Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Stability Indicating HPTLC Method for Sofosbuvir and Velpatasvir in Combination

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ARTICLE INFO

Article history:
Received: 07 January, 2020
Revised: 04 February, 2020
Accepted: 10 February, 2020
Published: 30 March, 2020

Keywords:
Hepatitis-C, High-performance thin-layer chromatography, Sofosbuvir, Stability-indicating method, Velpatasvir.

DOI:
10.25004/IJPSDR.2020.120206

ABSTRACT

The discovery of new direct-acting antiviral drugs gave rise to a leap forward in the treatment of hepatitis C viral (HCV) infections. For the first time since 1998, the Food and Drug Administration (FDA) approved interferon-free oral treatment paradigms. Among the new treatment regimens, the combinations of Sofosbuvir (SOF) and Velpatasvir (VEL) became ideal treatment regimens for being potent, highly tolerated, and used once daily. Hence accurate, precise, selective, and sensitive stability-indicating method for simultaneous estimation of SOR and VEL by high-performance Thin layer chromatography has been developed and validated. Chromatographic separation was achieved on TLC plates coated with silica gel 60 F_{254} as a stationary phase. Ethyl acetate: isopropyl alcohol (9:1 v/v) was used as a mobile phase. Densitometric scanning was carried out at 260 and 302 nm for SOF and VEL, respectively. The method was successfully validated as per the ICH Guideline. The linear concentration range was 100-2000 ng/band (r^2 = 0.991) and 100-500 ng/band (r^2 = 0.991) for SOF and VEL respectively. The LoD was 25.16 ng/band and 9.96 ng/band for SOF and VEL, LoQ were 76.25 ng/band and 30.19 ng/band for SOF and VEL. The method could be applied to the quality control and routine analysis of SOF and VEL in their pure forms and pharmaceutical formulations.

Introduction

Hepatitis C is a liver disease caused by the HCV. The fixed drug combination consists of sofosbuvir (SOF) (400 mg) and Velpatasvir (VEL) (100 mg), used in the treatment of hepatitis C. This is a new direct-acting antiviral drug combination, which is approved by United States Food and Drug Administration in June 2016. The combination of SOR and VEL became the ideal treatment regimen for being most potent, highly tolerated. This direct-acting antiviral was approved for the treatment of adults with chronic hepatitis C with or without compensated cirrhosis, and in combination with ribavirin for decompensated cirrhosis, for all 6 genotypes.

The IUPAC name of SOR is Isopropyl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]-methoxy-phenoxy-phosphoryl]-amino]-propanoate. It is inhibitor of the HCV NS5B ribonucleic acid (RNA) dependent RNA polymerase, which undergoes intracellular metabolism to form uridine analogue triphosphate and inhibits the viral replication by incorporating into HCV RNA and acts as a chain terminator.

VEL chemically is methyl((S)-1-((S)-2-(5-(6-(2((S)-1-((methoxycarbonyl)-L-valyl)pyrrolidin-2-yl)-1H-imidazol-4-yl)naphthalen-2-yl)-1H-benzo[d]imidazol-2-yl) pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate is an inhibitor of HCV NS5A protein, which blocks the action of the protein and inhibits the viral replication.

The main objective of the current work was to develop and validate the stability-indicating high-performance thin layer chromatography (HPTLC) method for the

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

simultaneous estimation of SOF and VEL. The method was validated as per the ICH guidelines. After a thorough literature survey, it is observed that there are many analytical methods reported for analysis of SOF and VEL, either single or in combination, but no SIM- HPTLC method was found for combination. The reported methods of SOF combination with VEL include analytical methods like LC-MS/MS,[1] HPLC,[2-5] UPLC MS/MS[6] and UPLC.[7]

Materials And Methods

Materials and Reagents

The SOF and VEL pure drug were received as a gift sample from NATCO, Hyderabad. Ethyl Acetate (HPLC grade) and Isopropyl alcohol (HPLC grade) were purchased from Loba Chemie Pvt. Ltd. (India). Methanol (HPLC grade). HPTLC pre-coated plates were purchased from Merck Pvt. Ltd.

Equipment

A CAMAG HPTLC system equipped with a Linomat 5 sample applicator operated under a gentle stream of nitrogen, coupled with a Hamilton microliter syringe (100 µL). CAMAG TLC SCANNER 3 controlled by WINCATS software was used for the application and detection of spots, respectively. Chromatographic separation was carried on TLC aluminum plates pre-coated with silica gel 60 F_{254}(10 × 10 cm), and development was carried out in CAMAG twin trough the TLC chamber. Chamber saturation is achieved by using ethyl acetate-isopropanol (9:1 v/v) as a developing system kept aside for saturation 30 minutes under room temperature. The plates were developed and air-dried at room temperature. Densitometric scanning was carried out at a dual-wavelength of 260 nm and 302 nm.

Preparation of the Standard Stock Solution I

An accurately weighed 10 mg of SOF, and 10 mg of VEL were transferred to two separate 10 mL volumetric flasks, and the volume was made up with methanol, to get two separate standard stock solution of SOF (1000 µg/mL) and VEL (1000 µg/mL), respectively.

Preparation of the Working Standard Solution

A 4 mL of standard stock solution of SOF (1000 µg/mL) was diluted with 10 mL methanol to get a standard working solution of SOF 400 µg/mL. A 1 mL of the standard solution of VEL (1,000 µg/mL) was diluted with 10 ml methanol to get 100 µg/mL working standard solution of VEL.

Preparation of Mixed Standard Solution (M)

1 mL solution of SOF 400 µg/mL and 1 mL solution of VEL 100 µg/mL were mixed and diluted with 10 mL methanol to get a mixed standard solution. This mixed standard solution (M) of containing SOF 40 µg/mL and VEL 10 µg/mL, respectively, was used for stability-indicating HPTLC analysis for simultaneous estimation of SOF and VEL.

Optimization of Chromatographic Method

The main objective was to obtain satisfactory resolution of SOF and VEL and their degradation products. Various combinations of solvents were tried as mobile phase. The optimized mobile phase was Ethyl acetate: isopropyl alcohol (9: 1 v/v). The detection was carried out at 260 nm and 302 nm for SOF and VEL, respectively. The retention factors were found to be 0.34 ± 0.02 and 0.54 ± 0.02 for SOF and VEL, respectively. The densitogram is shown in Fig. 1.

Forced Degradation Studies

To develop stability indicating method, forced degradation studies were carried out according to ICH guidelines. The drug substance was stressed by hydrolysis under different pH, thermal, oxidation, and photolysis. Stress conditions were optimized to achieve degradation of about 10–30%.

Acid-induced Hydrolysis Degradation

Acid degradation study was carried out by taking 1 mL of a standard solution of SOF and VEL (400 µg/mL and 100 µg/mL) and 1 mL of 0.1 N HCL and make up the volume up to 10 mL with methanol, shake and immediate application on TLC plate. The plate developed with a mobile phase and scanned at 260 and 302 nm.

Base Catalyzed Hydrolysis Degradation

Base degradation study was carried out by taking 1 mL of a standard solution of SOF and VEL (400 µg/mL and 100 µg/mL) and 1 mL of 0.1 N NaOH and makeup the volume up to 10 mL with methanol, kept for 20 minutes aside in dark place and then applicated on TLC plate. The plate developed with a mobile phase and scanned at 260 and 302 nm.

Neutral-hydrolysis Induced Degradation

Neutral hydrolysis study was carried out by taking 1 mL of a standard solution of SOF and VEL (400 µg/mL and 100 µg/mL) and 1 mL water and made up the volume up to 10 ml with methanol, shake and immediate application on TLC plate.

Hydrogen-peroxide Induced Degradation

The Hydrogen- peroxide degradation study was carried out by taking 1 mL SOF and VEL (400 and 100 µg/mL) and 1 mL from 30% H_2O_2 solution and made up the volume up to 10 mL with methanol. The solution kept aside for 1 hour in a dark place and then spotted on the TLC plate.

Thermal-induced Degradation

The sample for thermal degradation was made by exposing 50 mg of SOF and VEL separately at 80°C in hot air oven for 4 hours. The drug was dissolved and diluted 40 µg/mL and 10 µL/mL for SOF and VEL with methanol and applied on TLC plate.

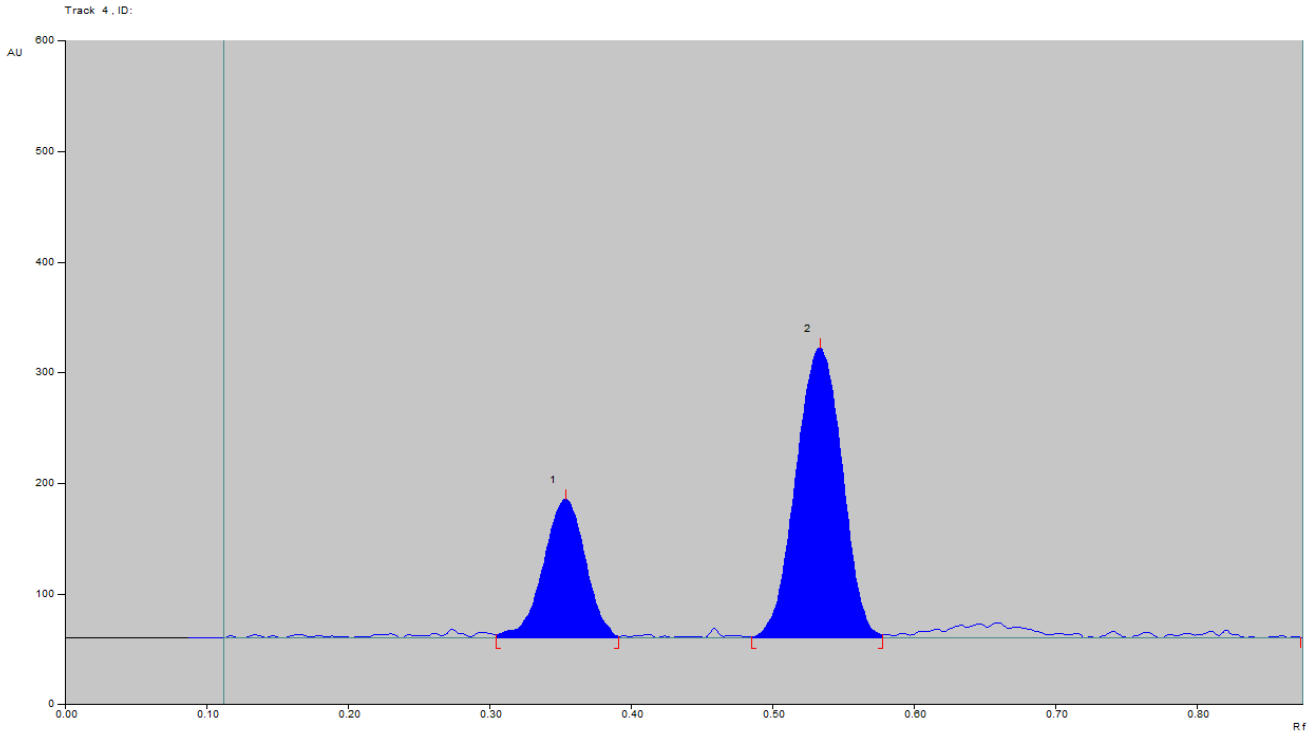

Fig. 1: Representative densitogram of *Sofosbuvir* (Rf 0.54) and VEL (Rf 0.34)

Photolytic Condition

For photolytic condition, the degradation sample was made by exposing 30 mg of SOF and VEL to fluorescence for 1.2 million lux hours/square meter and UV (320-400 nm) 200 Watt-hours/square meter. Solutions were made of 40 µg/mL and 10 µg/mL for SOF and VEL from the exposed SOF, and VEL, and spotting were done. The optimized stress condition was repeated to check reproducibility.

Result

For acid hydrolysis, 1 N was tried, but it led to complete degradation; hence 0.1 N HCl with immediate spotting was the optimized condition with the percent recovery of 83.70 and 76.04% for SOF and VEL. For base hydrolysis, the different time periods were optimized for an acceptable percent recovery result, which was found to be 83.56% and 92.16% for SOF and VEL. For neutral hydrolysis, the percent recovery was found to be 82.66 and 92.22% for SOF and VEL. For oxidation, the different strengths of H_2O_2 solutions with varying periods of time were studied, the percent recovery with 30% v/v H_2O_2 was found to be 78.03% and 85.95% for SOF and VEL. The percent recovery for thermal stress of 80°C for 4 hours was found to be 81.33 and 88.13% for SOF and VEL. For photolysis, the percent recovery after UV illumination was found to be 74.02 and 79.25% and after exposure to fluorescent light was found to be 87.01 and 93.24% for SOF and VEL, respectively. The summary is given in Table 1 and 2.

Table 1: Details of Recovery Studies

Drug	*% level*	*Initial amount (ng/band)*	*Amount added (ng/band)*	*% recovery*
	80	800	640	76.04%
SOF	100	800	800	92.16%
	120	800	960	85.95%
	80	200	160	88.13%
VEL	100	200	200	79.25%
	120	200	240	93.24%

Table 2: Intra-Day Precision Details of SOR and Velpatasvries

		System precision		*Method precision*	
Sr. No	*Precision studies*	*Sofosbuvir*	*Velpatasvir*	*Sofosbuvir*	*Velpatasvir*
1	Concentration (ng/band)	800	200	800	200
2	Standard deviation	76.54	78.79	71.33	53.34
3	% RSD	0.925	0.919	1.019	0.634

% RSD: Relative Standard Deviation.

Method Validation

The developed HPTLC method was validated as per the ICH guidelines Q2 (R1) for linearity, precision, accuracy, limit of detection (LoD), limit of quantification (LoQ), robustness, and specificity.

Linearity

A standard mixture solution containing SOF 40 µg/mL and VEL 10 µg/mL were spotted on TLC plate with spotting volume 10, 20, 30, 40, and 50 µL to achieve concentration range of 100–2,000 ng/band for SOF and 100–500 ng/band for VEL. The plate was developed as per optimized procedure. It was repeated for six times (n = 6). The calibration curves for SOF and VEL were obtained by plotting peak areas vs. amount spotted, as shown in Fig. 2 and 3.

Recovery Study

The accuracy of the method was determined by the method of standard addition. Application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analyzed have been added. The blend prepared for the assay was spiked with pure drug substance at 80, 100, and 120% level. This was assessed using three concentration levels covering the specified range, i.e., 3 concentrations/3 replicates of each one. Accuracy was reported as percent recovery by the assay of a known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence interval. Densitogram is shown in Fig. 4 and the results obtained are shown in Table 3.

Assay

A blend of commonly used excipient was spiked using SOF and VEL. The assay result is unaffected by the presence of these materials, i.e., by comparison with the assay result obtained on unspiked samples. The amount equal to the average weight of the tablet was taken and dispersed in methanol to obtain a concentration equivalent to 400 µg/mL and 100 µg/mL for SOF and VEL respectively, and it was analyzed by developed HPTLC method.

Precision

The precision of the method was demonstrated by intraday precision and inter-day precision studies. Intraday precision was assessed by application of six replicates of the 800 ng/band and 200 ng/band concentration for SOF and VEL on TLC plate on the same day and the consecutive day, respectively. The percentage of RSD was calculated. The %RSD values were found to be less than 2%. The results obtained are shown in Tables 4 and 5. The obtained densitogram shown in Fig. 5.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The detection and quantitation of drugs were calculated from calibration curves. The calculation is based on the standard

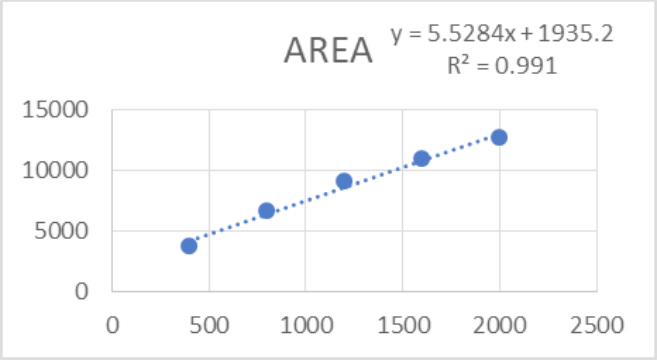

Fig. 2: linearity graph of *Sofosbuvir*

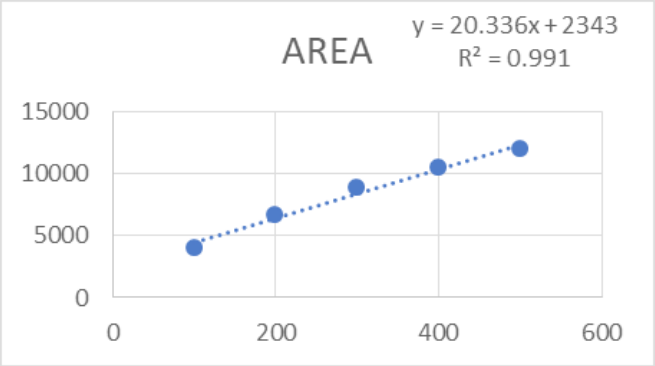

Fig. 3: linearity graph of *Velpatasvir*

Table 3: Inter-Day precision details of SOR and Velpatasvries

Sr. No.	*Precision studies*	*System precision*		*Method precision*	
		Sofosbuvir	*Velpatasvir*	*Sofosbuvir*	*Velpatasvir*
1	Concentration (ng/band)	800	200	800	200
2	Standard deviation	67.02	95.67	67.23	50.75
3	% RSD	0.817	1.148	0.915	0.616

% RSD: Relative Standard Deviation.

Table 4: LOD and LOQ details of SOR and Velpatasvries

Sr. No.	*Parameter*	*Sofosbuvir (ng/band)*	*Velpatasvir (ng/band)*
1	LOD	25.16	9.96
2	LOQ	76.25	30.19

Table 5: Robustness Details of Velpatasvries

		% RSD	
Sr. No.	*Parameter*	*Sofosbuvir*	*Velpatasvir*
1	Mobile phase composition (± 0.2 ml)	1.052	1.800
2	Saturation time (± 5 min)	1.310	1.714
3	Time from development to scanning (10 min, 20 min)	1.738	1.330
4	Time between development to scanning (15 min, 30 min)	1.533	1.554

RF: Retention factor; %RSD: Relative Standard Deviation

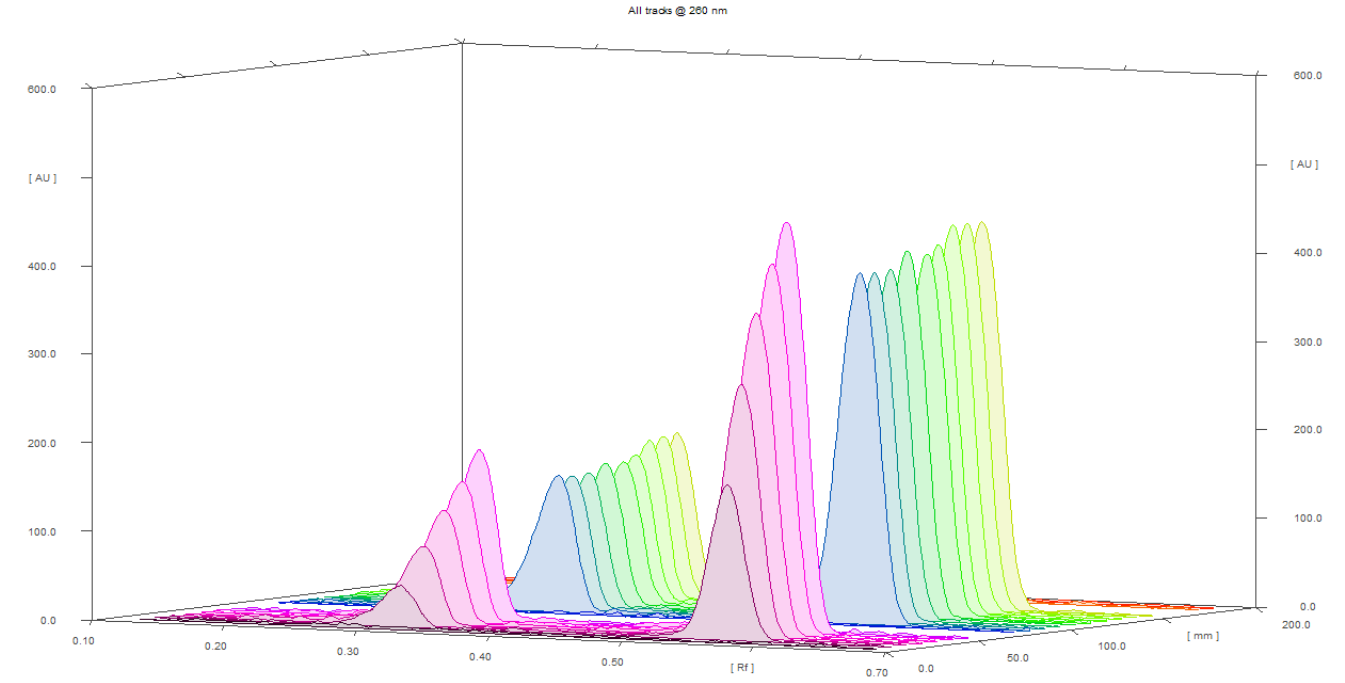

Fig 4: 3D densitogram of accuracystudy, 2-6 linearity, 10-19 std addition tracks

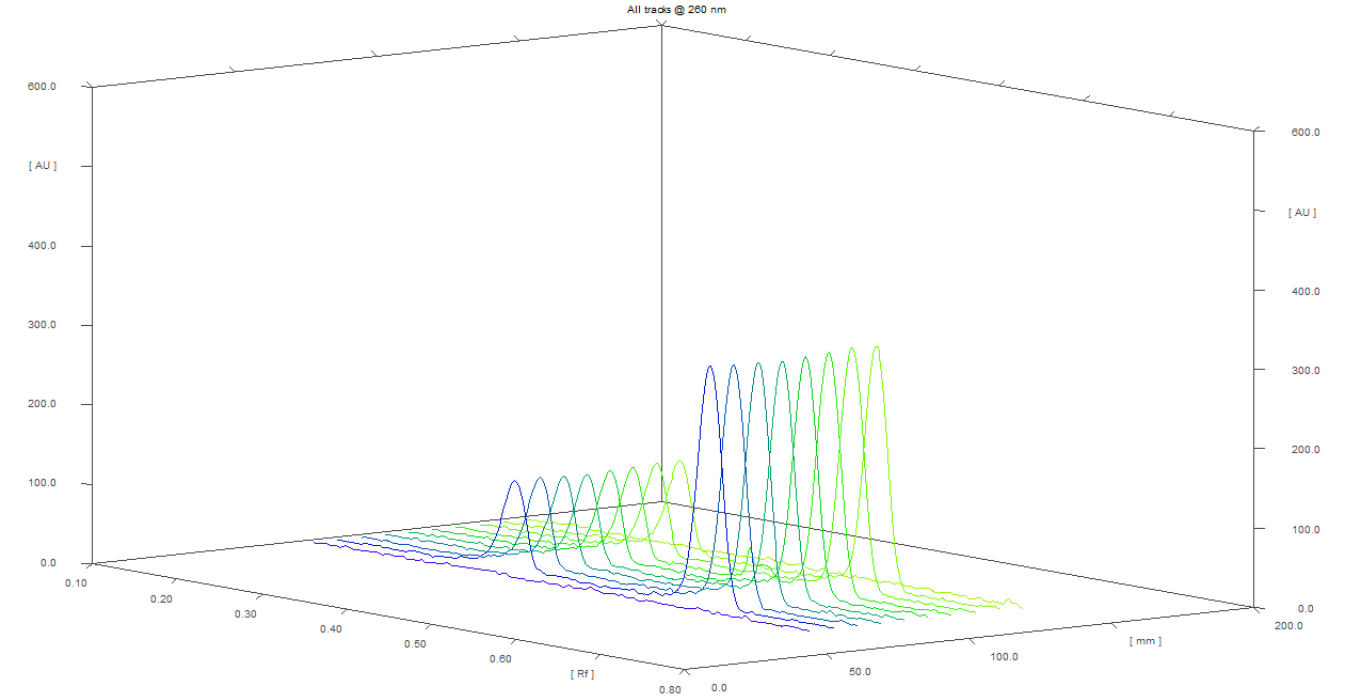

Fig 5: 3D densitogram of precision

deviation of the y-intercept and the slope of the calibration curves. The following equations were used to calculate LoD and LoQ. The results obtained are shown in Table 6.

LoD = 3.3 × σ/S

LoQ = 10 × σ/S

Where, σ = the standard deviation of y-intercept. S = slope of the calibration curve.

Robustness

Robustness studies were done by making small, deliberate changes in optimized conditions like mobile phase composition, saturation time, and time from spotting to development, time from development to scanning, to determine its effect on the result. The percentage of RSD was calculated given in Table 7. The summarized results are given in Table 8.

Discussion

The developed stability-indicating HPTLC method for SOR and VEL was validated as per ICH guidelines. The developed method was found to be linear within the range of 100-2000 ng/band (r^2 = 0.991) and 100-500 ng/spot (r^2 = 0.991) for SOF and VEL respectively. The summary of validation parameters is shown in Table 8. Degradation was observed for SOF and VEL under different stress conditions, but no degradation product was found in any stress condition. Non-interference by any degradant was confirmed by peak purity using spectral detection by software. The method was found to comply with all validation parameters as per ICH guidelines. The developed method is simple, highly sensitive, robust, economical, and ensures short run time compared to other reported analytical methods. The

Table 6: Summary of degradation parameters of SOR

				Peak purity	
Sr. No	*Parameter*	*Condition*	*% recovery*	*R (s, m)*	*r (m, e)*
1	Acid	0.1N HCl immediate	83.70%	0.999815	0.999517
2	Base	0.1N NaOH 20 min	83.56%	0.999165	0.999944
3	Neutral	1 mL water immediate	82.66%	0.998221	0.996192
3	Oxidation	30 % v/v H_2O_2	78.03%	0.999623	0.998231
4	Thermal	80°C 4 hour	81.33%	0.998345	0.998211
5	UV	200 Watt hours/square meter	74.02%	0.999712	0.999146
6	Fluorescence	1.2 million lux hours/square meter	87.01%	0.998128	0.998245

Table 7: Summary of degradation parameters of Velpatasvries

				Peak purity	
Sr. No	*Parameter*	*Condition*	*% recovery*	*R (s, m)*	*r (m, e)*
1	Acid	0.1 N HCl immediate	76.04%	0.998688	0.998328
2	Base	0.1 N NaOH 20 min	92.16%	0.998754	0.998727
3	Neutral	1 mL water immediate	92.22%	0.996881	0.996790
3	Oxidation	30% v/v H_2O_2	85.95%	0.997202	0.996091
4	Thermal	80°C 4 hours	88.13%	0.996431	0.999718
5	UV	200 Watt hours/square meter	79.25%	0.994652	0.994550
6	Fluorescence	1.2 million lux hours/square meter	93.24%	0.996159	0.996321

Table 8: Summary of validation parameters

Sr.No.	*Validation parameter*	*Sofosbuvir*	*Velpatasvir*
1	Linearity equation	Y = 5.5284x + 1935.2 R^2 = 0.991	Y = 20.336x + 2343 R^2 = 0.991
2	Precision	0.68–0.81%	0.66–0.96%
3	Assay	100.51%	101.47%
4	Accuracy	98.27–102.23%	100.40–102.90%
5	LOD	25.16 (ng/band)	9.96 (ng/band)
6	LOQ	76.25 (ng/band)	30.19 (ng/band)
7	Robustness		
	Mobile phase composition (± 0.2 mL)	1.052%	1.800%
	Saturation time (± 5 min)	1.310%	1.714%
	Time from development to scanning (10 min, 20 min)	1.738%	1.330%
	Time between development to scanning (15 min, 30 min)	1.533%	1.554%

method could be applied to the quality control and routine stability monitoring of SOR and VEL.

Acknowledgment

The authors are thankful to the Principal and the management of AISSMS College of Pharmacy, Pune, for providing all necessary facilities required carrying out this research work.

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HOW TO CITE THIS ARTICLE: Damle MC, Kalaskar PB. Stability indicating HPTLC method for sofosbuvir and velpatasvir in combination. Int. J. Pharm. Sci. Drug Res. 2020;12(2):129-135. **DOI:** 10.25004/IJPSDR.2020.120206