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ORIGINAL ARTICLE

An RP-UPLC Method Development and Validation for the Simultaneous Quantitation of Sofosbuvir, Velpatasvir and Voxilaprevir in a Dosage Forms

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ABSTRACT
A sensitive, rapid and accurate, stability-indicating RP-UPLC method for the simultaneous estimation of VXR, SFR and VLR in formulations was developed and validated as per the ICH guidelines. Retention times for VXR, SFR and VLR were achieved at 1.677 min, 0.926 min, and 1.259 min respectively. Mean percentage recovery of VXR, SFR and VLR were found to be 99.90%, 99.87%, and 99.91% respectively. LOD and LOQ values obtained from regression equations of VXR, SFR and VLR and were found to be 0.01 µg/ mL /0.02 µg/ mL, 0.13 µg/ mL /0.40 µg/ mL, and 0.01 µg/ mL /0.02 µg/ mL. Regression equation of VXR, SFR and VLR wer: y = 12132x + 144.43, y = 7805.4x + 358.26 and y = 11367x + 226.54 respectively. Stability studies of these drugs proven that the percentage degradation of analytes were found in between 0.32% to 5.90%. Retention time and total run times of analytes were decreased. Hence, the developed method was rapid and economical that can be applicable in routine analysis of these drugs in quality control department of pharmaceutical trades.
Keywords: Sofosbuvir, Velpatasvir, Voxilaprevir, Precision, Robustness.

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INTRODUCTION

Hepatitis C virus 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. In early stage 75% to 85% of the liver is persisted with the virus. These defects have been treated by use of an oral form of these combinational drugs respectively.

Sofosbuvir (Fig.-1a) is an antiviral drug in the treatment of chronic hepatitis C virus. It is chemically isopropyl (2s)-2[[[(2R,3R,4R,5R)-5-(2,4-dioxop[yrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl- tetra hydro furan-2-yl] methoxy- phenoxy-phosphoryl] amino] propanoate. Mainly Sofosbuvir is activated in the liver to the triphosphate by hydrolysis of the carboxylate ester [1-3]

Velpatasvir (Fig.-1b) is an NS5A inhibitor which acts on hepatitis C virus. Velpatasvir is chemically Methyl {(2 S) - 1 -[(2S, 5S) – 2 - (9 - { 2 -[(2S ,4S) – 1 - {(2R) - 2 -[(methoxycarbonyl) amino] -2 - phenylacetyl} - 4- (methoxymethyl) -2 - pyrrolidinyl] -1 H - 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} carbamate used as an anti-cholinergic and anti-spasmodic[4].

Voxilaprevir (Fig.-1c) it is also a protease inhibitor and acts as a transporter of polypeptide. Voxilaprevir is chemically (1R,18R,20R,24S,27S,28S)-N-[(1R,2R)-2-(Difluoromethyl)-1-{[(1-methylcyclopropyl) sulfonyl] carbamoyl} cyclopropyl]-28-ethyl-13,13-difluoro-7-methoxy-24-(2-methyl-2-propanyl)-22, 25-dioxo-2,21-dioxa-4,11,23,26-tetra aza penta cyclo nonacosa-3(12),4,6,8,10-pentaene-27-carboxamide [5].

Some of the literature was available for the combination of Sofosbuvir and Velpatasvir. No literature available for the estimation of Sofosbuvir, Velpatasvir and Voxilaprevir in a combined dosage form. Sofosbuvir(400mg), Velpatasvir(100mg) and Voxilaprevir(100mg) were available in a mixed dose form of VOSEVI. The US FDA was approved in 2017[6-8]. The HPLC technique was used for the development and validation of combinational drugs were reported. But no method was found for the estimation of Sofosbuvir (Sof), Velpatasvir(Vel) and Voxilaprevir(Vox) in pharmaceutical dosage forms in the literature.As per ICH guidelines the method was developed and validated.

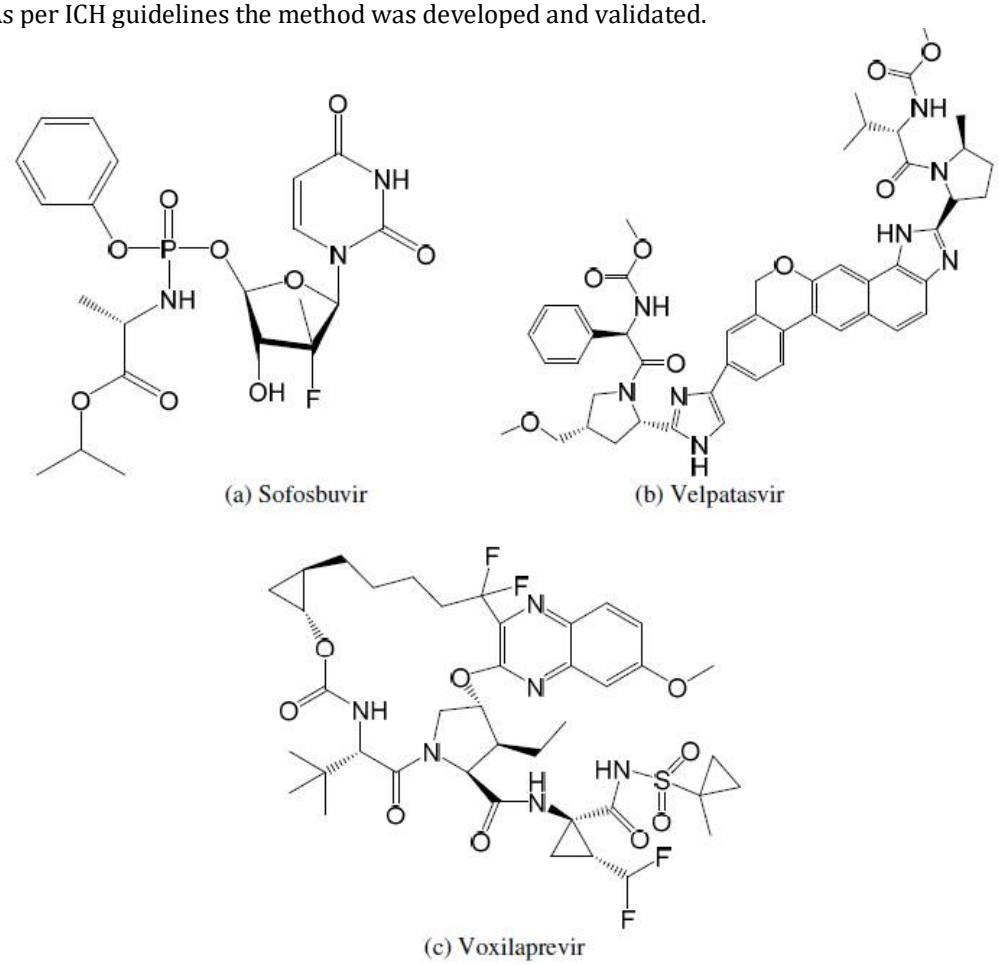

Fig. 1: Chemical Structures of analytes.

MATERIAL AND METHODS

Chemicals and Reagents

The standard components of VXR, SFR and VLR were provided as a gift sample from spectrum Pharma Research Solutions, Hyderabad. VOSEVI tablets labeled to contain VXR 100 mg, SFR 400 mg and VLR 100 mg were procured from the local market. HPLC grade acetonitrile, methanol were obtained from A.B enterprises, Mumbai, India. Orthophosphoric acid was bought from Ranchem, Mumbai, India. HPLC grade water was processed by utilizing Milli-Q Millipore water purification system used during the method development.

Method development

During the method development various mobile phase compositions consisting of methanol, acetonitrile, water, phosphate buffers and different stationary phases were executed to get fine chromatographic conditions like theoretical plates, resolution, tailing and peak shape[9-14].

Optimized Chromatographic Conditions

Chromatographic system of Waters UPLC system furnished with photodiode array detector, auto-sampler, and Phenomenex C18 column which have dimensions of 100 × 2.1mm, 2.0µ particle size. The output signal was monitored and integrated utilizing water Empower-2.0 software. The isocratic mobile consisting of 0.01N Potassium dihydrogen ortho phosphate (pH 4.8) and methanol in the proportion of 40:60%v/v, pumped through the Phenomenex C18 (100 × 2.1mm, 2.0µ) column at a fixed flow of 1 mL/

min. The injection volume of 1.00 µL was utilized to measure the chromatograms at 260 nm as wavelength maximum in the detection system.

Preparation of Buffer

Accurately weighed 1.36gm of Potassium dihydrogen orthophosphate in a 1000mL of Volumetric flask add about 900mL of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1mL of triethylamine then PH adjusted to 4.8 with dilute orthophosphoric acid solution[15-17].

Preparation of Stock and Standard Solution

Accurately Weighed and transferred 10mg of SFR and 2.5mg of VLR & 2.5mg of VXR working Standards into a 25mL clean and dry volumetric flask, add 3/4th volume of diluent (Water: Acetonitrile (50:50 v/v)), sonicated for 5 minutes and made up to final volume with diluent. 1.0 mL from the above stock solution was taken into a 10 mL volumetric flask and made up to 10 mL to get 40 µg/ mL of SFR and 10 µg/ mL of VLR and 10 µg/ mL of VXR[10-14].

Preparation of Sample Solution

Five tablets were weighed and calculated the average weight of tablets and then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask containing 50 mL of diluent and sonicated for 25.0 min. Further the volume made up with diluent and subjected for filtration. From the filtrate 1.0 mL solution was pipetted out into a 10.0 mL volumetric flask and made upto 10.0 mL with diluent.

Analytical Method Validation

The developed method for VXR, SFR and VLR was subjected for validation for the parameters like limit of detection (LOD), limit of quantification (LOQ), linearity, robustness, precision, system suitability and accuracy as per the guidelines of ICH.

RESULTS AND DISCUSSION

Method development

With different mobile phase compositions and stationary phases three different trials were executed and fourth trail was optimized[14-19]. In all the three trials: merged peaks were observed in trail-1, peak shape was poor and tailing in the trail-2 and base line was poor in the trial -3. Optimized chromatographic peaks were shown in Fig. 2.

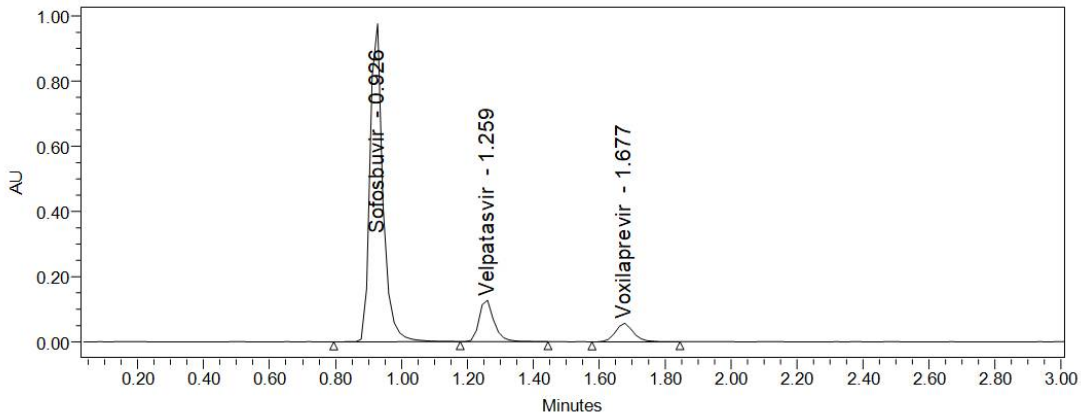

Fig. 2: Optimized Chromatogram

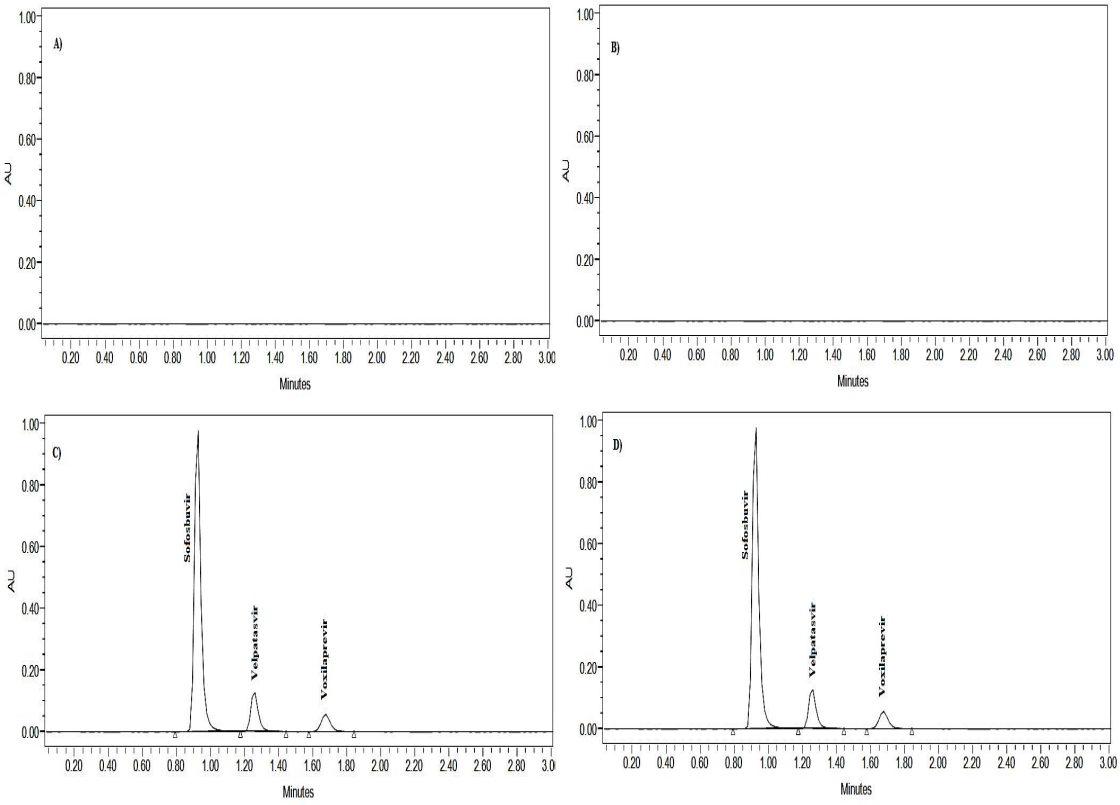

Fig. 3: Chromatograms of A) blank, B) Placebo, C) Standard and D) Formulation

Method Validation

Specificity

It is the ability of a method to unequivocally evaluate the analyte components in presence of other components like impurities, degradants and excipients etc. expected to be present. This parameter was estimated by injecting and evaluating the blank, placebo, standard and sample solutions and chromatograms respectively. Chromatograms of blank, placebo, and sample solution shown no peaks at the retaining time of VXR, SFR and VLR peaks. The chromatograms of VXR, SFR and VLR of standard, blank, formulation, and placebo were represented in Fig. 3.

Linearity

Aliquots of 0.25, 0.50, 0.75, 1.0, 1.25, and 1.50 mL of standard stock solution were pipetted out from the standard stock solution of concentration 100 µg/ mL of VXR, 400 µg/ mL of SFR and 100 µg/ mL of VLR and made up to 10.0 mL mark with diluent. The resulting solutions were came into 2.5 to 15.0 µg/ mL of VXR, 10.0 to 60 µg/ mL of SFR and 2.5 to 15.0 µg/ mL of VLR concentration range. The resulting linearity solutions were infused into a chromatographic system and form the chromatograms linearity graph was plotted by taking the peak area on Y-axis and concentration on X-axis. The calibration graphs were shown in Fig. 4 to 6 and Table 1.

Table 1: Calibration curve data of VXR, SFR and VLR

SFR		VLR		VXR	
Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**	**Concentration (µg/mL)**	**Peak area**
10	80570	2.5	29134	2.5	31001
20	154713	5	57385	5	60744
30	235700	7.5	84709	7.5	91775
40	311047	10	113309	10	120409
50	388511	12.5	143371	12.5	150447
60	471096	15	170419	15	183558
Regression equation					
y = 7805.4x + 358.26		y = 11367x + 226.54		y = 12132x + 144.43	
Correlation coefficient (R^2)					
0.9999		0.9999		0.9998	

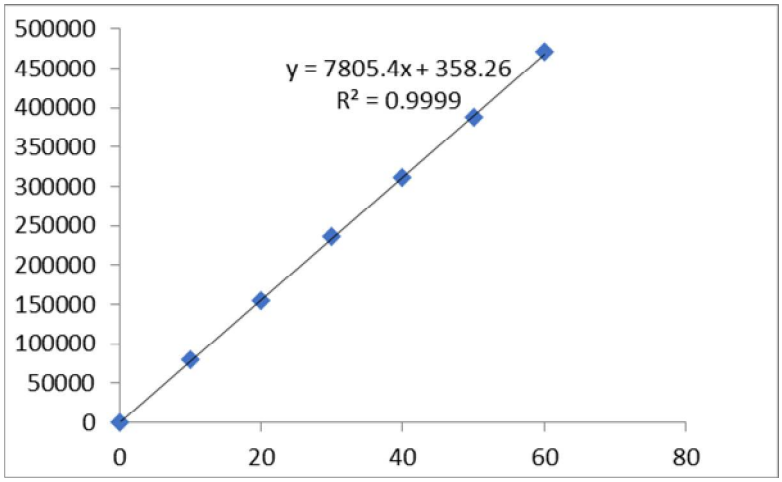

Fig. 4: Linearity of SFR

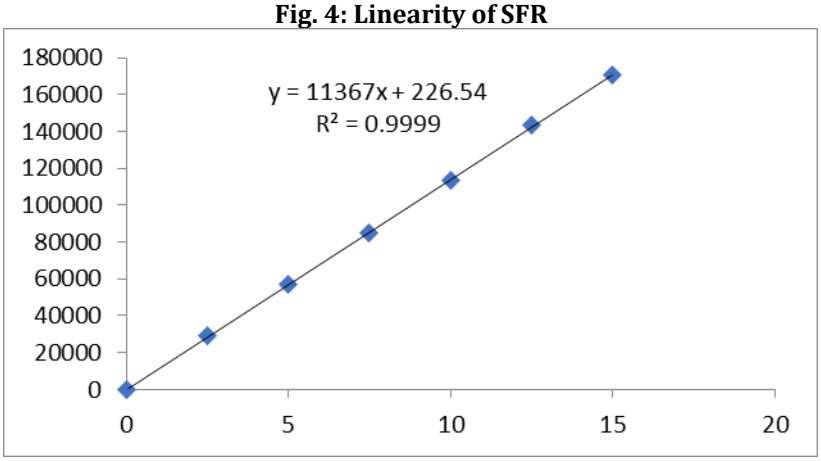

Fig. 5: Linearity of VLR

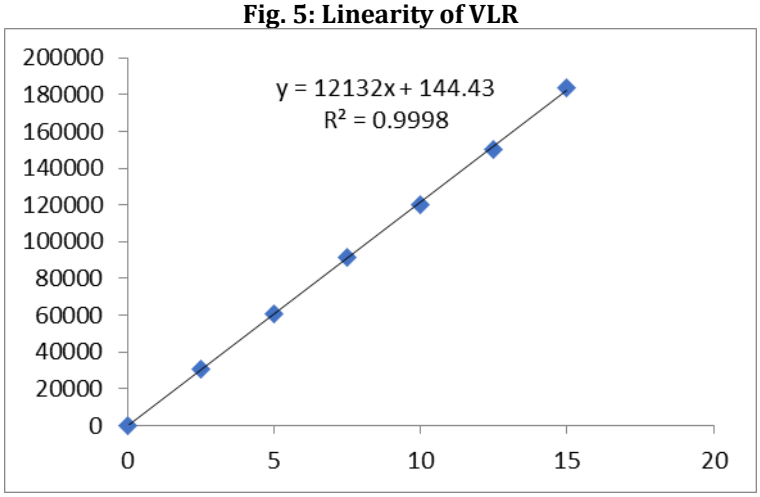

Fig. 6: Linearity of VXR

System Suitability

Six replicates of the standard reference solution were processed and infused to perform the system suitability parameter and the resulting chromatograms peak area, retention time, resolution, plate count, and tailing were measure[11-16]. The findings of system suitability parameter were shown in the Table. 2.

Table. 2: VXR, SFR and VLR system suitability results.

S No	Peak name	Peak area	Retention time	Plate count	Resolution	Tailing
1.	SFR	312779	0.926	4198		1.03
2.	VLR	112479	1.259	4247	5.7	1.25
3.	VXR	120173	1.677	5363	8.5	1.11

LOD and LOQ

LOD and LOQ parameters for VXR, SFR and VLR were calculated form the linear regression equation. Linearity values, graph and regression equation were got from the linearity study and the LOD and LOQ values were represented in the Table 3.

Table. 3: Limit of detection and limit of quantification results

Parameter	Measured concentration (µg/mL)		
	SFR	VLR	VXR
LOD	0.13	0.01	0.01
LOQ	0.40	0.02	0.02

Precision

Analytical method precision is defined as closeness of agreement between the replicate measurements of the analyte[9-14]. It is expressed as the percentage coefficient of correlation or relative standard deviation (RSD) of the replicate measurements.

System Precision

Working standard preparation of 1.0 µL solution was infused six times into the chromatographic system and chromatograms were obtained. %RSD of the peak area was calculated. The findings of system precision were shown in Table. 4.

Table. 4: System precision data

S. No.	Peak area response of drugs		
	SFR	VLR	VXR
1	312069	112479	120173
2	312779	113408	121013
3.	312238	110728	120615
4	312774	113342	120586
5	311121	113450	120450
6	307430	113258	120670
Average	311402	112778	120585
STDV	2038.5	1066.7	275.4
% RSD	0.7	0.9	0.2

Method Precision

Working sample solutions of 1.0 µL was infused 6 times into the chromatographic system and chromatograms were obtained. The %RSD of the assay result of six preparations was determined. The findings achieved for assay were represented in Table. 5.

Table. 5: Method precision results

S. No.	Peak area response of drugs		
	SFR	VLR	VXR
1	314292	113184	120333
2	311541	112188	121233
3.	311924	113675	120563
4	312765	113015	120619
5	310377	112819	120173
6	313315	112899	120932
Average	312369	112963	120642
STDV	1385.7	486.6	388.7
% RSD	0.4	0.4	0.3

Intermediate Precision

Working standard preparation of 1.0 µL was infused six times test preparations into the chromatographic system and chromatograms were obtained. The %RSD was evaluated for peak areas. The findings of intermediate precision study were represented in Table. 6.

Table. 6: Intermediate precision results

S. No.	Peak area response of drugs		
	SFR	VLR	VXR
1	310514	108619	117972
2	310229	109543	115023
3	306881	108648	116751
4	307498	107947	117905
5	301224	109878	118239
6	302657	108905	116389
Average	306501	108923	117047
STDV	3840.9	695.3	1235.3
% RSD	1.3	0.6	1.1

Accuracy

A known amount of VXR, SFR and VLR at each three concentration levels of 50%, 100%, and 150% was added to a pre-analyzed sample solution and injected in triplicate at each level into the chromatographic system [12-14]. The mean percentage recovery of VXR, SFR and VLR at each level was estimated. The findings were represented in Tables 7.

Table. 7: Percentage recovery results

	SFR				VLR				VXR			
Spiked level	spiked (µg/mL)	recovery (µg/mL)	% recovery	Mean % recovery	spiked (µg/mL)	recovery (µg/mL)	% recovery	Mean % recovery	spiked (µg/mL)	recovery (µg/mL)	% recovery	Mean % recovery
50%	20	19.9527	99.76	99.90	5	4.964415	99.29	99.87	5	4.969964	99.40	99.81
	20	20.15859	100.79		5	4.988168	99.76		5	4.999637	99.99	
	20	20.11874	100.59		5	4.988255	99.77		5	4.980762	99.62	
100%	40	40.14655	100.37		10	9.950955	99.51		10	9.977629	99.78	
	40	39.68761	99.22		10	9.99415	99.94		10	9.944576	99.45	
	40	40.02432	100.06		10	9.975939	99.76		10	10.03351	100.34	
150%	60	59.5714	99.29		15	15.05265	100.35		15	14.92924	99.53	
	60	59.66109	99.44		15	15.07553	100.50		15	15.102	100.68	
	60	59.73732	99.56		15	14.98993	99.93		15	14.929	99.53	

Robustness

Working standard solution prepared as per test method was infused into the chromatographic system at variable conditions such as flow rate at ±0.1 mL/min, mobile organic phase composition by ±10%, and column temperature [15-19] by ±5°C. The results of robustness study parameter like peak area, retention time, plate count and tailing factor were within the limits.

Forced Degradation studies

Acid Degradation Studies

To 1 mL of stock s solution VXR, SFR and VLR, 1 mL of 2N Hydrochloric acid was added and refluxed for 30mins at 60°C[17, 18]. The resultant solution was diluted to obtain 40 µg/ mL of SFR and 10 µg/ mL of VLR and 10 µg/ mL of VXR solution and 1.0 µL was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (Fig. 7 and Table 8).

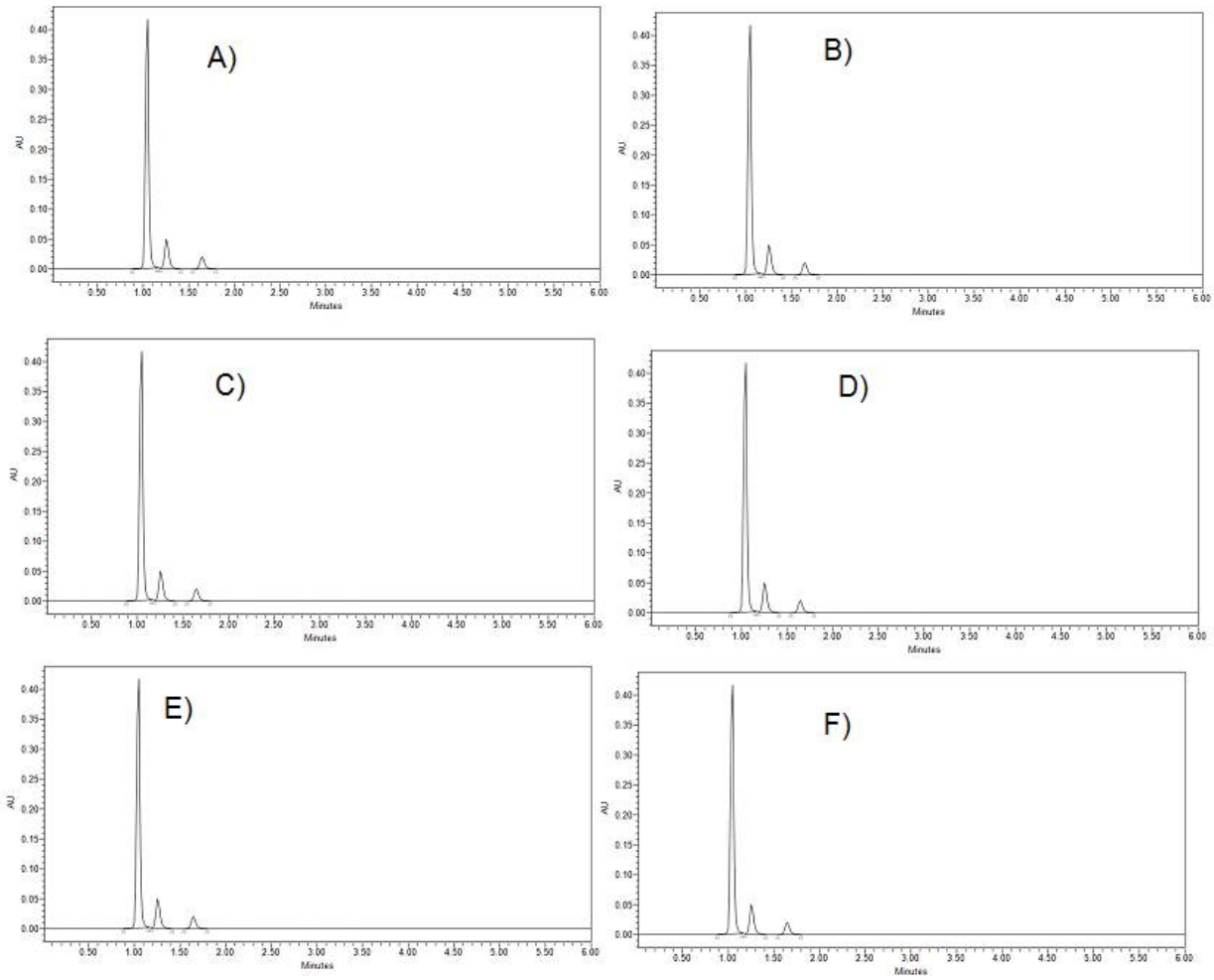

Fig. 7: Chromatogram for A) acid B) Oxidation C) alkali D) dry heat E) photo F) neutral degradation study.

Table. 8: Results of stress degradation study.

S.No	Degradation condition	SFR		VLR		VXR	
		% recovery	% Degraded	% recovery	% Degraded	% recovery	% Degraded
1	Acid hydrolysis	94.10	5.90	94.17	5.83	95.28	4.72
2	Base hydrolysis	95.56	4.44	95.69	4.31	96.36	3.64
3	Peroxide	96.81	3.19	96.90	3.10	96.42	3.58
4	Dry heat	97.12	2.88	97.74	2.26	97.04	2.96
5	Photo stability	98.82	1.18	98.42	1.58	98.49	1.51
6	Water sample	99.42	0.58	99.10	0.90	99.68	0.32

Oxidation

To 1 mL of stock solution of VXR, SFR and VLR, 1 mL of 20% hydrogen peroxide (H2O2) was added separately[71]. The solutions were kept for 30 min at 60°C. For UPLC study, the resultant solution was diluted to obtain 40 µg/ mL of SFR and 10 µg/ mL of VLR and 10 µg/ mL of VXR solution and 1.0 µL was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (Fig. 7 and Table 8).

Alkali Degradation Studies

To 1 mL of stock solution VXR, SFR and VLR, 1 mL of 2N sodium hydroxide was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 40 µg/ mL of SFR and 10 µg/ mL of VLR and 10 µg/ mL of VXR solution and 1.0 µL was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (Fig. 7 and Table 8).

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation[24]. For UPLC study, the resultant solution was diluted to get 40 µg/ mL of SFR and 10 µg/ mL of VLR and 10 µg/ mL of VXR solution and 1.0 µL was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (Fig. 7 and Table 8).

Photo Stability Studies

The photochemical stability of the drug was also studied by exposing the (100 µg/ mL, 400 µg/ mL and 100 µg/mL) solution to UV Light by keeping the beaker in UV Chamber for 3days or 200 Watt hours/m^2 in photo stability chamber. For UPLC study, the resultant solution was diluted to obtain 40 µg/mL of SFR and 10 µg/mL of VLR and 10 µg/mL of VXR solution and 1.0 µL was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (Fig. 7 and Table 8).

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60 °C. For UPLC study, the resultant solution was diluted to obtain 40 µg/ mL mL of SFR and 10 µg/ mL of VLR and 10 µg/ mL of VXR solution and 1.0 µL was injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample (Fig. 7 and Table 8).

Assay of Marketed Formulation

The marketed formulation of VOSEVI (film coated tablet) was evaluated by infusing 1.0 µL of reference and analyte solutions six times into the chromatographic system and the resulting chromatograms of analytes were documented. The quantity of anaytes existed in the marketed formulation were estimated by equating the peak area of reference and analyte. The % assay of VXR, SFR and VLR were found to be 99.0–101.0%.

In the literature all the methods were reported on the HPLC techniques with more retention time and run times. In the present work we selected UPLC to reduce the total run time. Method development was executed with different columns and mobile phases. Finally, the method was optimized with mobile phase of 0.01N Potassium dihydrogen orthophosphate (pH 4.8) and methanol in the proportion of 40:60%v/v utilizing a Phenomenex C18 column which has dimensions of 100 × 2.1mm, 2.0µ particle size and the flow rate of 1.0 mL/min. Further, the developed method was subjected for validation and forced degradation studies. Validation was executed as per the ICH Q2R1 guidelines for the parameters specificity, linearity, system suitability, LOD and LOQ, precision, accuracy and robustness. All the parameters were within the limits. Developed method was subjected for forced degradation studies as per the ICH like neutral degradation, photo stability, dry heat degradation, alkali degradation, oxidation and acid degradation. The degradation results also produced in the results section.

CONCLUSION

A sensitive, rapid and accurate, stability-indicating RP-UPLC method for the simultaneous estimation of VXR, SFR and VLR in formulations was developed and validated as per the ICH guidelines. Retention times for VXR, SFR and VLR were achieved at 1.677 min, 0.926 min, and 1.259 min respectively. Mean percentage recovery of VXR, SFR and VLR were found to be 99.90%, 99.87%, and 99.91% respectively. LOD and LOQ values obtained from regression equations of VXR, SFR and VLR and were found to be 0.01 µg/ mL /0.02 µg/ mL, 0.13 µg/ mL /0.40 µg/ mL, and 0.01 µg/ mL /0.02 µg/ mL. Regression equation of VXR, SFR and VLR wer: y = 12132x + 144.43, y = 7805.4x + 358.26 and y = 11367x + 226.54 respectively. Stability studies of these drugs proven that the percentage degradation of analytes were found in between 0.32% to 5.90%. Retention time and total run times of analytes were decreased. Hence, the developed method was rapid and economical that can be applicable in routine analysis of these drugs in quality control department of pharmaceutical trades.

REFERENCES

1. Berden FA, Kievit W, Baak LC, Bakker CM, Beuers U, Boucher CA, et al. (2014). Dutch guidance for the treatment of chronic hepatitis C virus infection in a new therapeutic era. The Netherlands Journal of Medicine. 72 (8): 388–400.
2. Cholongitas E, Papatheodoridis GV. (2014). Sofosbuvir: a novel oral agent for chronic hepatitis C. Annals of Gastroenterology. 27 (4): 331–337.
3. Tran TT. (2012). A review of standard and newer treatment strategies in hepatitis C. The American Journal of Managed Care. 18: S340–S349.
4. Heo YA, Deeks ED. (2018). Sofosbuvir/Velpatasvir/Voxilaprevir: A Review in Chronic Hepatitis C. Drugs. 78 (5): 577–587.

5. Sandhya, J.Sandhya Rani. (2018). Development and validation of RP-HPLC method for the simultaneous estimation of Sofosbuvir, Velpatasvir and Voxilaprevir in bulk and tablet dosage forms. Rasayan Journal of Chemistry. 11(2):452 – 459.
6. Zaman, B., Siddique, F. & Hassan, W. (2016). RP-HPLC Method for Simultaneous Determination of Sofosbuvir and Ledipasvir in Tablet Dosage Form and Its Application to In Vitro Dissolution Studies. Chromatographia 79, 1605–1613. https://doi.org/10.1007/s10337-016-3179-9.
7. Parimi U. (2017). Analytical Method Development and Validation for the Simultaneous Estimation of Sofosbuvir and Velpatasvir Drug Product by RP-HPLC Method. Indo American Journal of Pharmaceutical Research. 7(08).401-409.
8. Lee SC, Pinsonnault C, Ackad N, Landry P. (2018). A189 treatment of chronic hepatitis C genotype 1 in canada, real world experience with ombitasvir/paritaprevir/r and dasabuvir with or without ribavirin. J Canadian Association Gastroenterol;1:329–30.
9. Asselah T, Hezode C, Qaqish RB, ElKhashab M, Hassanein T. (2016). Ombitasvir, paritaprevir, and ritonavir plus ribavirin in adults with hepatitis C virus genotype 4 infection and cirrhosis (AGATE-I) a multicentre, phase 3, randomised open-label trial. Lancet Gastroenterol Hepatol;1:25-35.
10. Nicholas JB, Zameer M, Asmaa G, Mark WS, Nicola AC. (2017). Hepatitis C treatment where are we now. Int J Gen Med ;10:39–52.
11. Poordad F, Agarwal K, Younes Z, Cohen D, Xie W, Podsadecki T. (2015). Low relapse rate leads to high concordance of sustained viro-logic response (SVR) at 12 w with SVR at 24 w after treatment with ABT-450/ritonavir, ombitasvir, and dasabuvir plus ribavirin in subjects with chronic hepatitis C virus genotype 1 infection in the AVIATOR study. Clin Infect Dis;60:608-10.
12. Jensen SB, Serre SB, Humes DG, Ramirez S, Li YP, Bukh J, Gottwein JM. (2015). Substitutions at NS3 residue 155, 156, or 168 of hepatitis C virus genotypes 2 to 6 induce complex patterns of protease inhibitor resistance. Antimicrob Agents Chemoth-er;59:7426-36.
13. Kranthi Kiran K, Srinivasa Rao A, Gowri Sankar D. (2017). Development and validation of new stability indicating RP-HPLC method for the determination of selected combinational antiviral drugs in bulk and pharmaceutical dosage forms. Int J Med Chem Anal;7:63-73.
14. Jahnavi B, Ganapaty S. (2018). Stability indicating RP-HPLC method development and validation for the simultaneous determina-tion of ombitasvir, paritaprevir and ritonavir in tablet dosage forms. Asian J Pharm Edu Res;7:90-101.
15. Srinivas B, Yadagiriswamy P. (2017). Analytical method validation report for assay of ombitasvir, paritaprevir and ritonavir by RP-HPLC. Int J Anal Bio Chem;7:12-22.
16. International conference on harmonization ICH harmonised tripartite guideline Validation of analytical procedures, text and methodology Q2 (R1) ICH, Geneva; 2005.
17. International Conference on Harmonization (ICH), Stability testing of new drug substances and products, Q1A (R2); 2003.
18. Parbati K, Appala Raju N. (2018). Development and application of the liquid chromatographic method for simultaneous determination of ombitasvir, paritaprevir and ritonavir in fixed tablet do-sage form. Indo Am J Pharm Res ;8:1459-67.