



A NOVEL METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD AND STRESS DEGRADATION STUDY OF DETERMINATION OF SOFOSBUVIR AND VELPATASVIR IN BULK AND COMBINED TABLET DOSAGE FORM

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Velpatasvir and Sofosbuvir, in its pure form as well as in tablet dosage form. Chromatography was carried out on a spursil C18 (4.6 x 150mm, 5µm) column using a mixture of Acetonitrile: phosphate Buffer (pH 3.0) (50:50% v/v) as the mobile phase at a flow rate of 1.0mL/min, the detection was carried out at 255nm. The retention time of the Velpatasvir and Sofosbuvir was 3.472, 5.505min respectively. The method produce linear responses in the concentration range of 50-250µg/ml for Velpatasvir, and 200-1000µg/ml of Sofosbuvir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Velpatasvir and Sofosbuvir; RP-HPLC; UV Detection; validation.

INTRODUCTION

Sofosbuvir (Fig .1) is a medication used for the treatment of hepatitis C. It is only recommended with some combination of ribavirin, peginterferon-alfa, simeprevir, ledipasvir, or daclatasvir. Cure rates are 30 to 97% depending on the type of hepatitis C virus involved. Safety during pregnancy is unclear; while, some of the medications used in combination may result in harm to the baby. It is taken by mouth and chemically it is Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxypyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate Molecular formula $C_{22}H_{29}FN_3O_9P$ Molecular Weight 529.453 g/mol and Soluble in Methanol, Acetonitrile and water.

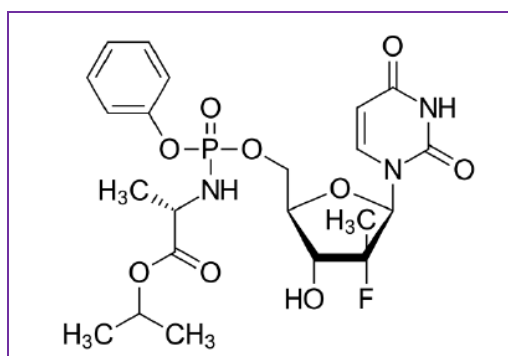


Fig. 1: Chemical structure of Sofosbuvir.

Velpatasvir (Fig.2) is an NS5A inhibitor which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.

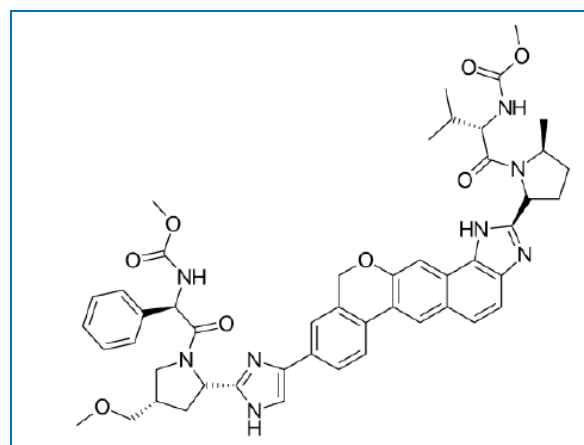


Fig. 2: Chemical Structure of Velpatasvir.

MATERIALS AND METHODS^[5-7]

Materials and Reagents

The reference standards of velpatasvir and sofosbuvir were procured from M/SEisai Pharmaceuticals indiapvt Ltd, Visakhapatnam, India. The branded tablet formulation Eplclusa (sofosbuvir 400 mg and velpatasvir 100 mg) was purchased from the local market. All the HPLC solvents and analytical reagent grade chemicals

were purchased from Qualigens Chemicals, Hyderabad, India

Instrumentation

A Waters HPLC system equipped with a 2695 binary pump, an auto sampler and a 2996 photo diode array detector was employed for the study. The output signal was monitored and processed with Empower software.

Preparation of Phosphate Buffer solution (pH 3.0)

6.8 grams of KH_2PO_4 was dissolved in a 1000 mL HPLC water, the pH of the solution was adjusted to 3.0 with Orthophosphoric acid.

Preparation of Mobile phase

500 mL of acetonitrile and 500 mL of phosphate buffer (pH3.0) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

Diluent

The above mobile phase was also used as the diluent for the preparation drug solution.

Preparation of stock and working standard solutions

100 mg of VEP and 400 mg of SOF were accurately weighed and transferred into 100 mL clean dry volumetric flask and added about 70 mL of the mobile phase and sonicated to dissolve it completely. The volume was made up to the mark with the mobile phase (Stock solution). Pipetted out 1.5 mL from the above stock solution was pipetted out into 10 mL volumetric flask and the contents diluted up to the mark with the diluent. The solution makes the working standard (150 μ g/mL of VEP and 600 μ g/mL of SOF respectively).

Chromatographic conditions

The separation of the drugs was achieved on a Spursil C18 HPLC Column (150 x 4.6 mm; 5 μ particle size) by running a mobile phase containing a 50:50 v/v mixture of Acetonitrile - Phosphate Buffer(pH 3.0) at a flow rate of 1.0 mL/min. The injection volume was 20 μ L. The column temperature was maintained at 27°C and the analytes in the eluates were monitored at 255 nm. The run time was 10.0 min. 500 mL of acetonitrile and 500 mL of phosphate buffer (pH3.0) were mixed and used as the diluent to prepare drug solutions.

Table 1: Optimized chromatographic conditions.

Column	Spursil C18 (150 x 4.6 mm; 5 μ m)
Mobile Phase	Acetonitrile - Phosphate Buffer(pH 3.0) (50: 50)
Injection volume	20 μ L
Detection wavelength	255 nm
Column Temperature	27°C
Flow rate	1.0 mL/min
Run time	10 min
Retention time	Velpatasvir 3.472 min, Sofosbuvir 5.505min

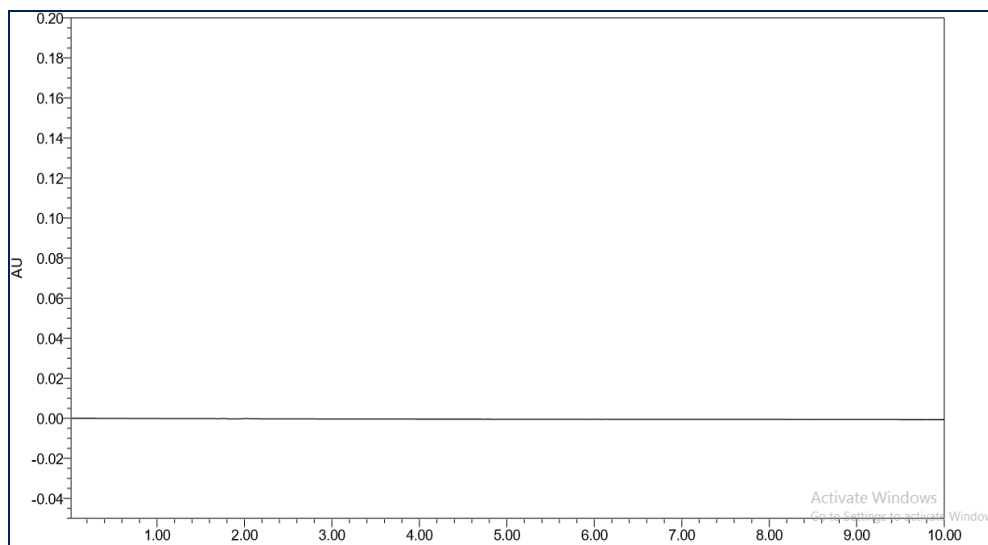


Fig. 3: Chromatogram of blank.

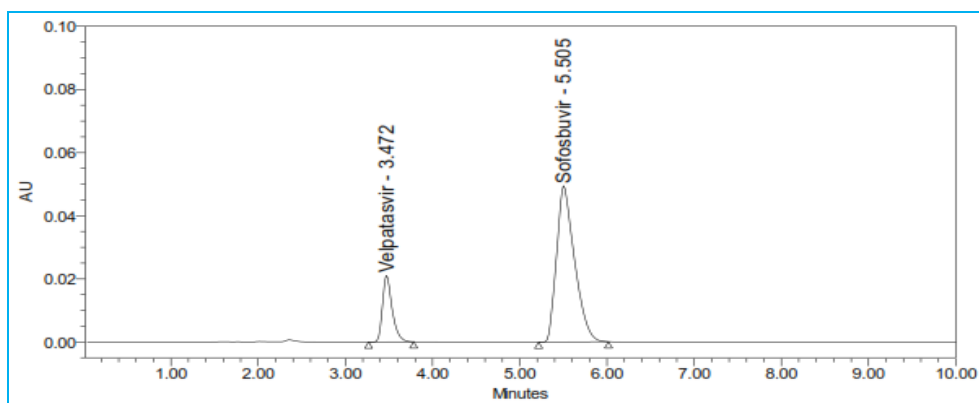


Fig. 4: Typical chromatogram of mixed standard solution of Velpatasvir and Sofosbuvir.

VALIDATION OF THE PROPOSED METHOD

The method was validated in compliance with ICH (Q2R1). The parameters for validation are specificity,

precision, ruggedness, accuracy, linearity, robustness, stability of analytical solution and forced degradation. System suitability parameters.

Table 2: Results of System Suitability Parameters.

Parameter	Acceptance criteria	Results	
		Velpatasvir	Sofosbuvir
USP Plate Count	> 2000	4822	4722
USP Tailing Factor	≤ 2.0	1.36	1.26
Resolution Factor	>2.0	-	6.35
% RSD for peak areas of both the drugs.	≤ 2.0	0.3	0.1

System Suitability

System suitability was established for initial evaluation of the method before running the sample for the validation parameters. The test was performed according to the USP.^[10] The standard solutions prepared as per the proposed method were analyzed. The results of the

system suitability study are presented in Figure 4. The acceptance criterion is % RSD ≤ 2.0 . A percent RSD of 0.3 and 0.1 indicates good system precision of the method. The tailing factor obtained from the standard injection is 1.43&1.35 and Theoretical plates obtained from the standard injection are 4822&4722 respectively.

Table 3: Result of system suitability parameters.

S.no	Parameter	Velpatasvir	Sofosbuvir
1	Retention time	3.472min	5.505 min
2	Theoretical plates	4822	4722
3	Tailing factor	1.35	1.43
4	Area	161345	747339

Specificity

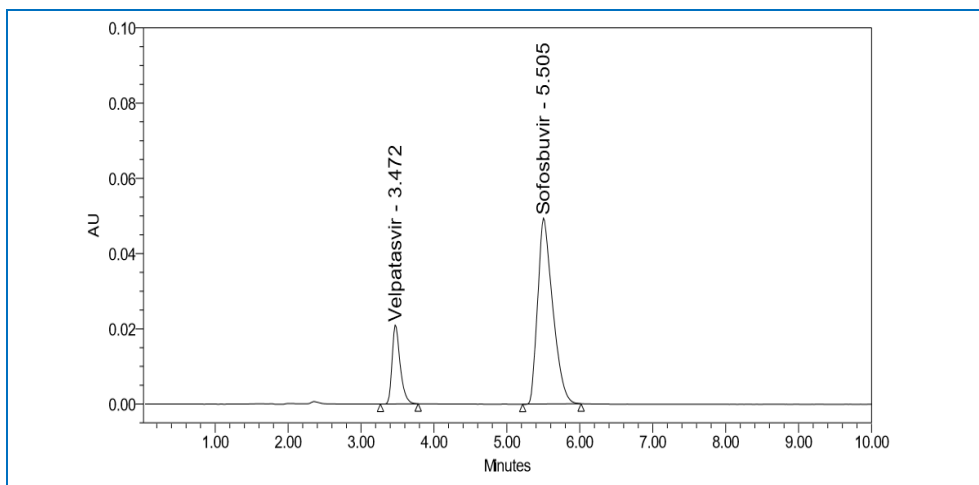


Fig. 5: Typical chromatogram of Velpatasvir and Sofosbuvir.

Linearity

The linearity were observed for in the concentration ranges from 50-250µg/mL for Velpatasvir and 200-1000 µg/mL. The Linearity of the method was demonstrated by preparing different concentrations of drug substance and analyzing as per the proposed method. A plot of the area

of the peak as a function of analyte concentration was prepared and its regression equation computed. The linearity data of the two drugs are given in Table 6. and Fig.6 and 7.

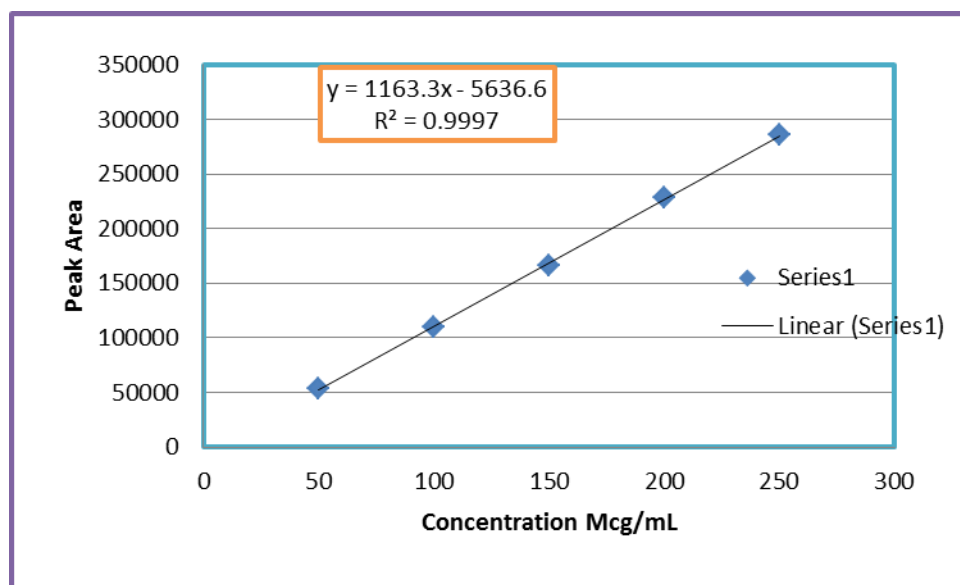


Fig. 6: Calibration Curve for Velpatasvir.

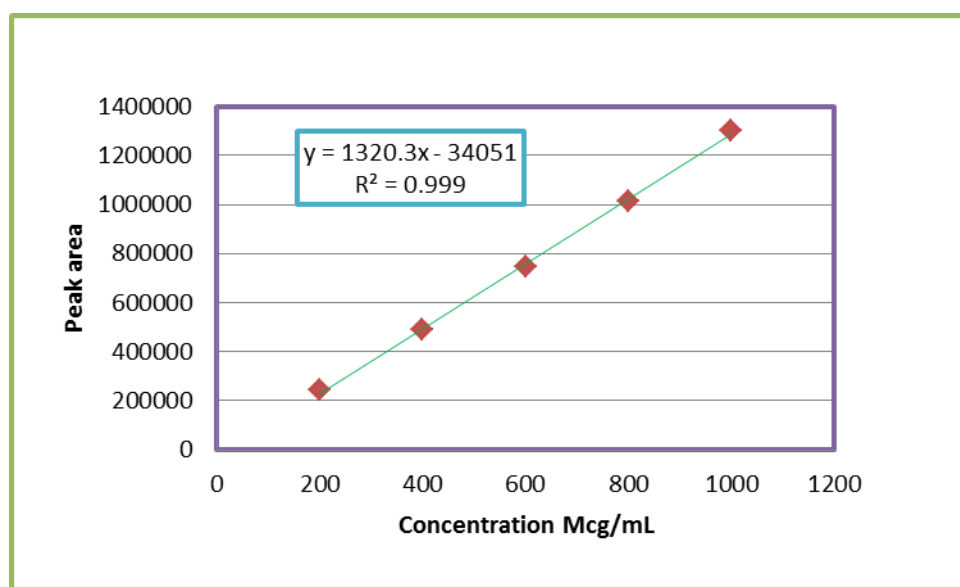


Fig. 7: Calibration Curve for Sofosbuvir.

Table 4: Linearity Results of Velpatasvir and Sofosbuvir.

Linearity level	Velpatasvir		Sofosbuvir	
	Conc. (µg/mL)	Mean Peak Area	Conc. (µg/mL)	Mean Peak Area
1	50	53953	200	243401
2	100	110011	400	488042
3	150	166601	600	744612
4	200	227887	800	1013904
5	250	285840	1000	1300811
Correlation co-efficient (r ²)		0.9997		0.999
Slope		1163.3		1320.3
Intercept		5636		-34051

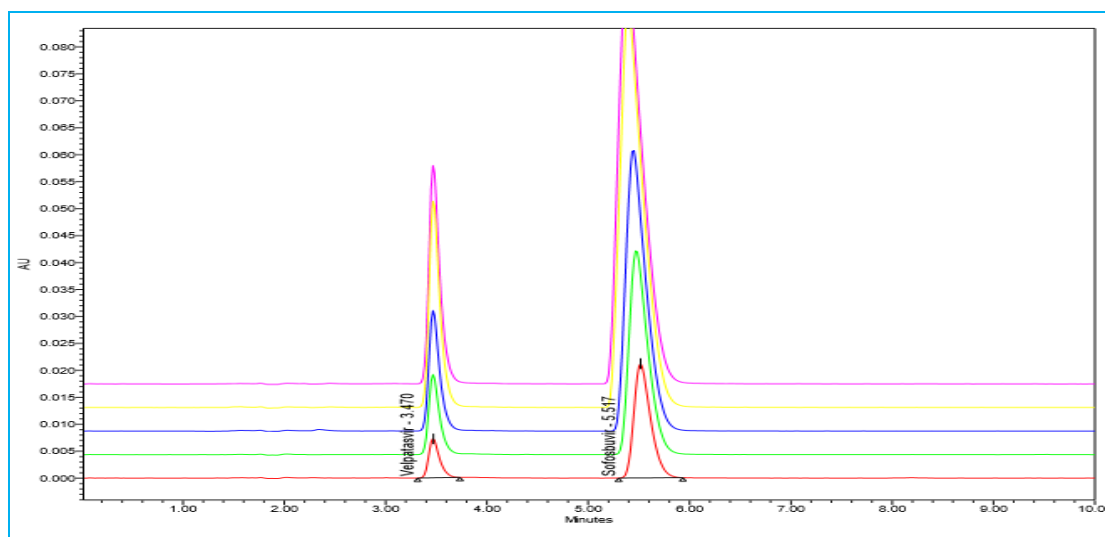


Fig. 8: Overlain chromatogram of both the drugs (linearity).

Limit of detection and Limit of quantitation

Limit of detection (LOD) & the limit of quantifications (LOQ) is evaluated by the serial dilutions of VEP and SOF stock solutions in order to obtain a signal to the noise ratio of 3:1 for the LOD & 10:1 for the LOQ. The LOD value for VEP and SOF were found to be 1.2 & 2.1 µg/mL respectively and the LOQ value were 4.05 µg/mL and 7.2 µg/mL respectively.

Table 5: Data of LOD and LOQ.

Drug	LOD	LOQ
Velpatasvir	1.2	4.05
Sofosbuvir	2.1	7.2

Precision

In the precision study, % RSD was found to be less than 2 % for Velpatasvir 0.8% and Sofosbuvir 0.1 which indicates the system has a good reproducibility for precision studies 6 replicate studies of Velpatasvir and Sofosbuvir formulation (method precision) was performed. % RSD was determined for peak areas of Velpatasvir and Sofosbuvir and the acceptance limits should be NMT 2% and the results were found to be within the acceptance limits. The chromatograms of precision were shown in Figs:7.22-7.26. results were reported in Table:6.

Table 6: Data of Precision.

Injection No.	Peak Area of Velpatasvir	Peak Area of Sofosbuvir
1	161345	747339
2	161232	746432
3	161671	747131
4	161999	747399
5	162898	747018
6	164679	747649
Mean	162304.0	747161.3
SD	1308.1	419.3
%RSD	0.8	0.1

Accuracy

The accuracy studies were shown as % recovery for Velpatasvir and Sofosbuvir at 50%, 100%, 150%, the limits of recovery should be in range of 98-102% the limits obtained for Velpatasvir and Sofosbuvir were found to be within the limits. Hence the method was found to be accurate. The accuracy studies show % recovery of the Velpatasvir 100% and Sofosbuvir and the limits of % recovery of drugs were 98-102% and from the above results it indicates that the method was accurate and also revealed that the commonly used excipients present in the pharmaceutical information do not interfere in the proposed method. The chromatograms shown in results were shown in Tables:7.

Table 7: Data of Accuracy.

Name of the drug	% Level	Amount Added (mg)	Amount Found (mg)	% Recovery	% Mean Recovery ± SD
Velpatasvir	50%	50	49.72	99.43	100.08 ± 0.87
	100%	100	99.82	99.82	
	150%	150	151.49	100.99	
Sofosbuvir	50%	200	199.69	99.84	100.02 ± 1.08
	100%	400	398.74	99.68	
	150%	600	603.2	100.52	

Robustness

Table 8: Data of Robustness.

Parameters		Adjusted to	USP Plate Count	USP Tailing	USP Resolution
Velpatasvir	Flow Rate As per method 1.0 mL/min	0.9 mL/min	4353.29	1.30	
		1.0 mL/min	4822.40	1.36	
		1.1 mL/min	4533.29	1.40	
	Mobile Phase (50:50) (ACN: Buffer)	10% Less	4543.29	1.40	
		50:50	4822.40	1.36	
		10% More	4537.29	1.40	
Sofosbuvir	Flow Rate As per method 1.0mL/min	0.9 mL/min	3433.94	1.42	9.50
		1.0 mL/min	4722.40	1.26	9.50
		1.1 mL/min	3863.94	1.44	9.50
	Mobile Phase (50:50) (ACN: Buffer)	10% Less	3863.94	1.44	9.50
		50:50	3815.92	1.12	9.50
		10% More	3863.94	1.44	9.50

FORCED DEGRADATION STUDIES

In order to determine whether the developed analytical method was stability indicating, the above tablet formulation of VEP and SOF was subjected to stress conditions like acidic, alkaline, oxidation, thermal and photolytic. The active pharmaceutical ingredients in the tablet formulation should be well resolved from degradation products obtaining from stress conditions.

For degradation studies, 10 tablets were accurately weighed and triturated to a fine powder form. From this tablet powder, a quantity of powder equivalent to 100 mg of VEP and 400 mg SOF transferred into a 100 mL clean dry volumetric flask. About 70 mL of diluent was added and sonicated for 30 minutes to dissolve completely. The

volume was made up to the mark with the diluent. Then, it is filtered through 0.45 μ filter (Stock solution).

Acid degradation

For acid degradation study, 1.5 mL of the degradation stock solution was pipetted in to 10 mL volumetric flask; to this 3 mL of 0.1N HCl was added to it, refluxed for 24 hr at 60 °C, cooled to room temperature. The solution was neutralized with 3 mL of 0.1N NaOH and diluted with the diluent. The sample solution was filtered through 0.45 μ filter and transferred in HPLC vials and injected in to the HPLC system. The chromatograms of acid degradation and spectrum index is shown in Fig.9 and Fig.10. In acid degradation study, it was found that 5.17 % of VEP and 3.78 % of SOF were degraded respectively.

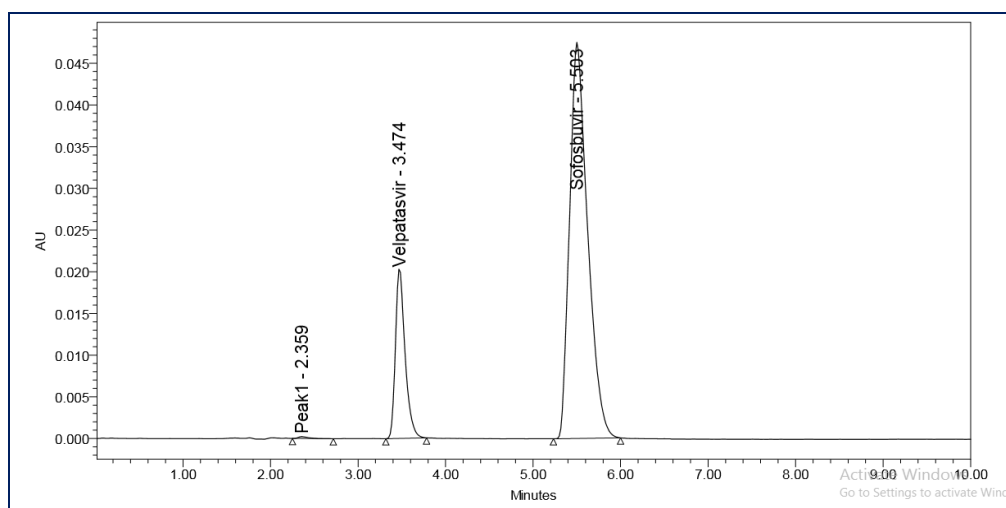


Fig. 9: Chromatogram showing effect of acidic degradation.

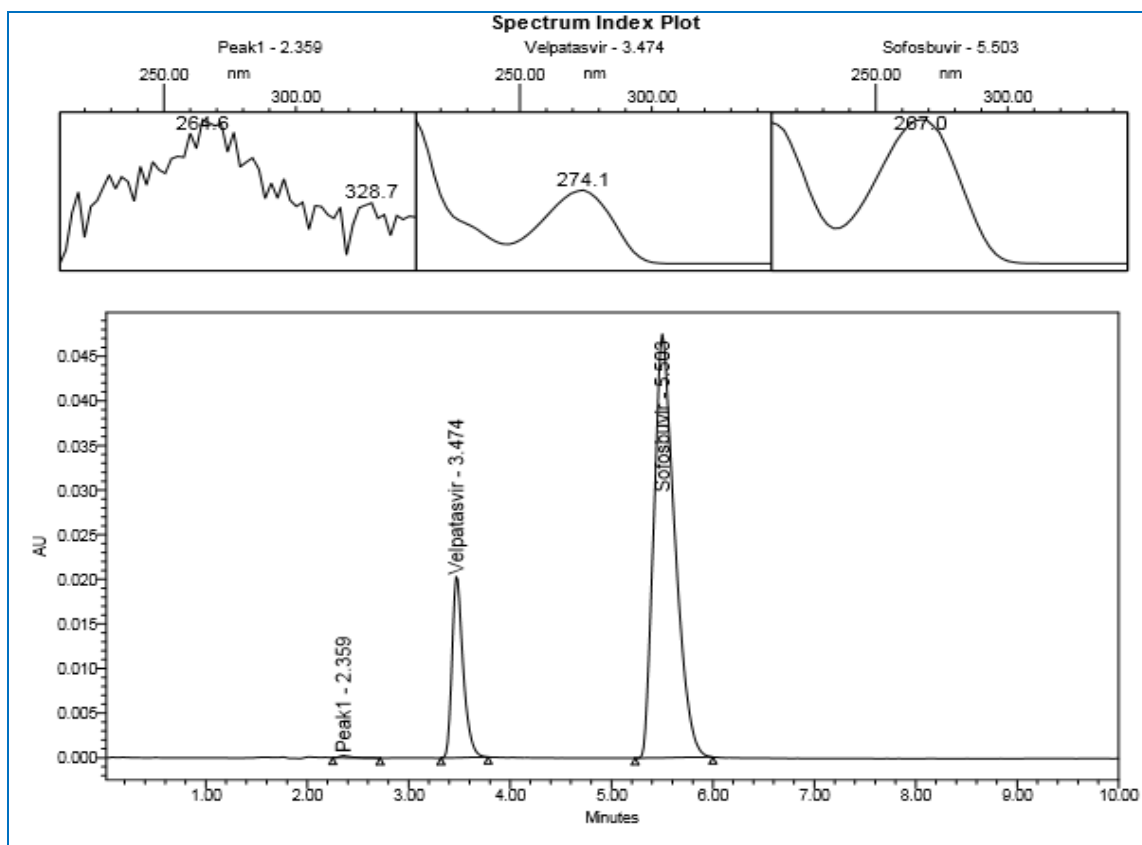


Fig. 10: Spectrum index of acidic degradation.

Alkaline degradation

For alkaline degradation study, 1.5 mL of the degradation stock solution was pipetted in to 10 mL volumetric flask; to this 3 mL of 0.1N NaOH was added to it, refluxed for 10 hr at 60°C and cooled to room temperature. The solution was neutralized with 3 mL 0.1N HCl and diluted with the diluent. The above sample

solution was filtered through 0.45µ filter and transferred in HPLC vials and injected in to the HPLC system. The chromatograms of alkaline degradation and spectrum index is shown in Fig. 11 and Fig.12. In alkaline degradation study, it was found that 5.00 % of the VEL and 3.77 % SOF were degraded respectively.

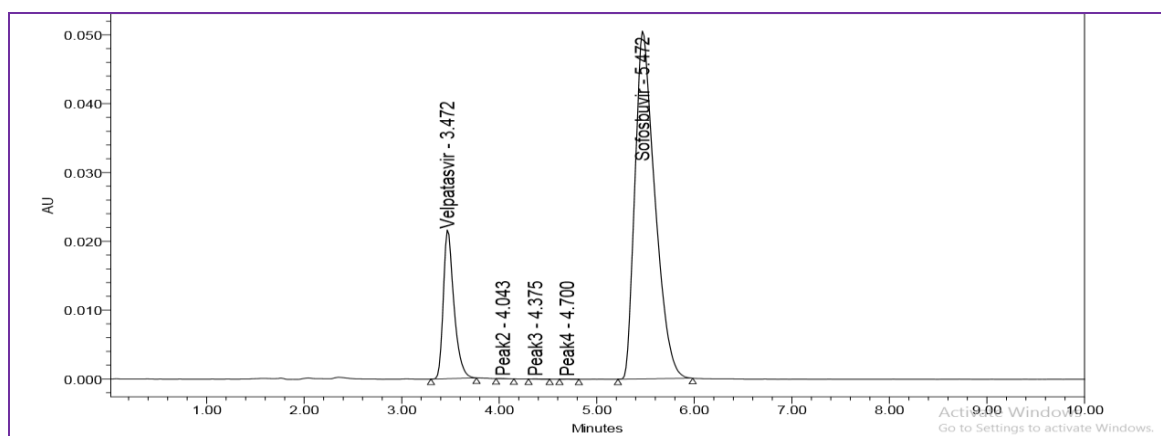


Fig. 11: Chromatogram showing effect of Alkaline degradation.

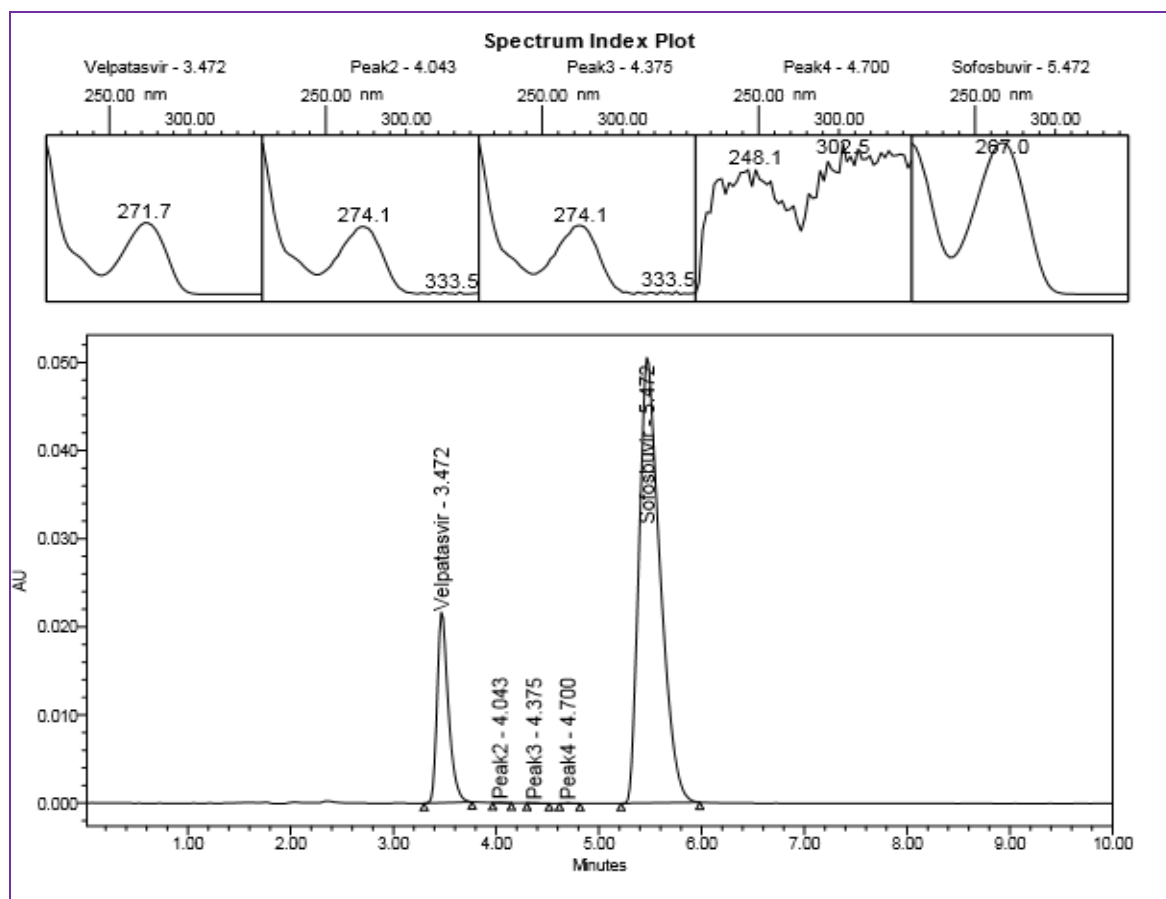


Fig. 12: Spectrum index of alkaline degradation.

Oxidative degradation

Inoxidative degradation study, 1.5mL of the degradation stock solution was pipetted in to 10 mL volumetric flask; to this 1 mL of 30 % hydrogen peroxide was added, kept for 10 hr and diluted with the diluent. The sample solution was filtered through a 0.45 μ filter and

transferred in HPLC vials and injected in to the HPLC system. The chromatograms of oxidative degradation and spectrum index is shown in Fig.13 to Fig.14. In oxidative degradation study, it was found that 5.80% of the VEL and 5.69 % SOF were degraded respectively.

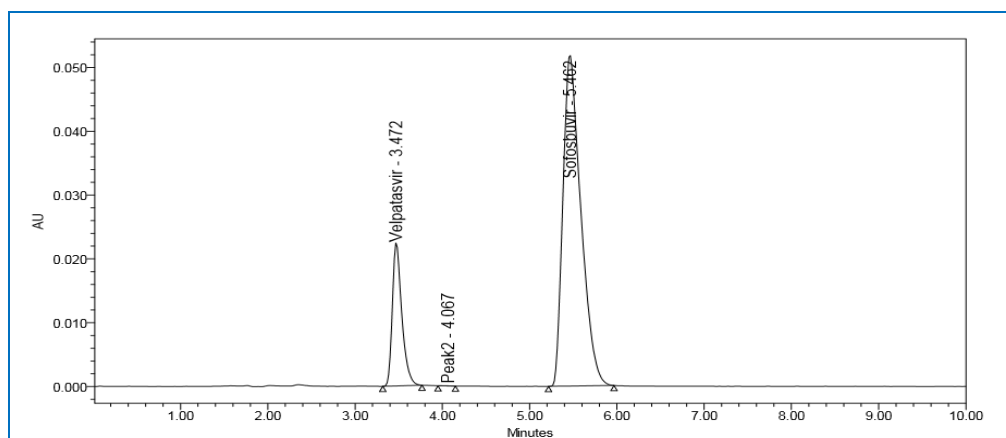


Fig. 13: Chromatogram showing effect of oxidative degradation.

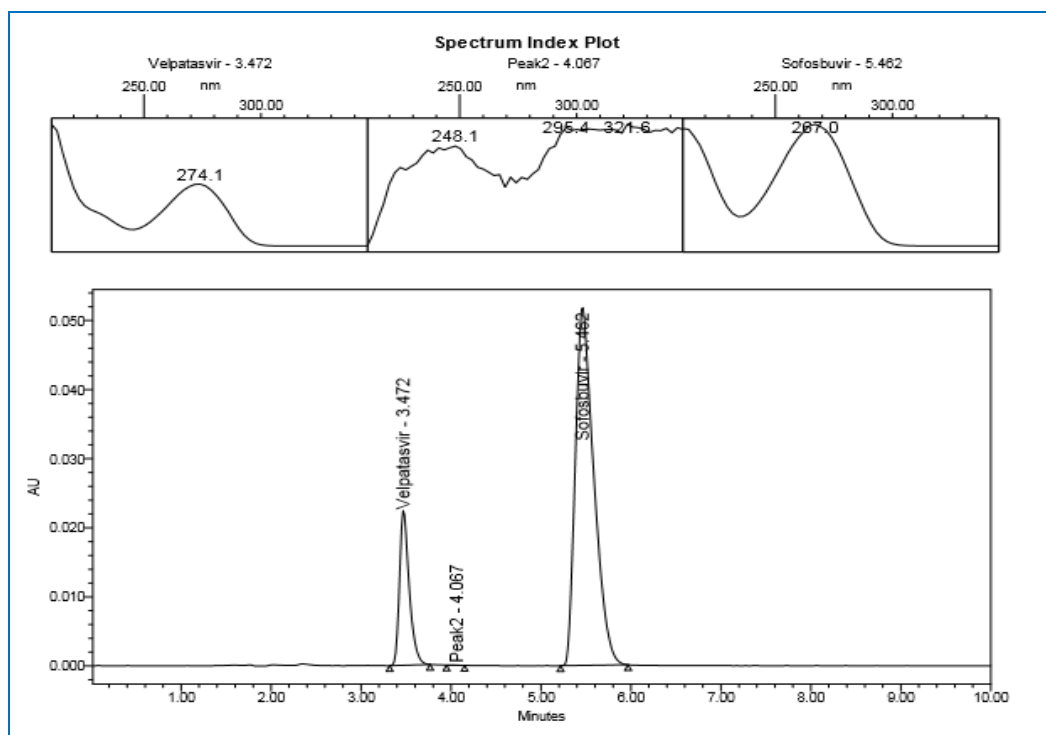


Fig. 14: Spectrum index of oxidative degradation.

Photolytic Degradation

For photolytic degradation study, ten tablets were accurately weighed and triturated it to a fine powder form and transferred into a petri dish. The petridish contained sample was subjected to stress condition in the U.V chamber at 1.2 million lux for one week. The Sample solutions were prepared as equivalent concentrations of 150 µg/mL of VEP and 600 µg/mL of

SOF. The sample solution was filtered through a 0.45 µ filter and transferred in HPLC vials and injected in to the HPLC system. The chromatograms of photolytic degradation and spectrum index is shown in Fig.15 and Fig.16. In photolytic degradation study, it was found that 3.19 % of the VEP and 4.38 % SOF were degraded respectively..

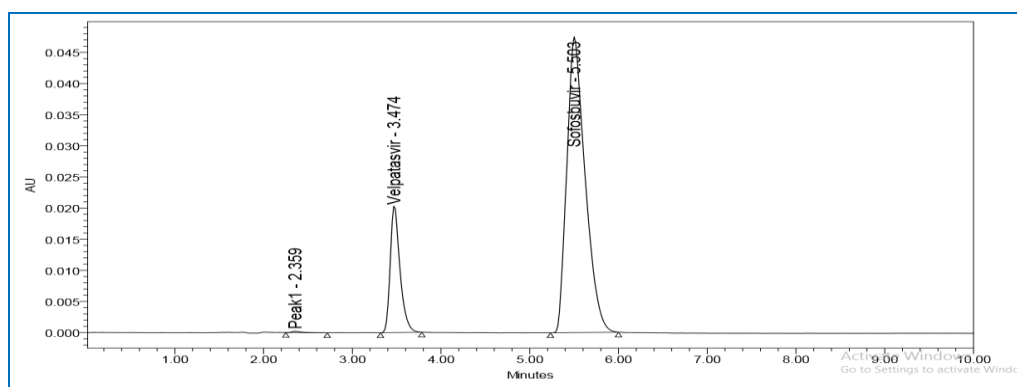


Fig. 15: Chromatogram showing effect of photolytic degradation.

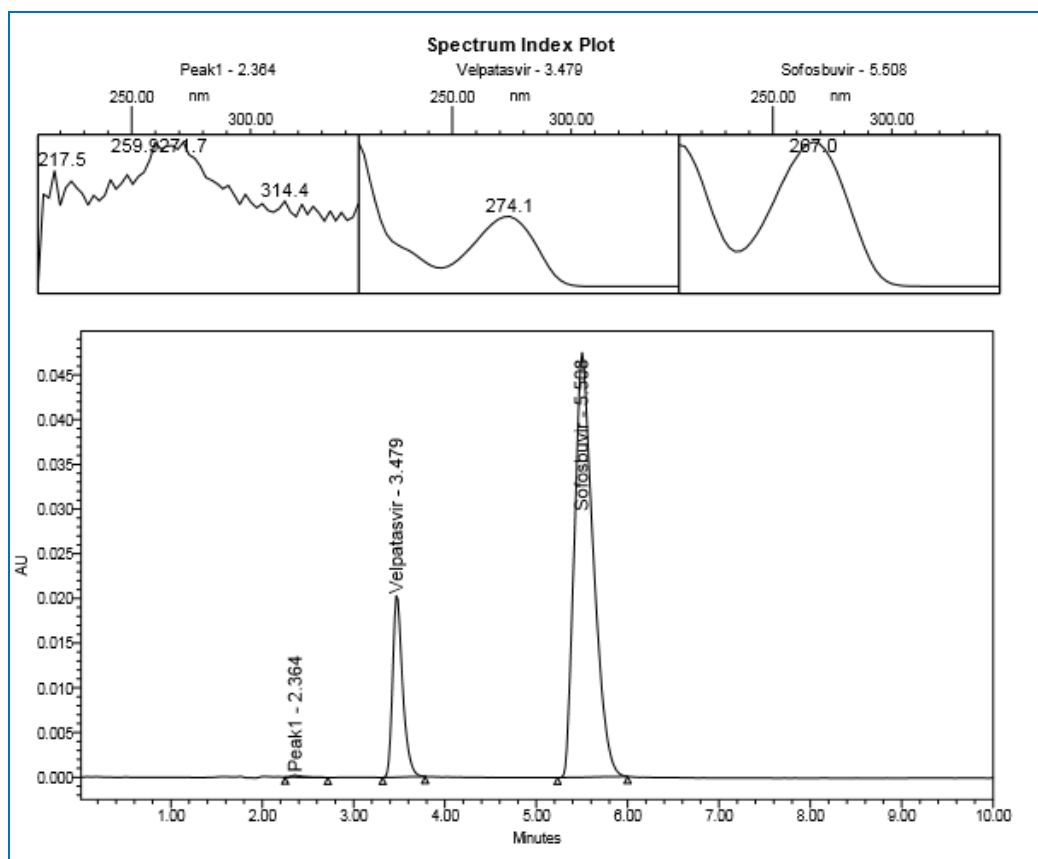


Fig. 16: Spectrum index of Photolytic degradation.

Thermal degradation

For thermal degradation study, ten tablets were accurately weighed and triturated it to a fine powder form and transferred into a petri dish. The petridish contained sample was subjected to thermal stress at 110 °C for about one week. The Sample solutions were prepared as equivalent concentrations of 150 µg/mL of

VEP, 600 µg/mL of SOF and transferred in vials and injected in to the HPLC system. The chromatogram of thermal degradation and spectrum index is shown in Fig.17 to Fig.18. In thermal degradation study, it was found that 5.60 % of the VEP and 4.37 % SOF were degraded respectively.

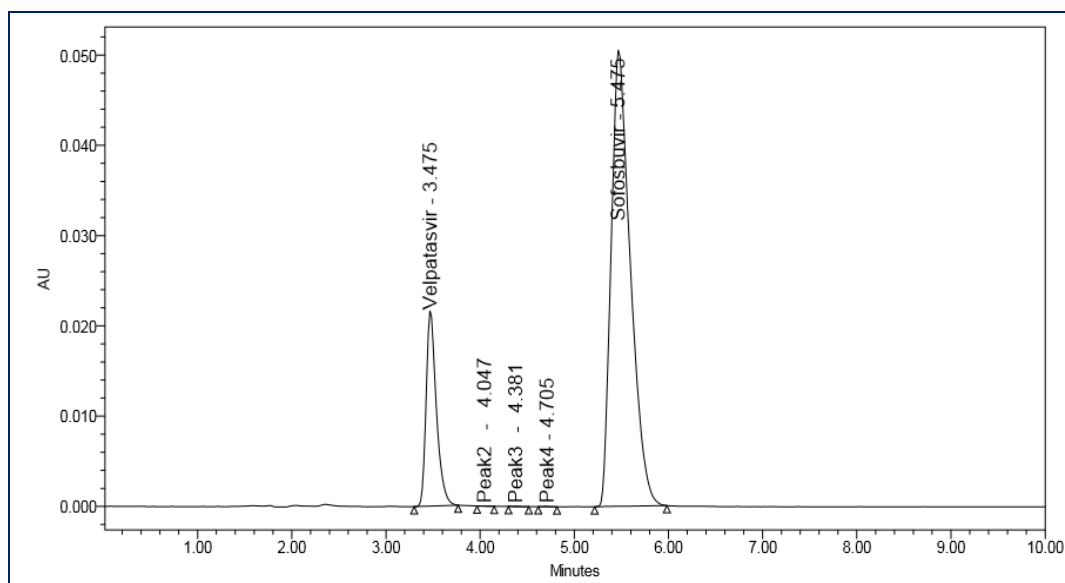


Fig. 17: Chromatogram of thermal degradation for VEP and SOF.

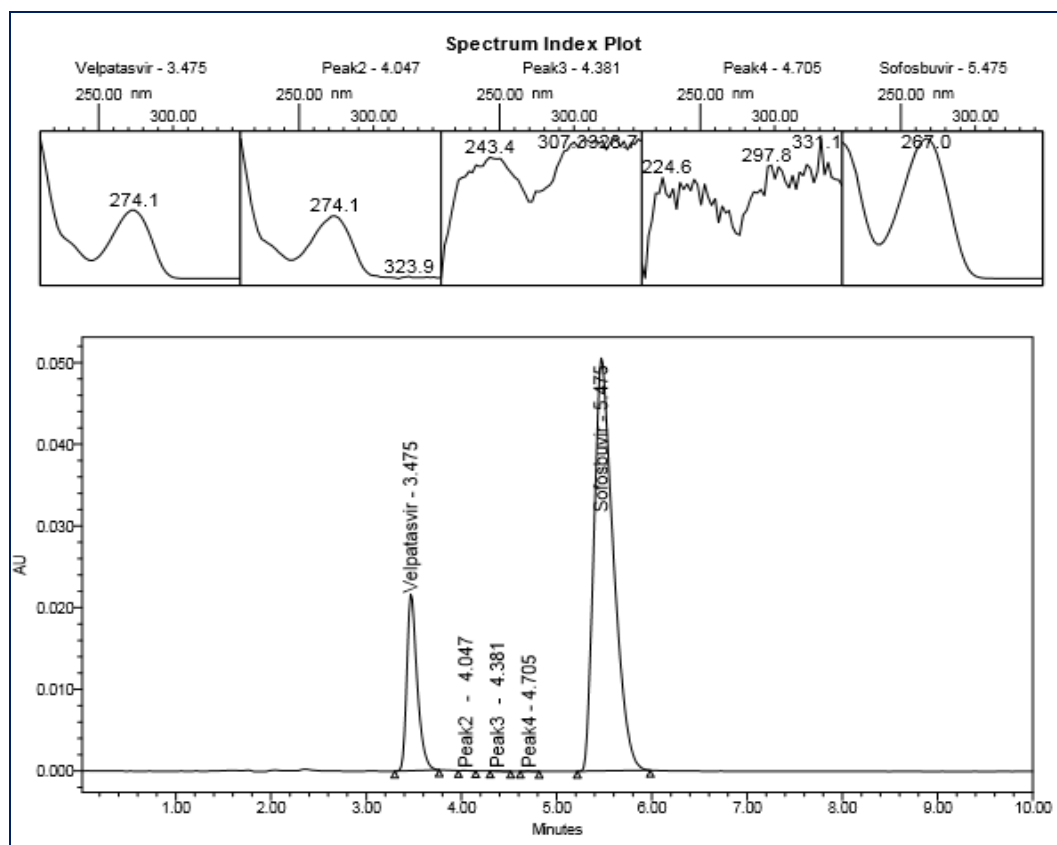


Fig. 18: Spectrum index of Thermal degradation.

Table 9: Peak purity results of Velpatasvir and Sofosbuvir.

Stress	Purity Angle		Purity Threshold	
Condition	VEP	SOF	VEP	SOF
Acid Degradation	34.257	1.029	63.605	2.151
Alkali Degradation	29.614	1.496	59.102	3.360
Oxidative Degradation	31.271	1.118	62.375	3.726
Thermal Degradation	31.075	0.664	62.060	1.282
Photolytic Degradation	37.667	2.008	72.196	3.540

Table 10: Percentage degradation of VEP and SOF.

	Velpatasvir		Sofosbuvir	
	Peak Area	% Degraded	Peak Area	% Degraded
Standard	161608.8		747503	
Acid	153252	5.17	719259	3.78
Base	153532	5.00	719321	3.77
Peroxide	152239	5.80	704978	5.69
Thermal	157552	5.60	714851	4.37
Photo	156452	3.19	714789	4.38

The purity angle value is less than the threshold value (as evident from purity plot) thereby indicating that Velpatasvir and Sofosbuvir were free from co-eluting peaks and method found to be stability indicating.

SUMMARY AND CONCLUSION

A simple and precise stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of sofosbuvir and velpatasvir in tablet dosage form. The method was developed using Spursil C18 (150 x 4.6 mm; 5 μ m) with mobile phase

consisting of Acetonitrile and Phosphate buffer (pH-3.0), 50:50 v/v with a flow rate of 1.0 mL/min in isocratic mode. Detection was carried out at 255 nm using UV detector. The retention time for VEP and SOF were found to be 3.472 and 5.505 min respectively. The proposed method was validated for linearity, range, accuracy, precision, robustness, LOD and LOQ. Linearity was observed over a concentration range 200-1000 μ g/ml for sofosbuvir (r^2 = 0.9999) and 50-250 μ g/ml for velpatasvir (r^2 = 0.9997). The percentage recoveries of both the drugs were 99.84-100.52% and

99.43-100.99 % for SOF and VEP respectively from the tablet formulation. The LOD and LOQ were found to be 2.1 µg/ml and 7.2 µg/mL for sofosbuvir and LOD and LOQ were found to be 1.2 and 4.05 µg/mL for velpatasvir respectively. Sofosbuvir and velpatasvir were subjected to stress conditions of degradation including acidic, alkaline, oxidative, thermal and photolysis. The degradation peaks were well resolved from the drug peaks indicating the stability indicating nature of the method. The method was applicable to simultaneous estimation of velpatasvir and sofosbuvir in tablet formulation.

CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Velpatasvir and Sofosbuvir in tablet dosage form. It was also proved to be convenient and effective for the determination of Velpatasvir and Sofosbuvir in the bulk and combined dosage form. It inferred the method found to be simple, accurate, precise and linear. The method was found to have a suitable application in routine laboratory analysis with high degree of accuracy and precision.

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