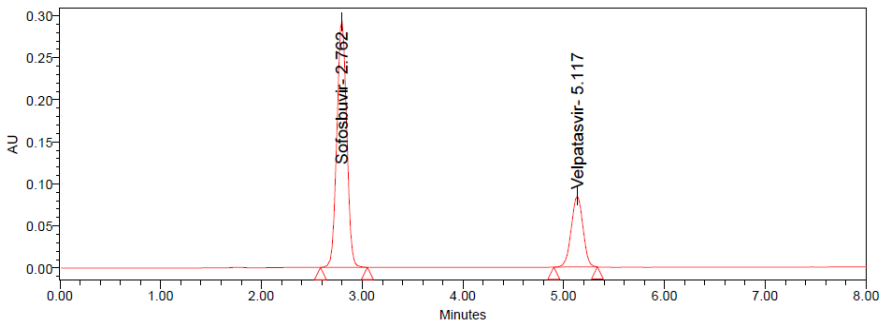

Figure 7: HPLC Chromatogram of Peroxide Degraded Sample.

In this manner, the system precision was passed because the precision limit was less than "2."

Accuracy (Recovery)

Tables 9, 10 show the percentage recoveries of sofosbuvir and velpatasvir from each concentration level

Discussion

For Sofosbuvir and Velpatasvir, the mean percentage recovery from spiked samples was determined to be within the acceptable range of 99.8-100.2% and 99.6-99.86%, respectively. The high accuracy of the suggested RP-HPLC technique was indicated by the outstanding mean recoveries and standard deviation.

Robustness

Tables 11, 12 present the system appropriateness characteristics that were assessed based on the acquired chromatograms.

Discussion

It was discovered that under all variable situations, the system suitability parameters were within the bounds. It is clear from the data that the suggested RP-HPLC process is resilient to minor changes.

Specificity (Interference studies)

Table 13 reports the findings of specificity investigations and the chromatograms obtained from the blank, placebo, standard, and sample solutions.

Discussion

The retention periods of the analytes in the standard and sample solutions were expected to be identical, and the chromatograms of blank and placebo solutions did not display any peaks at the Sofosbuvir and Velpatasvir retention times. Therefore, it was claimed that the suggested RP-HPLC technique was selective and unaffected by the excipients in the tablets.

Table 9: Data of Accuracy Studies of Sofosbuvir.

Sl. No.	% Level of test conc.	Peak Area	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean % Recovery	% RSD*
1.	50	1013482	40	40.2	100.5	100.2	0.25
2.	50	1011364	40	40.11	100.3		
3.	50	1008241	40	39.99	100.0		
4.	100	2011742	80	79.79	99.7	99.8	0.1
5.	100	2014576	80	79.9	99.9		
6.	100	2013781	80	79.87	99.8		
7.	150	3012871	120	119.5	99.6	99.9	0.35
8.	150	3023712	120	119.93	99.9		
9.	150	3034842	120	120.37	100.3		

*Mean of three determinations.

Table 10: Data of Accuracy Studies of Velpatasvir.

Sl. No.	% Level of test conc.	Peak Area	Amount Added(µg/mL)	Amount Recovered (µg/mL)	%Recovery	Mean%Recovery	% RSD*
1.	50	297822	10	10.03	100.3	99.7	0.53
2.	50	295478	10	9.95	99.5		
3.	50	294937	10	9.93	99.3		
4.	100	591571	20	19.91	99.6	99.86	0.25
5.	100	593241	20	19.97	99.9		
6.	100	594687	20	20.02	100.1		
7.	150	885617	30	29.81	99.4	99.6	0.31
8.	150	887314	30	29.87	99.6		
9.	150	891436	30	30.01	100.0		

Table 11: Data of Robustness studies of Sofosbuvir.

Parameter	Optimized condition	Used condition	Peak area	Retention Time	Plate Count	Tailing factor
Flow rate (±0.1mL/min)	1 mL/min	0.9 mL/min	2253564	3.445	5173	1.06
		1 mL/min	2263652	3.449	5161	1.06
		1.1mL/min	2243874	3.454	5157	1.06
Column temp. (±5°C)	30°C	25°C	1746481	2.312	5730	1.03
		30°C	1766214	2.317	5639	1.05
		35°C	1736325	2.319	5289	1.06
Mobile phase Composition (5%v/v)	40:60	35:65	2256501	3.252	4834	1.05
		40:60	2246812	3.261	4821	1.06
		45:55	224635	3.253	4834	1.05

Table 12: Data of Robustness studies of Velpatasvir.

Parameter	Optimized condition	Used condition	Peak area	Retention Time	Plate Count	Tailing factor
Flow rate(±0.1mL/min)	1mL/min	0.9 mL/min	668626	6.359	11264	1.0
		1 mL/min	669944	6.362	11239	1.01
		1.1mL/min	669762	6.368	11231	1.01
Column temp.(±5°C)	30°C	25°C	445472	4.235	10989	1.0
		30°C	446587	4.239	11131	1.01
		35°C	446896	4.242	11059	1.02
Mobile Phase Composition (5%v/v)	40:60	35:65	638954	7.379	7713	0.98
		40:60	643968	7.356	7213	0.97
		45:55	643972	7.364	7273	0.99

Table 13: Results of specificity studies.

Sl. No.	Solution	Sofosbuvir		Velpatasvir	
		Rt (mins)	Peak Area	Rt (mins)	Peak Area
1.	Blank	-	-	-	-
2.	Standard	2.770	2020286	5.118	595540
3.	Placebo	-	-	-	-
4.	Sample	2.791	2018119	5.124	592728

FORCED DEGRADATION STUDIES

As seen in the images 5-10, the chromatograms of stressed samples were assessed for peak purity.

These stress degradation results of Sofosbuvir and Velpatasvir are reported in Tables 14, 15.

Analytical Interpretation of Degradation Pathways and Mechanisms

The chemical behaviour of both analytes varied prominently under different environmental stresses:

- **Hydrolytic Degradation Pathways (Acid and Alkali):** The chemical framework of Sofosbuvir is highly prone to solution-state hydrolytic pathways. Under acidic (2N HCl) and alkaline (2N NaOH) reflux contexts, the molecule primarily undergoes ester hydrolysis at its isopropyl ester side chain, along with secondary cleavage of the phosphoramidate nucleoside linkage. This breakdown yields highly polar secondary degradation fragments that elute very early near the solvent front (between 1.2 and 2.1 min). Velpatasvir exhibits robust stability in acid, but undergoes structural modification under severe alkaline boiling conditions due to partial cleavage within its carbamate groups.

- **Oxidative Degradation Mechanisms:** In the presence of 20% H_20_2, oxidative degradation pathways occur predominantly at electron-rich functional centres. For Velpatasvir, the tertiary heterocyclic amine networks and the pyrrolidine core are susceptible to N-oxide formation. Sofosbuvir exhibits lower sensitivity to hydrogen peroxide, but forms minor oxidative impurities over long exposure windows.
- **Thermal and Photolytic Profiles:** Both drugs show high solid-state structural stability when exposed to intense dry heat (105ºC) and continuous short-wave UV illumination (241 nm), demonstrating that the therapeutic combination is structurally stable under standard processing and dry storage configurations.

4.x.2 Peak Purity Verification and Stability-Indicating Capacity

To rigorously confirm the stability-indicating capacity of this protocol, peak purity verification was executed across all stressed matrices spectral matching software.

In all chromatograms (Figures 5 through 10), the calculated peak purity angles for the remaining parent drug spots were consistently lower than their respective peak purity thresholds. This demonstrates that no co-eluting, process-related degradation impurities or secondary matrix artifacts occupy the specific retention windows of Sofosbuvir (Rt = 2.770 min) or Velpatasvir (Rt = 5.118 min). Complete spatial separation and baseline resolution were strictly maintained between the parent chemical peaks and all emerging degradation products. This proves the specificity, selectivity, and stability-indicating capability of the proposed method for accurate quality control tracking in commercial tablet batches.

Discussion

The peak purity of two medicines exceeded peak purity in all forced degradation samples, and the degradant peaks were observed to be well separated from the primary analyte peaks. Sofosbuvir and Velpatasvir were deteriorated below 5% without any substantial degradations in thermal, photolytic, and neutral degradation experiments.

Limit of Quantification (LOQ) and detection (LOD)

Sofosbuvir and Velpatasvir were found to have LOD values of 0.0801 µg/mL and 0.020 µg/mL, respectively, and LOQ values of 0.8 µg/mL, 0.20 µg/mL, and 0.05 µg/mL, respectively. The sensitivity of the suggested RP-HPLC technique is demonstrated by these low LOD and LOQ values (Tables 16 and 17).

Solution stability studies

From the above study, it was found that the stability of standard and test preparation is Table for a period of 24 hr at room temperature.

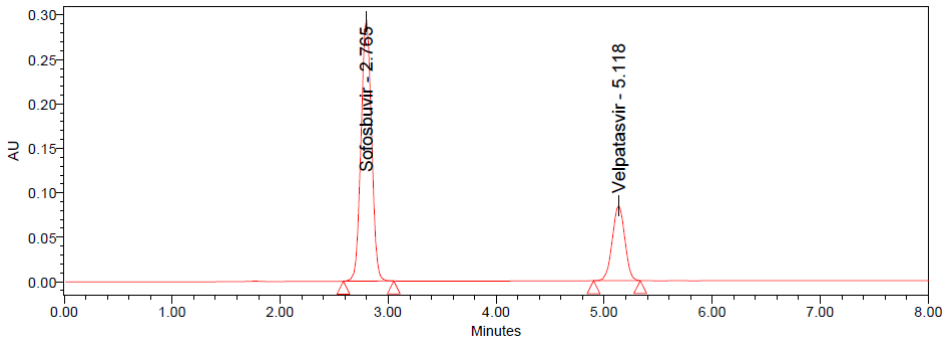

Figure 8: HPLC Chromatogram of Thermal Degraded Sample.

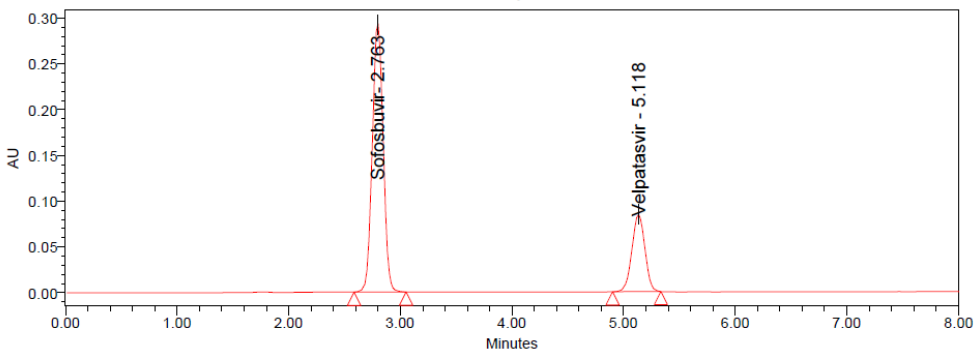

Figure 9: HPLC Chromatogram of UV Degraded sample.

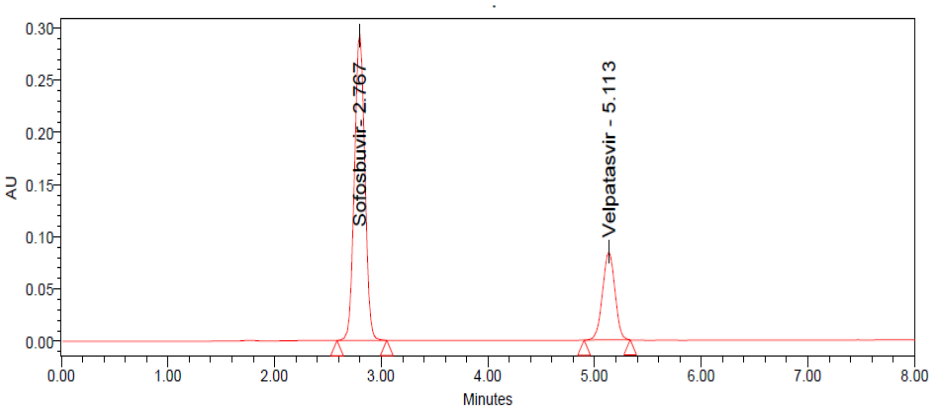

Figure 10: HPLC Chromatogram of Neutral Degraded sample.

Table 14: Forced Degradation studies of Sofosbuvir.

Sl. No.	Forced Degradation Condition	Purity Angle	Purity threshold	% Degradation	Peak Purity
1.	Acid stress	5.145	25.412	4.3	passes
2.	Alkali stress	5.412	25.421	5.5	passes
3.	Oxidation stress	5.311	25.741	6	passes
4.	Thermal stress	5.241	25.189	6.4	passes
5.	Photolytic Stress	5.356	25.321	6.8	Passes
6.	Neutral stress	5.412	25.415	6.7	Passes

Table 15: Forced Degradation studies of Velpatasvir.

Sl. No.	Forced Degradation Condition	Purity Angle	Purity threshold	% Degradation	Peak Purity
1.	Acid stress	15.231	40.397	5.8	passes
2.	Alkali stress	15.621	40.374	5.7	passes
3.	Oxidation stress	15.326	40.648	6.2	passes
4.	Thermal stress	15.741	40.748	6.9	passes
5.	Photolytic Stress	15.642	40.987	7.6	Passes
6.	Neutral stress	15.268	40.873	7.9	Passes

Table 16: Bench top Stability conditions of Sofosbuvir.

Time Interval	Standard Peak Area	Sample Peak Area	% Assay	% Assay Difference
Initial	2017132	2003146	100.1	NA
After 6 hr	1995652	1996563	99.4	0.7
After 12 hr	1991637	1974235	99.1	0.9
After 18 hr	1962365	1955684	99.1	0.9
After 24 hr	1998567	2003146	99.2	0.8

Table 17: Benchtop Stability Conditions of Velpatasvir

Time Interval	Standard Peak Area	Sample Peak Area	% Assay	% Assay Difference
Initial	589632	589632	99.2	NA
After 6 hr	586589	586324	98.6	0.6
After 12 hr	581384	581632	98.9	0.3
After 18 hr	578569	579625	98.5	0.7
After 24 hr	575368	577852	99.0	0.2

Proposed method for marked formulation

The amount of the drug found in marketed tablet was calculated from the following formula.

Calculation

% content of the drug in formulation:

AT: Peak area response from sample preparations; AS: Peak area response from standard preparations; WS: Weight of the working standard taken in mg; WT: Weight of the working sample taken in mg; DS: Dilution factor for standard solution; DT: Dilution factor for sample solution; P: Potency of working standard; Avg. Wt: Average weight of the capsule taken in mg; LA: Label claim of the drug in mg

CONCLUSION

The investigation's findings show that Sofosbuvir and Velpatasvir had retention periods of 2.770 and 5.135min, respectively, when the suggested RP-HPLC method was used. With a correlation coefficient of 0.999, quantitative linearity was seen in the concentration ranges of 10-60 µg/mL and 5-30 µg/mL. Additionally, it was discovered that the precision studies' %RSD values were less than 2, indicating a precise procedure". The high percentage of recoveries shows how precise the suggested approach was. The suggested method's strong sensitivity is indicated by the low LOD and LOQ values. The chromatograms of blank and placebo interference investigations show no interfering peaks, indicating a particular technique and degradants produced under stress. Additionally, degradation experiments were clearly segregated and did not affect the medications' quantification using the recommended stability-indicating RP-HPLC approach. The suggested stability-indicating RP-HPLC approach was found to be straightforward, accurate, exact, quick and helpful for the regular analysis of velpatasvir and sofosbuvir in pharmaceutical dosage forms and bulk. According to ICH criteria, the results were satisfactory.

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ABBREVIATIONS

ACN: Acetonitrile; HCV: *Hepatitis* C virus; HPLC: High-Performance Liquid Chromatography; ICH: International Council for Harmonisation; LOD: Limit of Detection; LOQ: Limit of Quantification; OPA: Orthophosphoric acid; RP-HPLC: Reverse Phase High-Performance Liquid Chromatography; RSD: Relative Standard Deviation; SD: Standard Deviation; S/N: Signal-to-Noise ratio; TFA: Trifluoroacetic Acid; UV: Ultraviolet.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

In this work, a rapid RP-UPLC method for concurrently measuring Sofosbuvir and Velpatasvir in pharmaceutical dose forms is developed and validated. Good separation with respectable peak shape and resolution was made possible by the enhanced chromatographic parameters. The approach demonstrated exceptional accuracy, precision, linearity, and robustness after being validated in accordance with ICH guidelines. The suggested method's pertinency for stability assessment was confirmed by its capacity to identify degradation products. As a result, the technique can be effectively used to regular quality control studies of these medications in pharmaceutical formulations.

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