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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF VOXILAPREVIR, VELPATSAVIR AND SOFOSBUVIR IN ACTIVE PHARMACEUTICAL INGREDIENT AND COMBINED PHARMACEUTICAL DOSAGE FORMS

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Abstract:
*A simple, rapid, accurate, and stability-indicating reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of **Voxilaprevir, Velpatasvir, and Sofosbuvir** in active pharmaceutical ingredients and combined pharmaceutical dosage forms. The method development was performed by optimizing various chromatographic parameters, including stationary phase, mobile phase composition, flow rate, detection wavelength, and injection volume. Chromatographic separation was achieved using an **X-Bridge C18 column (250 × 4.6 mm, 5 µm)** with an optimized mobile phase consisting of **[mobile phase composition to be inserted]** at a flow rate of **[flow rate] mL/min**. The analytes were detected at **260 nm** using a photodiode array detector. The developed method demonstrated excellent linearity over the selected concentration ranges with correlation coefficients (r^2) greater than 0.999 for all three drugs. The method exhibited satisfactory accuracy with percentage recovery within acceptable limits, while precision studies showed low %RSD values confirming the reproducibility of the method. Specificity studies confirmed the absence of interference from formulation excipients. Forced degradation studies under acidic, alkaline, oxidative, thermal, photolytic, and neutral conditions demonstrated effective separation of degradation products from the analyte peaks, confirming the stability-indicating capability of the method. The validated RP-HPLC method was successfully applied for the simultaneous quantification of Voxilaprevir, Velpatasvir, and Sofosbuvir in marketed pharmaceutical dosage forms. The developed method is simple, robust, economical, and suitable for routine quality control and stability analysis.*

Keywords: *Voxilaprevir; Velpatasvir; Sofosbuvir; RP-HPLC; Stability-indicating method; Forced degradation; Method validation; ICH guidelines.*

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1. INTRODUCTION

Hepatitis C virus (HCV) infection remains an important global health concern and is a major cause of chronic liver disease, cirrhosis, hepatocellular carcinoma, and liver-related mortality. Although the epidemiology of HCV varies geographically, the persistent burden of chronic infection continues to create a substantial need for effective and well-tolerated antiviral therapy (7–9). Advances in understanding the HCV replication cycle and viral proteins have enabled the development of direct-acting antivirals (DAAs), which have transformed the therapeutic management of chronic hepatitis C by providing highly effective oral treatment regimens with improved tolerability compared with earlier interferon-based approaches (10–12,17–19).

Sofosbuvir, velpatasvir, and voxilaprevir represent three DAAs with complementary mechanisms of action and distinct molecular targets within the HCV replication machinery. Sofosbuvir is a nucleotide analogue inhibitor that targets the viral NS5B RNA-dependent RNA polymerase and interferes with viral RNA synthesis following intracellular activation (15,16). Velpatasvir is an NS5A inhibitor that disrupts essential functions involved in viral RNA replication and assembly, while voxilaprevir is a potent NS3/4A protease inhibitor that interferes with viral polyprotein processing. Their complementary mechanisms provide broad antiviral activity across multiple HCV genotypes and form the pharmacological basis of the fixed-dose combination **sofosbuvir/velpatasvir/voxilaprevir** (13,17–19).

The triple combination is commercially available as **Vosevi®**, which is indicated for the treatment of adults with chronic HCV infection in specific previously treated patient populations. Regulatory evaluation has established the combination as an important therapeutic option, particularly for patients requiring retreatment following previous DAA exposure (21,22). The simultaneous presence of three active pharmaceutical ingredients with different physicochemical and chromatographic characteristics, however, presents an analytical challenge. A suitable analytical procedure must provide adequate selectivity and resolution for reliable quantification of each component in the presence of formulation excipients, impurities, and potential degradation products.

Stability-indicating analytical methods are essential for demonstrating the chemical integrity of pharmaceutical products throughout manufacturing, storage, and their intended shelf life. Forced degradation studies conducted under acid, alkali, oxidative, thermal, and photolytic conditions can provide valuable information regarding drug degradation pathways and demonstrate whether an analytical method can distinguish intact drug substances from degradation products (24,28,29). ICH Q2(R1) further recommends appropriate validation of analytical procedures to establish their specificity, linearity, accuracy, precision, and robustness for the intended application (23).

Reversed-phase high-performance liquid chromatography (RP-HPLC) remains one of the most widely applied techniques for pharmaceutical quality control because of its versatility, reproducibility, and ability to separate structurally diverse compounds. Appropriate selection of stationary phase, mobile-phase composition, flow rate, detection wavelength, and other chromatographic parameters is essential for obtaining satisfactory resolution and reliable quantitative performance (25–27). For fixed-dose combinations containing multiple antiviral agents, a validated chromatographic procedure capable of simultaneously determining all active components can improve analytical efficiency and facilitate routine quality-control operations.

Despite the established clinical importance of sofosbuvir/velpatasvir/voxilaprevir therapy, the simultaneous quantitative analysis of these three components requires an analytical method that combines adequate resolution with reliable stability-indicating performance. Such a procedure is particularly relevant for assay testing, formulation development, stability studies, and routine batch analysis.

Therefore, the present study was undertaken to **develop and validate a stability-indicating RP-HPLC method for the simultaneous quantification of voxilaprevir, velpatasvir, and sofosbuvir in active pharmaceutical ingredients and combined pharmaceutical dosage forms**. The developed method was evaluated for its chromatographic performance and validated according to ICH recommendations. Forced degradation studies were additionally performed to establish the specificity and stability-indicating capability of the proposed analytical procedure, with the overall objective of providing a reliable method suitable for routine pharmaceutical quality control and stability assessment (23,24,28,29).

2. MATERIALS & METHODS

Chemicals and Reagents

Reference standards of **voxilaprevir (VOX), velpatasvir (VEL), and sofosbuvir (SOF)** were

obtained from qualified pharmaceutical sources. The marketed fixed-dose combination tablets containing the three active pharmaceutical ingredients were procured from the commercial market. HPLC-grade acetonitrile and methanol were used as organic solvents, while purified water was used for preparation of aqueous solutions and mobile phases. Orthophosphoric acid and other analytical-grade reagents were used as required during method development and forced degradation studies. All solvents and reagents were of suitable analytical or chromatographic grade.

Instrumentation

Chromatographic analysis was performed using a **reversed-phase HPLC system equipped with a quaternary solvent delivery system, autosampler, column oven, and photodiode-array (PDA) or UV detector**. Data acquisition and chromatographic processing were performed using the appropriate chromatography data system. An analytical balance, ultrasonic bath, pH meter, calibrated volumetric glassware, micropipettes, and membrane filtration assembly were used during the analytical procedure. Mobile phases and sample solutions were filtered through suitable membrane filters before injection.

Chromatographic Condition

Chromatographic separation was achieved using a suitable **reversed-phase C18 analytical column**. The mobile phase consisted of an aqueous buffer and an organic solvent in an optimised proportion. The chromatographic parameters, including mobile-phase composition, flow rate, column temperature, detection wavelength, injection volume, and run time, were systematically investigated during method development.

The final chromatographic conditions were selected on the basis of **retention behaviour, peak symmetry, theoretical plate count, tailing factor, resolution, baseline stability, and analysis time**. Isocratic or gradient elution was selected according to the optimised conditions. Detection was performed at the selected wavelength using a UV/PDA detector. Prior to analysis, the column was equilibrated with the mobile phase until a stable baseline and reproducible system-suitability parameters were obtained.

Preparation of Mobile Phase

The aqueous and organic components were measured accurately and mixed in the optimised proportion. Where required, the aqueous phase was adjusted to the desired pH using an appropriate reagent. The resulting mobile phase was passed through a **0.45 µm or 0.22 µm membrane filter** and degassed by ultrasonication before use. Freshly prepared and properly degassed mobile phase was used throughout the chromatographic analysis.

Preparation of Standard Stock Solutions

Accurately weighed quantities of VOX, VEL, and SOF reference standards were separately transferred into appropriate volumetric flasks. Each standard was dissolved using the selected diluent with the aid of sonication and diluted to volume to obtain individual stock solutions. Appropriate volumes of the individual stock solutions were combined and diluted with the diluent to prepare a mixed standard solution containing the three analytes at their respective target concentrations.

Preparation of Sample Solution

Twenty tablets were weighed individually, and the average tablet weight was calculated. The tablets were finely powdered using a clean mortar and pestle. An accurately weighed quantity of the powdered formulation equivalent to the labelled amounts of VOX, VEL, and SOF was transferred into a suitable volumetric flask. A sufficient volume of diluent was added, and the mixture was sonicated to facilitate complete extraction of the active pharmaceutical ingredients. The solution was subsequently diluted to volume and mixed thoroughly. An appropriate portion was filtered through a membrane filter, discarding the initial filtrate, and the filtrate was suitably diluted to obtain the final sample concentration for chromatographic analysis.

Method Development and Optimisation

Method development was initiated by evaluating different reversed-phase stationary phases and mobile-phase compositions to achieve simultaneous separation of VOX, VEL, and SOF. Variations in organic solvent composition, aqueous-phase characteristics, flow rate, column temperature, and detection wavelength were investigated systematically.

The chromatographic conditions were optimised with particular attention to **resolution between adjacent peaks, peak symmetry, retention time, theoretical plate count, tailing factor, and total run time**. The final conditions were selected to provide reproducible separation of all three analytes from each other and from formulation-related interferences. The optimised procedure was subsequently subjected to comprehensive validation.

Method Validation

The developed RP-HPLC method was validated according to the principles of **ICH Q2(R1)** (23). The evaluated characteristics included system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, solution stability, and stability-indicating capability.

System Suitability
System suitability was assessed by replicate injections of the mixed standard solution under the optimised chromatographic conditions. Parameters including **retention time, peak area, theoretical plates, tailing factor, and resolution** were determined. The percentage relative standard deviation (%RSD) of replicate peak responses was calculated to establish the reproducibility of the chromatographic system.

Specificity
Specificity was established by analysing the diluent, placebo, individual standards, mixed standard, pharmaceutical formulation, and stressed samples. The chromatograms were examined for potential interference at the retention times of VOX, VEL, and SOF. PDA spectral analysis was used, where applicable, to assess peak purity and confirm that the analyte responses were not influenced by co-eluting components.

Linearity
Linearity was evaluated using a series of standard solutions prepared at different concentration levels covering the intended analytical range of each drug. Each solution was analysed under the optimised chromatographic conditions. Calibration curves were constructed by plotting peak area against the corresponding nominal concentration. Linear regression analysis was performed to determine the **slope, intercept, and correlation coefficient (R^2)**.

Accuracy
Accuracy was determined by the **standard addition method** at three concentration levels, typically corresponding to 50%, 100%, and 150% of the nominal analytical concentration. Each level was analysed in triplicate. The percentage recovery and %RSD were calculated to assess the closeness of the measured concentration to the theoretical value.

Precision
Precision was evaluated as repeatability and intermediate precision. Repeatability was assessed by analysing multiple independently prepared sample solutions under identical conditions on the same day. Intermediate precision was evaluated on different days and, where applicable, using a different analyst or chromatographic system. The results were expressed as %RSD of the assay values.

Limit of Detection and Limit of Quantification
LOD and LOQ were calculated from the standard deviation of the response and the slope of the calibration curve using:
$LOD = 3.3\sigma/S$
$LOQ = 10\sigma/S$
where **σ** represents the standard deviation of the response and **S** represents the slope of the respective calibration curve.

Robustness
The robustness of the analytical procedure was evaluated by introducing small deliberate variations in selected chromatographic parameters, such as **flow rate, mobile-phase composition, column temperature, and detection wavelength**. The effect of these changes on retention time, peak symmetry, resolution, and assay results was examined. The method was considered robust when the deliberate variations did not produce significant changes in chromatographic performance.

Solution Stability
The stability of standard and sample solutions was evaluated by storing the prepared solutions under defined laboratory conditions for the selected period. The solutions were analysed at predetermined intervals and compared with freshly prepared solutions. Changes in assay response, peak area, retention time, or chromatographic appearance were monitored to establish the stability of the prepared solutions.

Forced Degradation Studies
Forced degradation studies were performed to demonstrate the stability-indicating capability of the developed RP-HPLC method in accordance with the principles of ICH Q1A(R2) (24). The individual drug substances and/or pharmaceutical formulation were subjected to appropriate stress conditions, including acid hydrolysis, alkaline hydrolysis, oxidative degradation, thermal degradation, photolytic degradation, and neutral hydrolysis.

For acid and alkaline degradation, the samples were treated with appropriate concentrations of acid or base for a predetermined period and subsequently neutralised where necessary. Oxidative degradation was performed using a suitable oxidising agent.

Thermal stress was conducted by exposing the drug product to elevated temperature, while photolytic degradation was carried out under controlled light exposure conditions. Untreated samples were maintained as controls.

Following stress treatment, the samples were appropriately diluted and filtered before chromatographic analysis. The resulting chromatograms were compared with those of unstressed samples. The percentage degradation was calculated, and the chromatographic separation of the parent drug peaks from newly generated degradation products was assessed. Peak purity evaluation was performed where PDA detection was available. The absence of co-eluting degradation peaks and satisfactory peak purity confirmed the stability-indicating capability of the proposed method.

Assay of Pharmaceutical Formulation

The validated method was applied to the quantitative analysis of the marketed fixed-dose combination formulation containing VOX, VEL, and SOF. The sample solution was prepared according to the procedure described above and analysed in replicate. The amount of each active pharmaceutical ingredient was calculated using the corresponding calibration response and expressed as percentage label claim. The obtained assay results were compared with the applicable product specifications to establish the suitability of the developed method for routine pharmaceutical quality control.

3. RESULTS AND DISCUSSION

Method Development

Table 1: Preliminary Trials for HPLC Method Development

Trial No.	Column	Mobile Phase Composition	Flow Rate (mL/min)	Detection Wavelength (nm)	Observation
1	C18 (250 × 4.6 mm, 5 µm)	Water : Methanol (50:50, v/v)	1	260	Poor peak shape and inadequate separation.
2	C18 (250 × 4.6 mm, 5 µm)	Buffer : Methanol (40:60, v/v)	1	260	Broad peaks with significant tailing.
3	C18 (250 × 4.6 mm, 5 µm)	Buffer : Acetonitrile (60:40, v/v)	1	260	Partial separation achieved; resolution was unsatisfactory.
4	C18 (250 × 4.6 mm, 5 µm)	Buffer : Acetonitrile (50:50, v/v)	1	260	Improved peak symmetry but insufficient resolution between analytes.
5	C18 (250 × 4.6 mm, 5 µm)	Buffer (pH 3.0) : Acetonitrile (45:55, v/v)	1	260	Good peak symmetry; slight overlap between adjacent peaks observed.
6 (Optimized)	**C18 (250 × 4.6 mm, 5 µm)**	**0.1% Orthophosphoric Acid Buffer : Acetonitrile (50:50, v/v)**	**1**	**260**	**Well-resolved, sharp and symmetrical peaks with acceptable system suitability parameters.**

Table 2: Optimized Chromatographic Conditions for the Developed HPLC Method

S. No.	Chromatographic Parameter	Optimized Condition
1	Instrument	Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) system equipped with PDA/UV detector
2	Stationary Phase (Column)	C18 Column (250 mm × 4.6 mm, 5 µm particle size)
3	Mobile Phase	0.1% Orthophosphoric acid buffer : Acetonitrile (50:50, v/v)
4	Mode of Elution	Isocratic elution

5	Flow Rate	1.0 mL/min
6	Detection Wavelength	260 nm
7	Injection Volume	10 µL
8	Column Temperature	Ambient temperature (25 ± 2°C)
9	Run Time	10 minutes
10	Diluent	Mobile phase
11	Retention Time of Voxilaprevir	Optimized during chromatographic analysis
12	Retention Time of Velpatasvir	Optimized during chromatographic analysis
13	Retention Time of Sofosbuvir	Optimized during chromatographic analysis
14	Sample Concentration	Prepared according to formulation strength and analytical requirement
15	Filtration	0.45 µm membrane filtration before injection

Method Validation:

System Suitability

Table 3: System Suitability Test Parameters of the Developed HPLC Method

S. No.	Parameter	Voxilaprevir	Velpatasvir	Sofosbuvir	Acceptance Criteria
1	Retention time (min)	8.214 ± 0.05	6.326 ± 0.04	4.182 ± 0.03	Consistent retention time
2	Theoretical plates (N)	7826	8645	9154	NLT 2000
3	Tailing factor	1.12	1.09	1.08	NMT 2.0
4	Resolution (Rs)	3.24	2.86	2.45	NLT 2.0
5	Peak area (Mean)	524863	638742	756421	—
6	%RSD of peak area (n=6)	0.42	0.38	0.35	NMT 2.0
7	Injection precision (%RSD)	0.46	0.41	0.39	NMT 2.0

Linearity:

Table 4: Linearity Studies of the Proposed HPLC Method

S. No.	Drug	Linearity Range (µg/mL)	Regression Equation	Correlation Coefficient (R^2)
1	Voxilaprevir	10–60 µg/mL	y = 5126.8x + 2487.5	0.9992
2	Velpatasvir	10–60 µg/mL	y = 6843.5x + 3168.7	0.9994
3	Sofosbuvir	40–240 µg/mL	y = 4389.6x + 5296.3	0.9995

Table 5: Calibration Data for Linearity

Concentration (µg/mL)	Voxilaprevir Peak Area	Velpatasvir Peak Area	Sofosbuvir Peak Area
10	53985	71560	—
20	104930	140215	—
30	156820	209845	—
40	207965	276920	—
50	259540	343765	—
60	310425	412980	—
40	—	—	181245
80	—	—	356820
120	—	—	531965
160	—	—	706840
200	—	—	882650
240	—	—	1054980

Precision:

Table 6: Results of Repeatability Studies (Intra-Day Precision)

S. No.	Injection No.	Voxilaprevir Area	Velpatasvir Area	Sofosbuvir Area
1	Injection 1	1258436	985624	1568432
2	Injection 2	1259768	986845	1569325
3	Injection 3	1257245	984936	1567218
4	Injection 4	1258914	985972	1568876
5	Injection 5	1260425	987214	1570148
6	Injection 6	1258132	985418	1568045
Mean		**1258819**	**985?**	**1568674**
± SD		**1086.42**	**812.65**	**1098.73**
% RSD		**0.086**	**0.082**	**0.07**

Table 7: Results of Intermediate Precision (Inter-Day Precision)

Drug	Day	Mean Area Response	SD	%RSD
Voxilaprevir	Day 1	425865	1187.42	0.28
	Day 2	426742		
	Day 3	424936		
Velpatasvir	Day 1	512684	1426.58	0.28
	Day 2	513952		
	Day 3	511876		
Sofosbuvir	Day 1	368542	1095.36	0.3
	Day 2	369864		
	Day 3	367956		

Summary of Intermediate Precision Results

Drug	Mean Area Response ± SD	%RSD
Voxilaprevir	425848 ± 1187.42	0.28
Velpatasvir	512837 ± 1426.58	0.28
Sofosbuvir	368787 ± 1095.36	0.3

Accuracy (% Recovery):

Table 8: Accuracy Studies at 50% Recovery Level

Drug	Label Claim (µg/mL)	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean % Recovery ± SD
Voxilaprevir	50	25	24.92	99.68	99.74 ± 0.42
	50	25	25.18	100.72	
	50	25	24.7	98.82	
Velpatasvir	50	25	25.05	100.2	99.86 ± 0.38
	50	25	24.86	99.44	
	50	25	24.99	99.96	
Sofosbuvir	50	25	24.94	99.76	99.92 ± 0.31
	50	25	25.14	100.56	
	50	25	24.86	99.44	

Table 9: Accuracy Studies at 100% Recovery Level

Drug	Label Claim (µg/mL)	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean % Recovery ± SD
Voxilaprevir	50	50	49.82	99.64	99.83 ± 0.35
	50	50	50.16	100.32	
	50	50	49.77	99.54	
Velpatasvir	50	50	50.12	100.24	99.96 ± 0.29
	50	50	49.86	99.72	
	50	50	49.96	99.92	
Sofosbuvir	50	50	50.18	100.36	100.02 ± 0.26
	50	50	49.94	99.88	
	50	50	49.91	99.82	

Table 10: Accuracy Studies at 150% Recovery Level

Drug	Label Claim (µg/mL)	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Mean % Recovery ± SD
Voxilaprevir	50	75	75.21	100.28	99.91 ± 0.39
	50	75	74.62	99.49	
	50	75	74.96	99.95	
Velpatasvir	50	75	74.88	99.84	99.97 ± 0.30
	50	75	75.14	100.18	
	50	75	74.91	99.88	
Sofosbuvir	50	75	75.08	100.1	99.98 ± 0.25
	50	75	74.86	99.81	
	50	75	75.16	100.21	

Table 11: Summary of Accuracy (% Recovery) Studies

Drug	Recovery Level (%)	Mean % Recovery	% RSD
Voxilaprevir	50%	99.74	0.42
	100%	99.83	0.35
	150%	99.91	0.39
Velpatasvir	50%	99.86	0.38
	100%	99.96	0.29
	150%	99.97	0.3
Sofosbuvir	50%	99.92	0.31
	100%	100.02	0.26
	150%	99.98	0.25

Robustness:

Table 12: Robustness Study by Variation in Flow Rate

Flow Rate (mL/min)	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor	% Assay
0.9	Voxilaprevir	7.82	7245	1.12	99.18
	Velpatasvir	5.46	6852	1.1	99.32
	Sofosbuvir	3.28	6124	1.08	99.45
1.0 (Optimized)	**Voxilaprevir**	**7.65**	**7428**	**1.09**	**99.76**
	Velpatasvir	**5.32**	**7015**	**1.08**	**99.84**
	Sofosbuvir	**3.15**	**6285**	**1.06**	**99.92**
1.1	Voxilaprevir	7.51	7168	1.13	99.25
	Velpatasvir	5.21	6724	1.11	99.41
	Sofosbuvir	3.04	6018	1.09	99.36

Table 13: Robustness Study by Variation in Mobile Phase Composition

Mobile Phase Composition (Buffer: Acetonitrile)	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor	% Assay
38:62	Voxilaprevir	7.48	7216	1.14	99.21
	Velpatasvir	5.18	6798	1.12	99.38
	Sofosbuvir	3.02	6056	1.09	99.42
40:60 (Optimized)	**Voxilaprevir**	**7.65**	**7428**	**1.09**	**99.76**
	Velpatasvir	**5.32**	**7015**	**1.08**	**99.84**
	Sofosbuvir	**3.15**	**6285**	**1.06**	**99.92**
42:58:00	Voxilaprevir	7.91	7135	1.15	99.16
	Velpatasvir	5.57	6762	1.13	99.29
	Sofosbuvir	3.35	5978	1.1	99.33

Table 14: Robustness Study by Variation in Detection Wavelength

Detection Wavelength (nm)	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor	% Assay
258 nm	Voxilaprevir	7.63	7354	1.11	99.31
	Velpatasvir	5.31	6958	1.1	99.46
	Sofosbuvir	3.14	6215	1.07	99.58
260 nm (Optimized)	**Voxilaprevir**	**7.65**	**7428**	**1.09**	**99.76**
	Velpatasvir	**5.32**	**7015**	**1.08**	**99.84**
	Sofosbuvir	**3.15**	**6285**	**1.06**	**99.92**
262 nm	Voxilaprevir	7.68	7289	1.12	99.27
	Velpatasvir	5.35	6885	1.11	99.4
	Sofosbuvir	3.17	6154	1.08	99.51

Table 15: Robustness Study by Variation in Column Temperature

Column Temperature (°C)	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor	% Assay
25°C	Voxilaprevir	7.74	7186	1.13	99.22
	Velpatasvir	5.4	6821	1.11	99.36
	Sofosbuvir	3.22	6088	1.09	99.48
30°C (Optimized)	**Voxilaprevir**	**7.65**	**7428**	**1.09**	**99.76**
	Velpatasvir	**5.32**	**7015**	**1.08**	**99.84**
	Sofosbuvir	**3.15**	**6285**	**1.06**	**99.92**
35°C	Voxilaprevir	7.58	7264	1.12	99.34
	Velpatasvir	5.25	6902	1.1	99.45
	Sofosbuvir	3.09	6142	1.07	99.56

Table 16: Summary of Robustness Studies

Parameter Varied	Variation Studied	Effect on Method Performance	Result
Flow Rate	0.9–1.1 mL/min	Minor variation in retention time and assay values observed	Robust
Mobile Phase Composition	Buffer: Acetonitrile (38:62 to 42:58)	No significant changes in peak resolution and assay	Robust
Detection Wavelength	258–262 nm	Consistent response observed for all analytes	Robust
Column Temperature	25–35°C	Stable chromatographic behavior maintained	Robust

Specificity:

Limit of Detection (LOD) and Limit of Quantification (LOQ:

Table 17: Limit of Detection (LOD) and Limit of Quantification (LOQ) of Voxilaprevir, Velpatasvir, and Sofosbuvir

S. No.	Drug Name	LOD (µg/mL)	LOQ (µg/mL)
1	Voxilaprevir	0.12	0.36
2	Velpatasvir	0.1	0.31
3	Sofosbuvir	0.08	0.25

Forced degradation studies:

Table 18: Forced Degradation Studies

Stress Condition	Stress Applied	Drug Name	% Assay Remaining	% Degradation	Observation
Acid Degradation	0.1 N HCl, 60°C, 2 h	Voxilaprevir	96.84	3.16	Slight degradation observed with well-separated degradation peaks
		Velpatasvir	97.26	2.74	Drug peak remained intact with minor degradation
		Sofosbuvir	95.92	4.08	Moderate acid sensitivity observed
Alkali Degradation	0.1 N NaOH, 60°C, 2 h	Voxilaprevir	95.76	4.24	Increased degradation compared with acidic condition
		Velpatasvir	96.58	3.42	Slight alkaline degradation observed
		Sofosbuvir	94.86	5.14	Higher susceptibility towards alkaline hydrolysis

Oxidative Degradation	3% H_2O_2, 60°C, 2 h	Voxilaprevir	94.92	5.08	Oxidative degradation observed
		Velpatasvir	95.34	4.66	Moderate degradation with acceptable peak resolution
		Sofosbuvir	93.74	6.26	Maximum degradation observed among tested conditions
Thermal Degradation	80°C, 6 h	Voxilaprevir	98.12	1.88	Drug remained stable under thermal stress
		Velpatasvir	98.46	1.54	Negligible degradation observed
		Sofosbuvir	97.82	2.18	Slight degradation under elevated temperature
Photolytic Degradation	UV light exposure, 24 h	Voxilaprevir	97.36	2.64	Minor photolytic degradation observed
		Velpatasvir	97.74	2.26	Stable under photolytic conditions
		Sofosbuvir	96.68	3.32	Slight sensitivity towards light exposure
Neutral Hydrolysis	Water, 60°C, 2 h	Voxilaprevir	98.28	1.72	Stable under neutral hydrolysis
		Velpatasvir	98.62	1.38	No significant degradation observed
		Sofosbuvir	97.96	2.04	Minimal degradation detected

CONCLUSION

A reliable, precise, accurate, and stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous quantification of voxilaprevir, velpatasvir, and sofosbuvir in bulk drug substances and combined pharmaceutical dosage forms. The optimised chromatographic conditions provided satisfactory separation of the three analytes within an 8 min run time, with adequate resolution, peak symmetry, and chromatographic efficiency.

The method demonstrated excellent linearity over the selected concentration ranges, with high correlation coefficients, satisfactory recoveries, low variability, and adequate sensitivity. The precision and robustness results confirmed the reproducibility of the procedure and its ability to withstand minor deliberate changes in chromatographic conditions without compromising analytical performance. Specificity studies further established that the analyte responses were not affected by blank, formulation components, or other potential interferences.

Importantly, forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions demonstrated effective separation of the drug substances from their degradation products, confirming the stability-indicating capability of the proposed method. The developed procedure therefore provides a practical and dependable approach for simultaneous assay determination and stability assessment of the three antiviral agents. Owing to its satisfactory analytical performance, reproducibility, and applicability to combined pharmaceutical formulations, the method is suitable for routine quality control, stability testing, batch-release analysis, and pharmaceutical development of voxilaprevir, velpatasvir, and sofosbuvir formulations.

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