ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 2, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR, VELPATASVIR, AND VOXILAPREVIR IN BULK AND TABLET DOSAGE FORMS

VENKATA PADMINI M*, GOWRI SANKAR D

Department of Pharmaceutical Analysis and Quality Assurance, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India. Email: padmajapriya39@gmail.com

Received: 01 February 2019, Revised and Accepted: 10 August 2019

ABSTRACT

Objective: The present study was aimed to develop a novel, simple, rapid accurate and precise, stability-indicating reversed-phase high-performance liquid chromatography method for the simultaneous estimation of sofosbuvir, velpatasvir, and voxilaprevir in bulk and tablet dosage forms.

Methods: The chromatographic elution was achieved in isocratic mode using the combination of sofosbuvir, velpatasvir, and voxilaprevir in the ratio of acetonitrile and water (65:35%v/v) using a Phenomenex C18 column which has specification (150 × 4.6 mm, 5 µ particle size) and the flow rate of 1.0 ml/min and wavelength (ultraviolet) detection at 220 nm.

Results: The retention time obtained for sofosbuvir, velpatasvir, and voxilaprevir was 2.213 min, 2.568 min, and 2.917 min, respectively. Sofosbuvir, velpatasvir, and voxilaprevir and their combination drug product were exposed to acidic, alkali, thermal, photolytic, and oxidative stress conditions. The current method was validated according to the ICH guidelines for accuracy, precision, linearity, specificity, and sensitivity.

Conclusion: The method developed is more sensitive, accurate, precise, and robust then the methods reported earlier. Retention time and run time were decreased; hence, the method is economical simple and precise. Forced degradation studies indicated for the suitability of the method for stability studies of sofosbuvir, velpatasvir, and voxilaprevir. The proposed method can be used for routine quality control analysis test in pharmaceutical industries.

Keywords: Sofosbuvir, Velpatasvir, and voxilaprevir, Reversed-phase high-performance liquid chromatography, Stability indicating, Validation.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i2.36000

INTRODUCTION

Hepatitis C virus (HCV) was found to be a commonly attacking disease to human beings and was increased day by day. The literature reveals that 72% of the patients were suffered from chronic HCV [1]. In early stage, 75–85% of the liver is persisted with the virus. These defects have been treated by the use of oral form of these combinational drugs, respectively [2].

Sofosbuvir is an antiviral drug in the treatment of chronic HCV [3,4]. It is chemically isopropyl(2S)-2-[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-Fluoro-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propionate [5,6]. Sofosbuvir is a white-to-white crystalline powder with a molecular formula of $C_{22}H_{29}FN_3O_9P$ and molecular weight of 529.4 g/mole. Its chemical structure is given in Fig. 1. Velpatasvir is an NS5A inhibitor which acts on HCV. Velpatasvir is chemically Methyl {(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxycarbonyl) amino]-2-phenylacetyl]-(methoxymethyl)-2-pyrrolidinyl]-1H-imidazol-4-yl}-1,-11-dihydroisochromeno[4', 3:6,7]naphtha[1,2-d] imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbonate [7,8]. It is a white to light yellow solid powder with a molecular formula of $C_{49}H_{54}N_8O_8$ and a molecular weight of 883.02 g/mole. Its chemical structure is given in Fig. 2.

Voxilaprevir is also a protease inhibitor and acts as a transporter of polypeptide. It is chemically (1R,18R,20R,24S,27S,28S)-N-[(1R,2R)-2-(Difluoromethyl)-1-{[(1-methylcyclo propyl)sulfonyl]carbamoyl} cyclopropyl]-28-ethyl-13,13-difluoro-7-methoxy-24-(2-methyl-2-propanyl)-22,25-dioxo-2,21-dioxa-4,11,23,26tetraazapentacyclo dioxanonacosa-3(12),4,6, 8,10-pentaene-27-carboxamide [9,10]. It is a white to light yellow solid powder with a molecular formula $C_{40}H_{52}F_4N_6O_9S$ and a molecular weight of 868.94 g/mole. Its chemical structure is given in Fig. 3. The literature survey reveals that a very few spectroscopic and liquid chromatographic procedures have been reported for the determination of sofosbuvir, velpatasvir, and voxilaprevir by ultraviolet (UV) and high-performance liquid chromatography (HPLC) [11,12]. Therefore, there is a need to develop a rapid, sensitive reversed-phase (RP)-HPLC method for simultaneous estimation of sofosbuvir, velpatasvir, and voxilaprevir in bulk and pharmaceutical dosage form.

EXPERIMENTAL

Materials and methods

The reference samples of sofosbuvir, velpatasvir, and voxilaprevir were provided as a gift sample from spectrum Pharma Research Solutions, Hyderabad. VOSEVI tablets labeled to contain sofosbuvir 400 mg, velpatasvir 100 mg, and voxilaprevir 100 mg were purchased from the local pharmacy store. HPLC grade acetonitrile was purchased from Ramkem, Haryana, India. Orthophosphoric acid was purchased from Fischer Scientific, Mumbai, India. HPLC grade water was prepared using Millipore Milli-Q water purification system used throughout the process.

Instrumentation and chromatographic conditions

The present assay was carried out on a Waters HPLC system model 2695 equipped with 2996 photodiode array detector, autosample injector, and column Phenomenex C18 (150 × 4.6 mm, 5 µm), respectively. The output signal was monitored and integrated using water Empower 2 software. The isocratic mobile phase consisted of acetonitrile and water (65:35%v/v), flowing through the Phenomenex C18 (150 × 4.6 mm, 5 µm) column at a constant flow rate of 1.0 ml/min at ambient temperature. The mobile phase was pumped through

the column at a flow rate of 1.0 ml/min with a sample injection volume of 5 µl. Detection of the analytes was carried out at a wavelength of 220 nm.

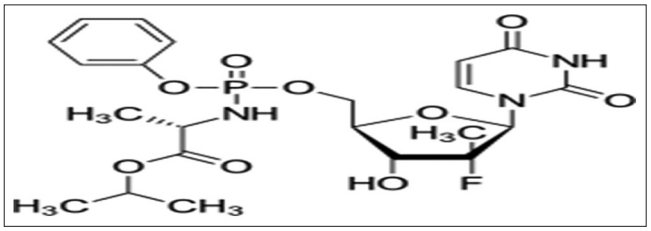

Fig. 1: Chemical structure of sofosbuvir

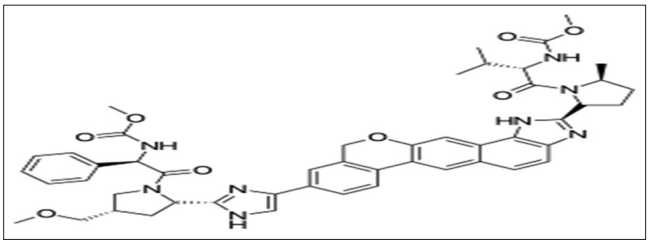

Fig. 2: Chemical structure of velpatasvir

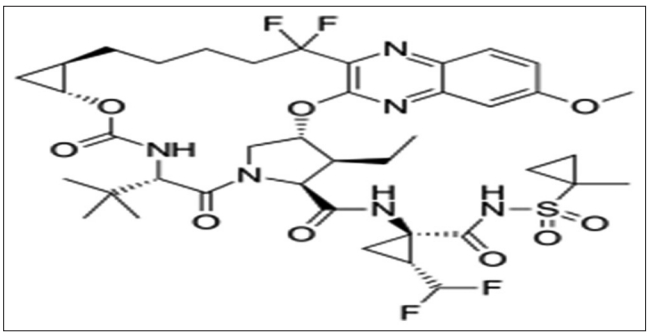

Fig. 3: Chemical structure of voxilaprevir

Preparation of stock and standard solution

Accurately weighed and transferred 100 mg of sofosbuvir, 25 mg of velpatasvir, and 25 mg of voxilaprevir working standards into a 25 ml clean dry volumetric flask and 15 ml of diluents was added to these flasks and sonicated for 30 min and make up to the final volume with diluents and labeled as standard stock solution. From the above solution**,** 1 ml from each stock solution was pipette out and taken into a 10 ml volumetric flask and made up with diluents.

Preparation of sample and standard solution

Ten tablets were accurately weighed and crushed to powder in a motor using pestle and weight equivalent to one tablet was transferred to 100 ml volumetric flask, about 50 ml of diluents was added and sonicated for 25 min, further the volume mark up with diluents and filtered. From above solution, 1.0 ml of filtered sample stock solution was transferred into 10 ml volumetric flask and made up with diluents.

Method validation

The developed method for sofosbuvir, velpatasvir, and voxilaprevir was validated for parameters such as system suitability, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), and solution stability as per ICH guidelines.

RESULTS AND DISCUSSION

Optimized chromatographic conditions

After systematic and detailed study of the various parameters involved in the method, the following conditions were employed:

Instrument	**Waters 2695 high-performance liquid chromatography**
Mobile phase	Acetonitrile and water taken in the ratio of 65:35%v/v
Flow rate	1.0 ml/min
Column	Phenomenex C18 (150×4.6 mm, 5 µ)
Detector wavelength	220 nm
Column temperature	30°C
Injection volume	5 µl
Run time	6 min
Retention time	2.213 min; 2.568 min; and 2.917 min
Diluents	Acetonitrile: Water 65:35%v/v

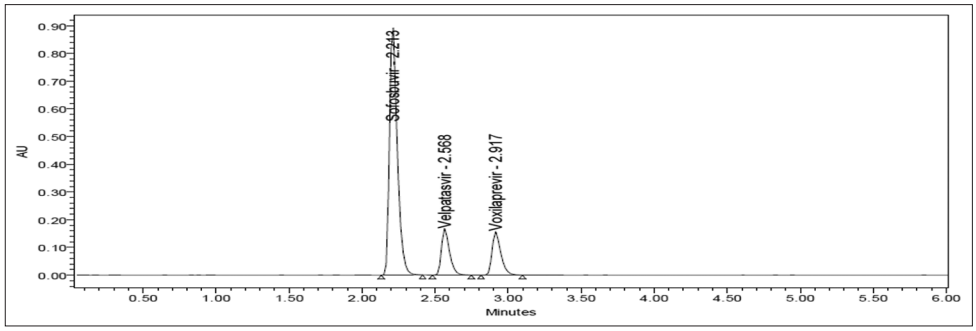

Fig. 4: Typical optimized chromatogram of sofosbuvir, velpatasvir, and voxilaprevir

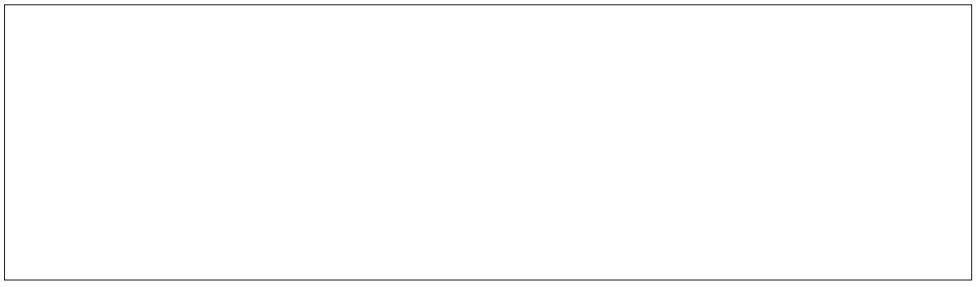

Fig. 5: Typical chromatogram of system suitability solution

Table 1: System suitability test parameter

S.No.	Peak name	Retention time	Peak area	USP plate count	USP resolution	USP tailing
1.	Sofosbuvir	2.213	3,340,127	8009.6		1.3
2.	Velpatasvir	2.568	639,849	10,285.5	3.4	1.3
3.	Voxilaprevir	2.917	645,351	11,637.0	3.1	1.4

Table 2: Linearity data results

Sofosbuvir		Velpatasvir		Voxilaprevir	
Concentration (µg/ml)	**Peak area**	**Concentration (µg/ml)**	**Peak area**	**Concentration (µg/ml)**	**Peak area**
100	406,350	25	75,773	25	83,481
200	814,431	50	160,339	50	160,535
300	1,220,190	75	235,035	75	240,410
400	1,645,779	100	314,221	100	325,656
500	2,034,377	125	386,712	125	406,542
600	2,438,433	150	457,706	150	482,784
Regression equation y=4072.4x+1076.3 Square of correlation coefficient (R^2)=0.9999		Regression equation y=3069.8x+2589.8 Square of correlation coefficient (R^2)=0.9994		Regression equation y=3228x+674.39 Square of correlation coefficient (R^2)=0.9998	

System suitability

Six replicates of the working standard solution were prepared and injected to carry out system suitability parameters such as retention time, peak area, United States Pharmacopeia (USP) plate count, USP resolution, and USP tailing. The system suitability parameters are given in Table 1 and system suitability chromatograms are given in Fig. 5.

Specificity

It is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might be including impurities, degradants, or matrix.

Blank placebo interferences

A study to establish the interferences of blank and placebo was conducted. Analysis was performed on solution placebo and formulation as per the test method. Chromatograms of blank, placebo, and formulation solution showed no peaks at the retention time of sofosbuvir, velpatasvir, and voxilaprevir peaks. The chromatograms of sofosbuvir, velpatasvir, voxilaprevir of standard, formulation, blank, and placebo using the proposed method are shown in Figs. 6-9.

Linearity

Diluting aliquots of 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 ml of standard working solution were pipette out from the standard stock solution of concentration 100 µg/ml of sofosbuvir, 25 µg/ml of velpatasvir, and 25 µg/ml of voxilaprevir and these diluted up to mark with diluents. Such that, the final concentrations were in the range of 100–600 µg/ml sofosbuvir, 25–150 µg/ml velpatasvir, and 25–150 µg/ml voxilaprevir. The calibration standard solution of sofosbuvir, velpatasvir, and voxilaprevir was injected using a 5 µl injector and the chromatograms were recorded at 220 nm and calibration curve was constructed by plotting the peak area versus drug concentration. The linearity data are presented in Figs. 10-12 and Table 2, and all parameter results are within the limits.

LOD and LOQ

A study to establish the LOD and LOQ for sofosbuvir, velpatasvir, and voxilaprevir was conducted. Series of very dilute LOD and LOQ solutions were prepared as per the test method and injected triplicate into the HPLC system. The LOD and LOQ were established based on signal-to-noise ratio. LOD was established by identifying the concentration which given s/n ratio 3, whereas LOQ was established by identifying the concentration which gives s/n ratio of about 10. The sensitivity results are shown in Table 3. Chromatograms of LOD and LOQ values are given in Table 3.

Table 3: Limit of detection and limit of quantification results

S.No.	Parameter	Measured values (µg/ml)		
		Sofosbuvir	**Velpatasvir**	**Voxilaprevir**
1.	Limit of detection	0.77	0.17	0.23
2.	Limit of quantification	2.32	0.53	0.70

Table 4: System precision results

Injection No.	Peak area response of drugs		
	Sofosbuvir	**Velpatasvir**	**Voxilaprevir**
1.	1,647,661	314,647	321,504
2.	1,642,234	319,461	321,969
3.	1,654,419	320,401	320,505
4.	1,639,212	314,874	322,831
5.	1,645,086	316,321	323,427
6.	1,643,523	316,300	327,833
Mean	1,644,895	316,967	3230.2
Standard deviation	5260.8	2406.0	2572.7
% Relative standard deviation	0.3	0.8	0.8

Table 5: Method precision results

Injection No.	Peak area response of drugs		
	Sofosbuvir	**Velpatasvir**	**Voxilaprevir**
1.	1,655,141	317,440	321,302
2.	1,648,499	319,234	321,134
3.	1,642,259	315,111	320,983
4.	1,645,985	320,011	322,422
5.	1,640,340	318,746	325,725
6.	1,645,991	313,611	321,137
Mean	1,646,369	317,359	32,117
Standard deviation	5196.5	2513.2	1843.2
% Relative standard deviation	0.3	0.8	0.6

Precision

It is an analytical method and is defined as agreement between the replicate measurements of the sample. It is expressed as the percentage coefficient of correlation or relative standard deviation (RSD) of the replicate measurements.

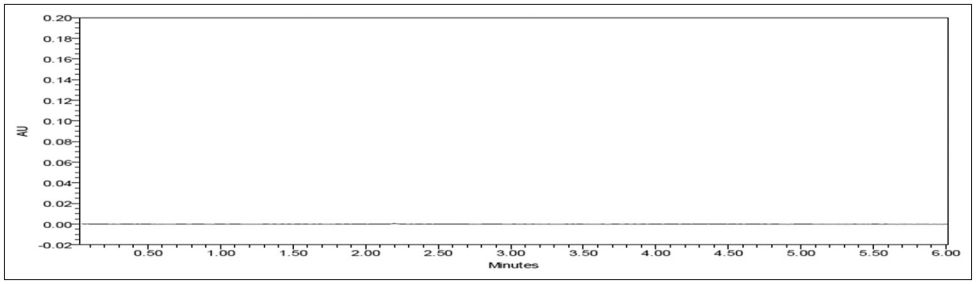

Fig. 6: Chromatogram of blank

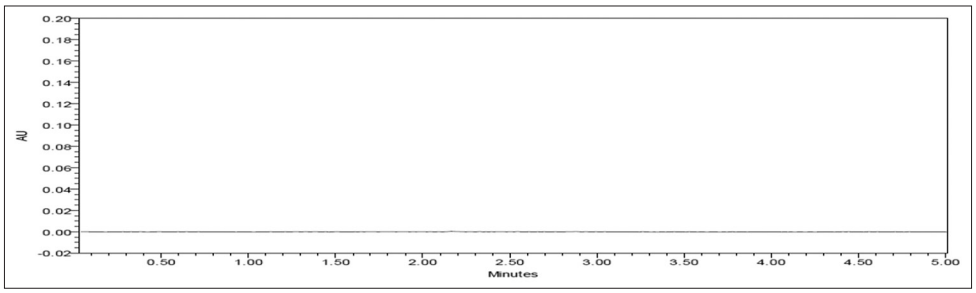

Fig. 7: Chromatogram of placebo

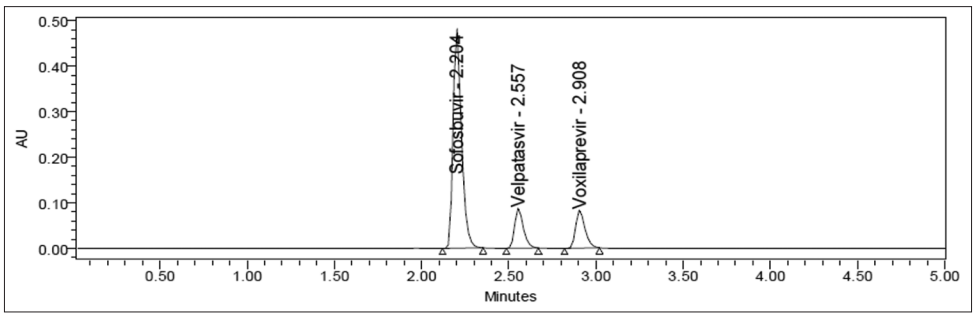

Fig. 8: Chromatogram of standard solution

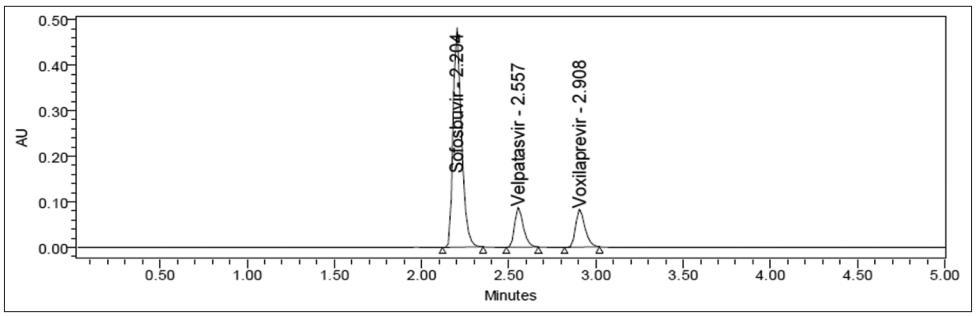

Fig. 9: Chromatogram of formulation

System precision (intraday precision/repeatability)
Working standard preparation of 5 µl solution was injected 6 times into the HPLC system and chromatograms were obtained. %RSD of the peak area was calculated. The results of system precision studies are shown in Table 4.

Method precision
Working sample solutions of 5 µl were injected six preparations into the HPLC system and chromatograms were obtained. The %RSD of the assay result of six preparations was calculated. The results obtained for assay are presented in Table 5.

Intermediate precision/interday precision
Working standard preparation of 5 µl was injected 6 times test preparations into the HPLC system and chromatograms were obtained. %RSD was determined for peak areas. The results of intermediate precision study are reported in Table 6.

Accuracy
A known amount of sofosbuvir, velpatasvir, and voxilaprevir at each three concentration levels 50%, 100%, and 150% was added to a pre-analyzed sample solution and injected in triplicate at each level into the HPLC system. The mean percentage recovery of sofosbuvir, velpatasvir,

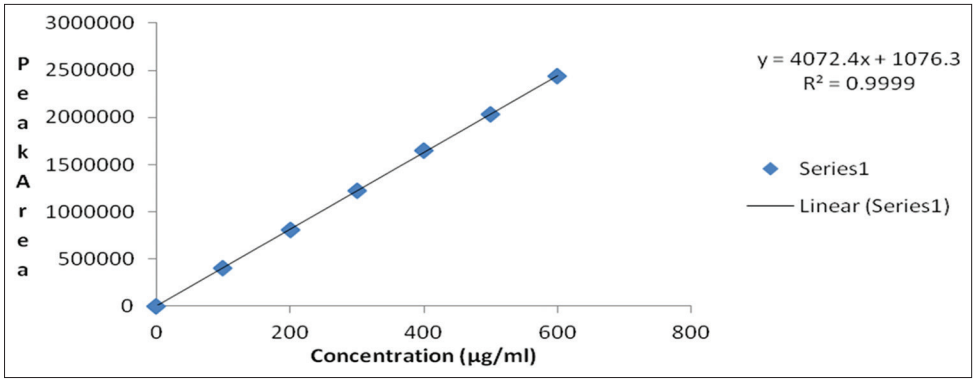

Fig. 10: Standard calibration graph of sofosbuvir

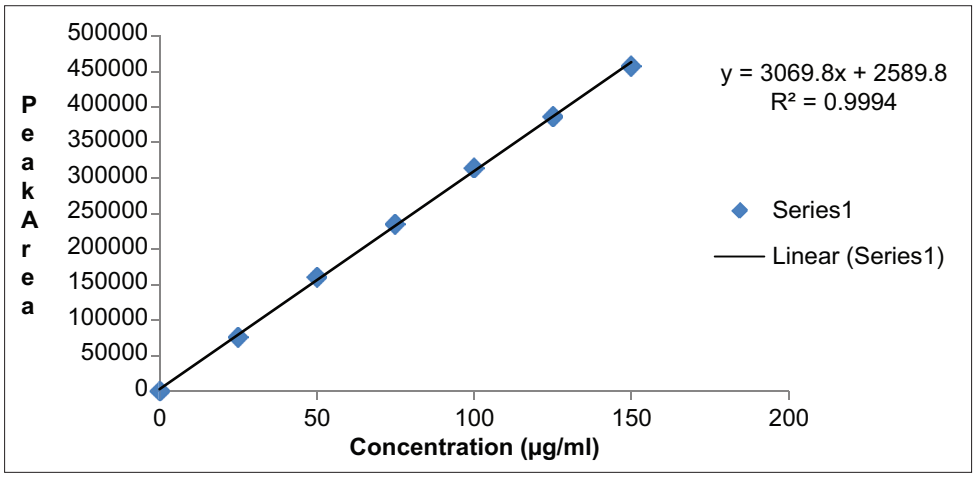

Fig. 11: Standard calibration graph of velpatasvir

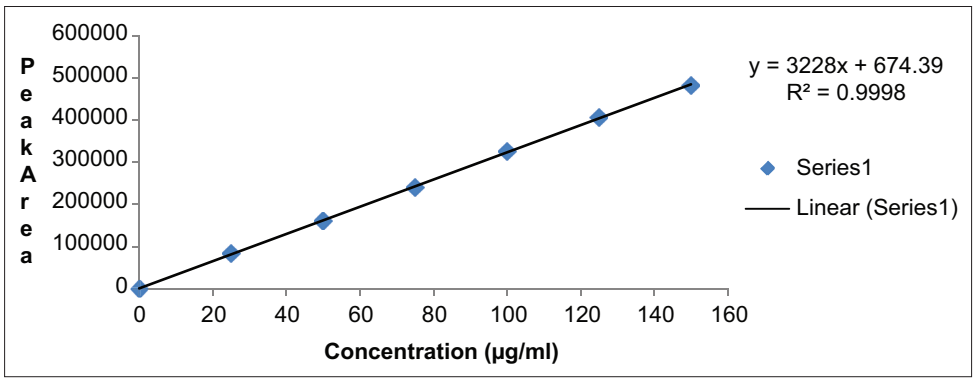

Fig. 12: Standard calibration graph of voxilaprevir

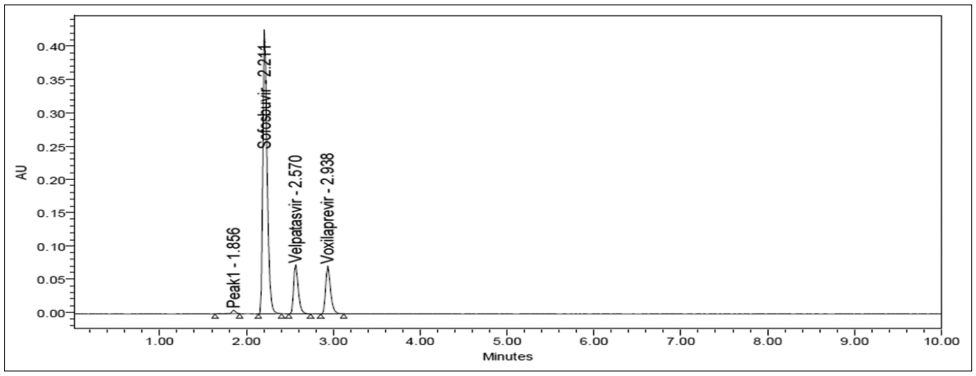

Fig. 13: Chromatogram of acid degradation

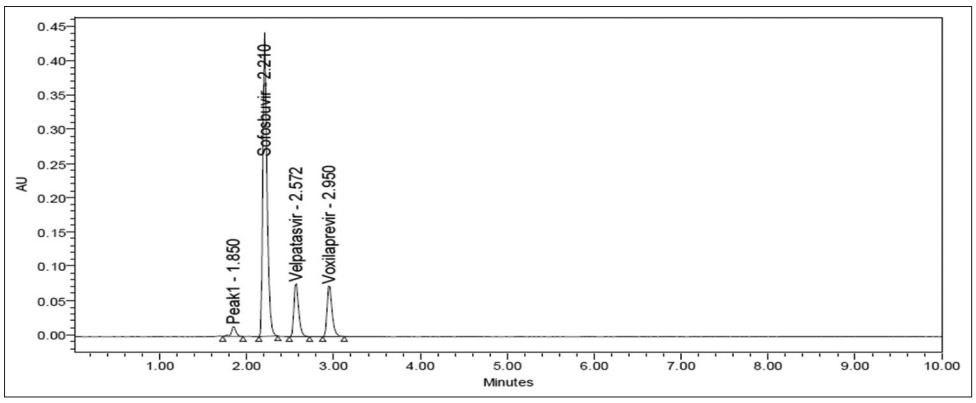

Fig. 14: Chromatogram of base degradation

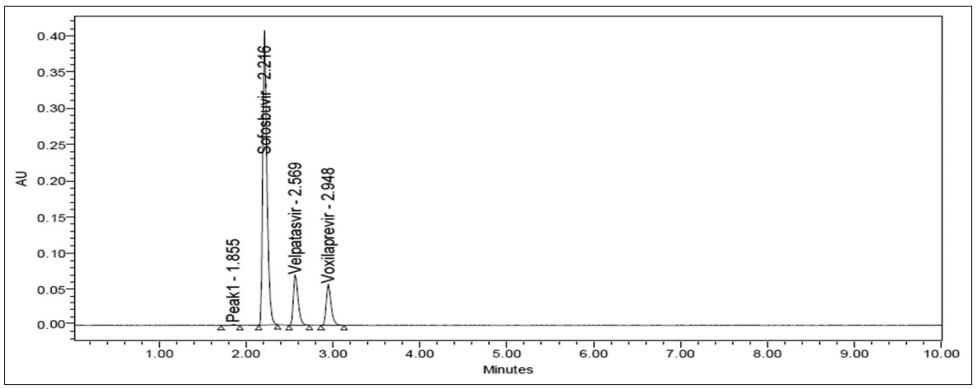

Fig. 15: Chromatogram of peroxide degradation

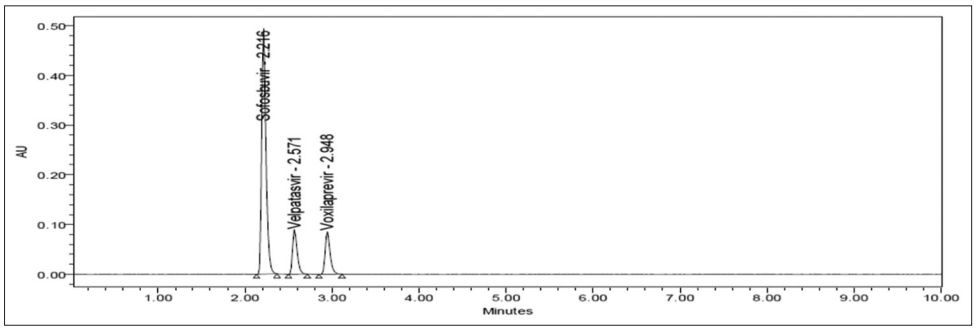

Fig. 16: Chromatogram of thermal degradation

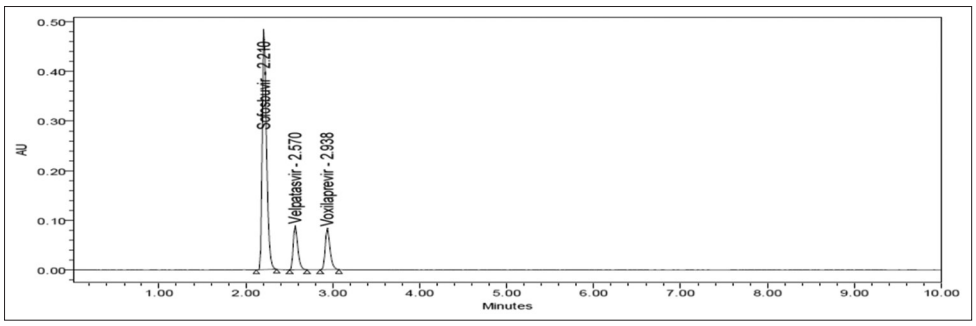

Fig. 17: Chromatogram of ultraviolet degradation

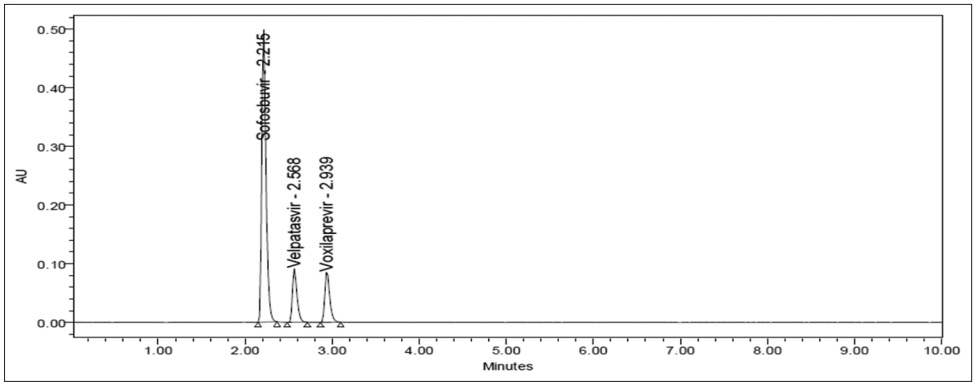

Fig. 18: Chromatogram of water degradation

and voxilaprevir at each level was calculated. The results are presented in Tables 7-9.

Robustness

Working standard solution prepared as per test method was injected into the HPLC system at variable conditions such as flow rate at ±0.1 ml/min, mobile organic phase composition by ±10%, and column temperature by ±5°C. The results of robustness study are shown in Tables 10-12.

Forced degradation studies

Acid degradation studies

To 1 ml of standard stock solution of sofosbuvir, velpatasvir, and voxilaprevir, 1 ml of 1 N hydrochloric acid was added and sonicated for 30 min at 60°C. The resultant solution was diluted to obtain 400 µg/ml, 100 µg/ml, and 100 µg/ml solutions and 5 µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample (Fig. 13).

Base degradation studies

To 1 ml of standard stock solution of sofosbuvir, velpatasvir, and voxilaprevir, 1 ml of 1 N sodium hydroxide was added and sonicated for 30 min at 60°C. The resultant solution was diluted to obtain 400 µg/ml, 100 µg/ml, and 100 µg/ml solutions and 5 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample (Fig. 14).

Oxidation

To 1 ml of standard stock solution of sofosbuvir, velpatasvir, and voxilaprevir, 1 ml of 20% hydrogen peroxide was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 400 µg/ml, 100 µg/ml, and 100 µg/ml solutions and 5 µl solution was injected into the system and the chromatogram was recorded to assess the stability of sample (Fig. 15 and Tables 13-15).

Dry heat degradation studies

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 400 µg/ml, 100 µg/ml, and 100 µg/ml solutions and 5 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample (Fig. 16).

Photostability studies

The photochemical stability of the drug was also studied by exposing the 400 µg/ml, 100 µg/ml, and 100 µg/ml solutions to UV light by keeping the beaker in UV chamber for 3 days or 200 Watt h/m^2 in photostability chamber. For HPLC study, the resultant solution was diluted to obtain 400 µg/ml, 100 µg/ml, and 100 µg/ml solutions and 5 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Table 6: Intermediate precision results

Injection No.	Peak area response of drugs		
	Sofosbuvir	Velpatasvir	Voxilaprevir
1.	1,599,406	290,435	305,906
2.	1,589,699	286,001	306,112
3.	1,607,788	289,084	303,179
4.	1,603,244	288,304	303,904
5.	1,616,783	293,396	300,619
6.	1,604,584	291,041	298,809
Mean	1,603,584	289,710	303,088
Standard deviation	8980.6	2530.2	2904.0
% Relative standard deviation	0.6	0.9	1.0

Table 7: % recovery results of sofosbuvir

Spiked level	Amount spiked (µg/ml)	Amount recovery (µg/ml)	% recovery	Mean % recovery
50%	200	199.903	99.95	100.10%
	200	201.101	100.55	
	200	200.845	100.42	
100%	400	400.714	100.18	
	400	399.891	99.97	
	400	200.845	100.15	
150%	600	600.435	100.07	
	600	599.777	99.96	
	600	597.567	99.59	

Table 8: % recovery results of velpatasvir

Spiked level	Amount spiked (µg/ml)	Amount recovery (µg/ml)	% recovery	Mean % recovery
50%	50	49.898	99.80	99.91%
	50	49.751	99.50	
	50	49.877	99.75	
100%	100	100.860	100.86	
	100	100.659	100.66	
	100	100.650	100.65	
150%	150	148.824	99.22	
	150	149.035	99.36	
	150	149.145	99.43	

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 400 µg/ml, 100 µg/ml, and 100 µg/ml solutions and 5 µl solutions were injected into the system and the

Table 9: % recovery results of voxilaprevir

Spiked level	Amount spiked (µg/ml)	Amount recovery (µg/ml)	% recovery	Mean % recovery
50%	50	50.461	100.92	100.26%
	50	50.398	100.80	
	50	50.473	100.95	
100%	100	99.240	99.24	
	100	99.194	99.19	
	100	99.332	99.33	
150%	150	151.472	100.98	
	150	150.790	100.53	
	150	150.580	100.39	

chromatograms were recorded to assess the stability of the sample (Fig. 17).

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 400 µg/ml, 100 µg/ml, and 100 µg/ml solutions and 5 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample (Fig. 18).

Proposed procedures for marketed pharmaceutical formulation

The commercial tablet (VOSEVI) was analyzed separating by injecting 5 µl of standard and sample preparation solutions injected 6 times into

Table 10: Robustness studies of sofosbuvir

Parameter	Optimized conditions	Used condition	Peak area	Retention time	Plate count	Tailing factor
Flow rate±0.1 ml/min	1 ml/min	0.9 ml/min	1,653,051	2.36	9952	1.32
		1.1 ml/min	1,621,845	2.05	9689	1.37
Column temperature (5°C)	30°C	25°C	1,685,187	2.29	9454	1.27
		35°C	1,656,297	2.19	9309	1.26
Mobile phase composition (5%v/v)	65:35	70:30	1,657,274	2.23	9725	1.32
		60:40	1,585,079	2.18	9187	1.27

Table 11: Robustness studies of velpatasvir

Parameter	Optimized conditions	Used condition	Peak area	Retention time	Plate count	Tailing factor
Flow rate±0.1 ml/min	1 ml/min	0.9 ml/min	303,267	2.74	10,815	1.30
		1.1 ml/min	294,415	2.38	10,649	1.25
Column temperature (5°C)	30°C	25°C	307,054	2.59	10,952	1.26
		35°C	306,720	2.53	11,617	1.27
Mobile phase composition (5%v/v)	65:35	70:30	310,173	2.61	11,907	1.27
		60:40	295,196	2.50	10,894	1.29

Table 12: Robustness studies for sofosbuvir, velpatasvir, and voxilaprevir

Parameter	Optimized conditions	Used condition	Peak area	Retention time	Plate count	Tailing factor
Flow rate±0.1 ml/min	1 ml/min	0.9 ml/min	317,961	3.12	12,216	1.30
		1.1 ml/min	326,991	2.72	12,661	1.27
Column temperature (5°C)	30°C	25°C	315,986	3.00	13,356	1.32
		35°C	318,434	2.86	12,231	1.28
Mobile phase composition (5%v/v)	65:35	70:30	320,049	3.06	14,599	1.25
		60:40	305,351	2.79	12,826	1.23

Table 13: Forced degradation studies results of sofosbuvir

S.No	Degradation conditions	% peak area	Purity angle	Purity threshold	% degradation	Peak purity
1.	Acid	72.02	0.164	0.292	8.90	Passes
2.	Base	69.50	0.262	0.289	7.97	Passes
3.	Peroxide	73.70	0.253	0.290	6.11	Passes
4.	Thermal	71.92	0.270	0.291	3.84	Passes
5.	Ultraviolet	72.28	0.253	0.287	1.56	Passes
6.	Water	71.92	0.264	0.288	0.67	Passes

Table 14: Forced degradation results of velpatasvir

S.No	Degradation condition	% peak area	Purity angle	Purity threshold	% degradation	Peak purity
1.	Acid	13.31	0.129	0.360	4.39	Passes
2.	Base	13.75	0.329	0.350	3.55	Passes
3.	Peroxide	14.21	0.264	0.349	3.06	Passes
4.	Thermal	13.85	0.341	0.354	2.37	Passes
5.	Ultraviolet	13.84	0.233	0.282	1.51	Passes
6.	Water	13.86	0.238	0.363	0.55	Passes

Table 15: Forced degradation results of voxilaprevir

S.No	Degradation condition	% peak area	Purity angle	Purity threshold	% degradation	Peak purity
1.	Acid	13.73	0.325	0.332	7.72	Passes
2.	Base	14.18	0.273	0.321	6.39	Passes
3.	Peroxide	11.89	0.341	0.347	4.54	Passes
4.	Thermal	14.23	0.323	0.338	2.31	Passes
5.	Ultraviolet	13.88	0.312	0.320	1.28	Passes
6.	Water	14.23	0.313	0.324	0.75	Passes

Table 16: Results of assay in tablet dosage form

Formulation	Label claim (mg)	% assay
Sofosbuvir, velpatasvir, and voxilaprevir tablets IP-400mg/ 100 mg/100 mg	Sofosbuvir-400 mg Velpatasvir-100 mg Voxilaprevir-100 mg	99.89 99.92 99.52

the HPLC system and chromatograms were recorded. The amount of the drug present in marketed tablets was calculated by comparing the peak area of standard and sample. The % assay of sofosbuvir, velpatasvir, and voxilaprevir was found to be 99–100%. The typical chromatograms of standard and sample solutions using the proposed method are shown in Table 16.

CONCLUSION

A simple, accurate, precise, and robust method was developed for the stability-indicating RP-HPLC method for the simultaneous estimation of sofosbuvir, velpatasvir, and voxilaprevir in tablet dosage forms. Retention time of sofosbuvir, velpatasvir, and voxilaprevir were found to be 2.213 min, 2.568 min, and 2.917 min. %RSD of the sofosbuvir, velpatasvir, and voxilaprevir was found to be 0.2 µg/ml, 0.9 µg/ml, and 0.6 µg/ml, respectively. Percentage recovery was obtained as 100.01%, 99.91%, and 100.26% for sofosbuvir, velpatasvir, and voxilaprevir, respectively. System precision values were found to be 0.3 µg/ml, 0.8 µg/ml, and 0.8 µg/ml, and method precision values were found to be 0.3 µg/ml, 0.8 µg/ml, and 0.6 µg/ml. LOD and LOQ values obtained from regression equations of sofosbuvir, velpatasvir, and voxilaprevir were 0.7 µg/ml, 0.17 µg/ml, 0.23 µg/ml, 2.32 µg/ml, 0.53 µg/ml, and 0.70 µg/ml. Regression equation of sofosbuvir y=4072.4x+1076.3, velpatasvir y=3069.8x+2589.8, and voxilaprevir y=3228x+974.39, respectively. Stress degradation studies were found to be within limits. Retention times were decreased and run time was decreased. Hence, the method development was simple and economical that can be adopted in regular quality control analysis test in pharmaceutical industries.

AUTHORS' CONTRIBUTIONS

The authors are thankful to Spectrum Pharma Research Solutions, Hyderabad, for providing facilities to carry out the research work.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of the paper.

REFERENCES

1. Rani JS, Devanna N. Development and validation of RP-HPLC method for the simultaneous estimation of sofosbuvir, velpatasvir and voxilaprevir in bulk and tablet dosage forms. Rasayan J Chem 2018;11:452-9.
2. Balaswami B, Ramana PV, Rao BS, Sanjeeva P. A new simple stability indicating RP-HPLC-PDA method for simultaneous estimation of triplicate mixture of sofosbuvir, voxilaprevir and velpatasvir in tablet dosage form. Res J Pharm Technol 2018;11:4147-56.
3. Sofosbuvir, Drug. Available from: https://www.drugbank.ca/drugs/db08934.
4. Velpatasvir, Drug. Available from: https://www.drugbank.ca/drugs/db11613.
5. Voxilaprevir, Drug. Available from: https://www.drugbank.ca/drugs/db12026.
6. Lalitha KV, Reddy JR, Devanna N. Stability indicating RP-HPLC method development and validation for estimation of sofosbuvir in pharmaceutical dosage forms. Pharma Innov 2018;7:656-62.
7. Sathar MD, Suneetha A. RP-HPLC method development and validation for velpatasvir and voxilaprevir by simultaneous determination in bulk and their pharmaceutical dosage forms. Int J Chem Pharm Sci 2018;6:36-42.
8. Devi LM, Reddy TR, Abbul K. Simultaneous determination and validation of third generation antiviral drugs by RP-HPLC method. Int J Pharm Anal Res 2019;8:1-8.
9. Rani JS, Devanna N. A new RP-HPLC method development and validation for simultaneous estimation of sofosbuvir and velpatasvir in pharmaceutical dosage forms. Int J Eng Technol Sci Res 2017;4:145-52.
10. Nalla S, Rao JV. A stability indicating RP-HPLC method for simultaneous estimation of velpatasvir and sofosbuvir in combined tablet dosage forms. World J Pharm Pharm Sci 2017;6:1596-611.
11. ICH Guidelines Q_2 (R1), Validation of Analytical procedures, Text and Methodology; 1995.
12. ICH Guidelines Q_1A (R2), Stability Testing of New Drug Substances and Products, International Conference on Harmonization; 2003.
13. Madhavi S, Ravi AP. Method development and validation for the determination of sofosbuvir from human plasma. Int J Pharm Pharm Sci 2017;9:1.
14. Vanitha C, Reddy B, Satyanarayana SV. Quality-by-design approach to selective stability indicating RP-HPLC method development and validation of estimation of sofosbuvir in bulk drug. Int J Res Pharm Sci 2018;9:298-308.