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A Stability Indicating RP-HPLC Method for Simultaneous Estimation of Velpatasvir and Sofosbuvir in its Bulk and Tablet Dosage Form

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ABSTRACT

A Stability indicating isocratic liquid chromatographic method with UV detection at 255 nm is described for simultaneous determination of sofosbuvir and velpatasvir in its bulk and tablet dosage form. Chromatographic separation of two drugs was achieved on a YMC column (4.6×15mm,5μ) using a mobile phase consisting of a binary mixture of acetonitrile and 0.025M KH_2PO_4 adjusted to pH3..0 with orthophosphoric acid in ratio 50:50. The developed Liquid Chromatographic method offers symmetric peak shape, good resolution and reasonable retention time for both drugs. Linearity, accuracy and precision were found to be acceptable over the concentration range of 50-250 µg/ml for velpatasvir and 200-1000µg/ml for sofosbuvir and R^2 found to be 0.999. Accuracy was measured via recovery studies and found to be acceptable, and the percentage recoveries were found in the range of 97-103%.Method precision results obtained are 0.1%RSD for sofosbuvir and 0.8%RSD for velpatasvir. Forced degradation studies were also conducted, and the drugs were subjected to various stress conditions such as acid hydrolysis, base hydrolysis, oxidative, photolytic and thermal degradation. The proposed method was successfully validated and applied for the quantitative estimation of these drugs in both bulk and tablet dosage forms. The LC method can be used for the quality control of formulated products containing sofosbuvir and velpatasvir.

Keywords: liquid chromatography**;** sofosbuvir, velpatasvir, forced degradation studies.

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INTRODUCTION

Sofosbuvir and velpatasvir are the nonstructural protein 5A (NS5A) inhibitors belong to a class of antiviral drugs called protease inhibitors. They are direct acting antiviral agents that target viral proteins and their development was a culmination of increased understanding of the viral life cycle. However, their mechanism of action is complex and not fully understood. Despite the recent introduction of numerous new antiviral drugs, resistance is still concern and these inhibitors are therefore always used in combination with other drugs. The NS5A protein plays an important role in viral complex interactions with cellular functions. The protein has been implicated in the modulation of host defenses, apoptosis, the cell cycle, and stress-responsive pathways. However, its function and complete structure have yet to be elucidated NS5A seems to be key in triggering the formation of the membranous web in the absence of other similar nonstructural proteins. Many proteins within the host cell can be affected by NS5A, e.g. phosphatidylinositol 4-kinase required for the replication of HCV. This kinase takes part in the biosynthesis of phosphateidylinositol 4-phosphate (PI4P) by interacting with NS5A, which stimulates its activity and appears to improve the integrity of the membranous web. Recently, the central role of NS5A in viral proliferation has made it the target for Drug development as a result new antiviral agents have been introduced**.**

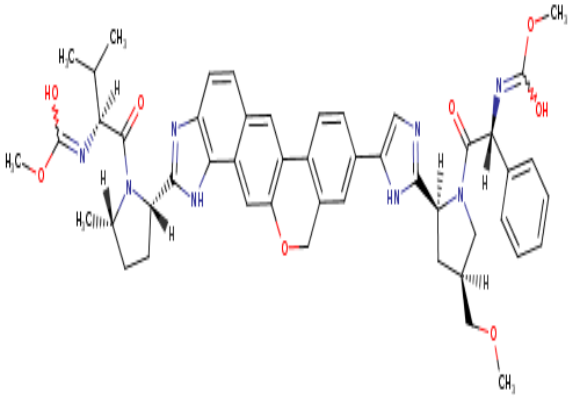

Figure 1: Chemical structure of velpatasvir

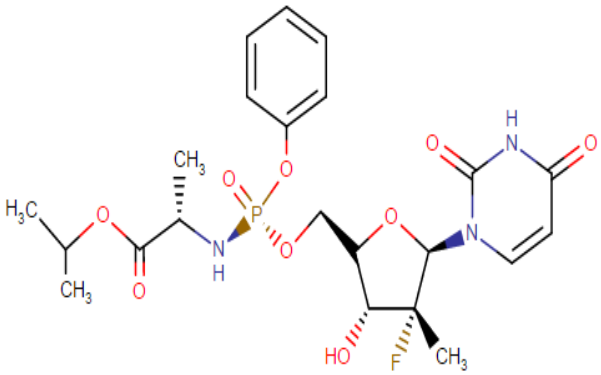

Figure 2: Chemical structure of sofosbuvir

MATERIALS AND METHOD

Chemicals and reagents

A standard sample of sofosbuvir and velpatasvir was obtained as a gift sample from HiQ pharma limited (Hyderabad, India), and the fixed dose combination of Sofosvel was purchased from a local market. Acetonitrile (ACN) and water of HPLC grade were supplied by Merck (Mumbai, India). Potassium dihydrogen orthophosphate (KH_2PO_4) and Ortho phosphoric acid of analytical

grade were obtained from Merck (Mumbai, India). All other reagents of analytical grade were purchased from Rankem (Mumbai, India).

Instrument and chromatographic separation

Chromatography was performed on a WATERS 2695 HPLC column (Waters Corporation, Milford, USA) with an autosampler and equipped with UV detector. Components were detected using 255 nm, and data processing was achieved by Empower 2 software. A hot air oven was used for thermal degradation of the samples, and a UV cross inker, with series of 234100 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 and 300 nm was selected for photolytic degradation. The chromatographic separation was performed on an YMC Column(4.6×150mm, 5 µm) column at an ambient column temperature. The samples were eluted using 0.025M (KH_2PO_4) buffer (pH 3.0):ACN (50:50% v/v) as the mobile phase at a flow rate of 1ml/min. The mobile phase was filtered through a 0.45-µm membrane filter, and it was degassed before use. Then, 20µL of sample solutions were injected into the HPLC system.

Preparation of solutions:

Standard Solution I:

Accurately weigh and transfer 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Standard solution II:

Pipette 1.5 ml of the above standard solution I into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer tablet powder equivalent to 100 mg of Velpatasvir and 400 mg of Sofosbuvir into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION:

System suitability:

Six injections of standard solution of sofosbuvir and velpatasvir were injected and results obtained were found to be within limit shown in Table 1 & 2:

Table 1: System suitability of Velpatasvir

S. No	Name	RT(min)	Area	USP tailing	USP plate count
1	Velpatasvir	3.472	162345	1.38	4903.68
2	Velpatasvir	3.471	162432	1.36	4870.60
3	Velpatasvir	3.472	162971	1.39	4493.09
4	Velpatasvir	3.472	162899	1.38	4973.70
5	Velpatasvir	3.473	162898	1.39	4896.79
6	Velpatasvir	3.472	162333	1.38	4521.41

S. No	Name	RT(min)	Area	USP tailing	USP plate count	USP Resolution
1	sofosbuvir	5.479	747229	1.48	2927.44	6.59
2	sofosbuvir	5.474	746432	1.47	3011.18	6.57
3	sofosbuvir	5.472	747131	1.43	2911.64	6.61
4	sofosbuvir	5.466	747399	1.47	3057.53	6.65
5	sofosbuvir	5.467	747018	1.45	2895.18	6.49
6	sofosbuvir	5.505	74764.9	1.44	2930.36	6.62

Analytical method validation:

Specificity:

Blank Standard and sample solution were injected and it was found to be, no interference of analytical peak with that of blank peak.

Linearity:

Linearity of an analytical method was evaluated over the concentration range of standard solutions. A minimum of six standard drug concentrations ranging between 50-250µg/ml for velpatasvir and 200-1000µg/ml for sofosbuvir were prepared, and their peak areas were recorded. The linearity of the calibration curve was checked by constructing a plot of area versus concentration. The LOD and LOQ were calculated using S/N ratio. The LOD value of velpatasvir, and sofosbuvir was found to 0.24µg/ml and 0.16µg/ml and 0.14µg/ml and 0.38µg/ml respectively**.**

Table 3: Statistical linearity data

Parameter	Velpatasvir	Sofosbuvir
Linearity range	50-250 µg/ml	200-1000 µg/ml
Regression equation	Y=1163.x+5636	Y=1320.x+34051
Limit of detection	0.24 µg/ml	0.16 µg/ml
Limit of quantification	0.14 µg/ml	0.38 µg/ml

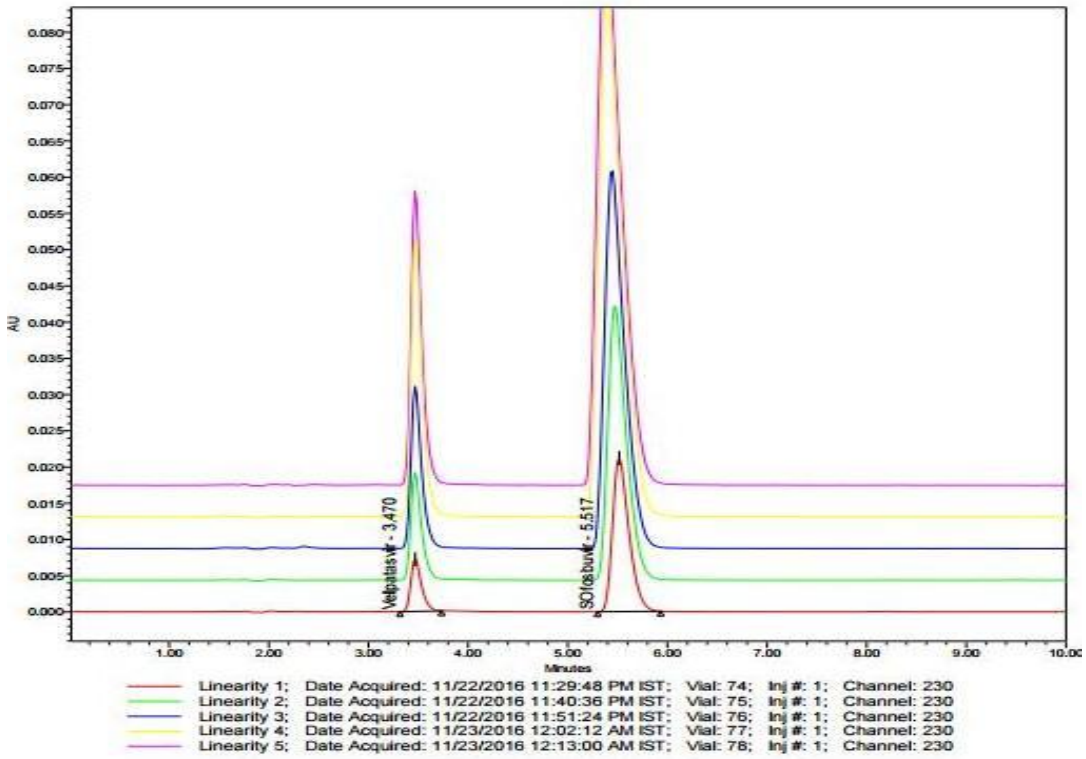

Figure 3: Over lay spectrum of linearity

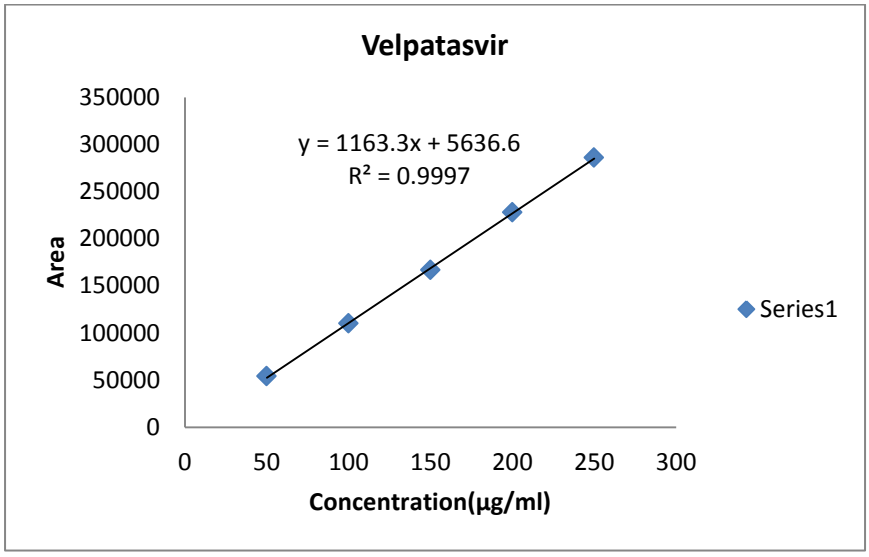

Figure 4: Calibration graph of velpatasvir

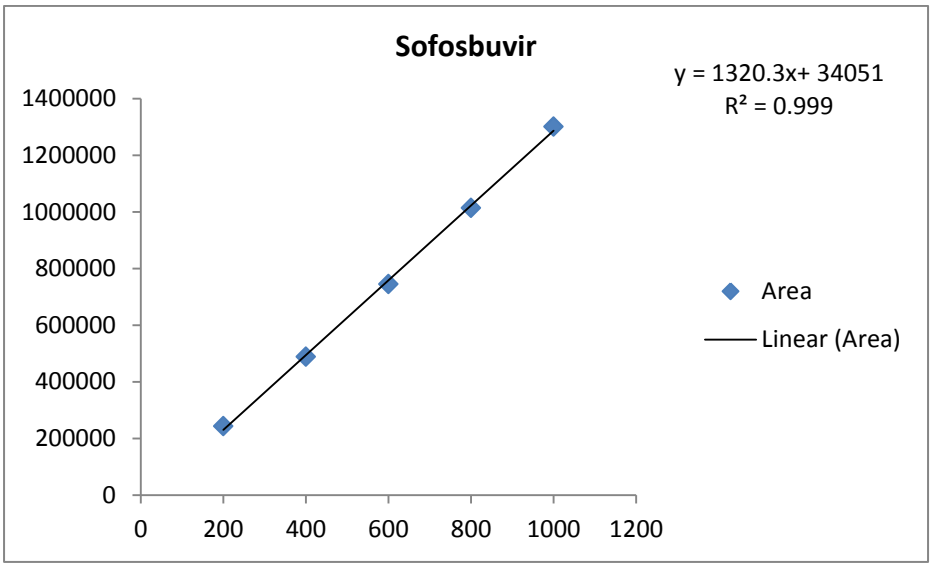

Figure 5: Calibration graph of sofosbuvir

Recovery:

The percentage recovery was calculated by preparing standard drug concentrations of sofosbuvir and velpatasvir with concentration levels of 50%, 100% and 150%. A known amount of the standard drug was added at each level. Good recovery of the drugs was obtained at each added concentration, and the mean percentage recovery of sofosbuvir and velpatasvir is 98-102% . The results are given in Table 4.

Table 4: Recovery results of sofosbuvir and velpatasvir

Compound	%Concentration at specific level	Amount added	Amount found(mg)	%recovery	Mean recovery
sofosbuvir	50	200	199.69	99.84	100.02
	100	400	398.74	99.68	
	150	600	603.12	100.52	
velpatasvir	50	50	49.72	99.43	100.08
	100	100	99.82	99.82	
	150	150	151.49	100.99	

Precision:

Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of velpatasvir (150 µg/mL) and sofosbuvir of (600µg/mL) have been analyzed by injecting them into a HPLC column on the same day and on consecutive days. From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision and intermediate precision are given in Table 5 & 6.

Table 5: Precision results of Sofosbuvir and Velpatasvir

Sofosbuvir				Velpatasvir		
S. No	**Concentration (µg/ml)**	**Peak Area**	**% Assay**	**Concentration (µg/ml)**	**Peak Area**	**% Assay**
1	600	747229	99.50	150	161345	100.10
2	600	746432	99.48	150	161232	100.07
3	600	747131	99.49	150	161671	100.14
4	600	747399	99.57	150	161999	100.21
5	600	747018	99.52	150	162898	100.18
6	600	747649	99.54	150	164679	100.16
Average		747161.3	99.55	Average	162304.0	100.2
SD		419.3	0.15	SD	1208.1	0.18
%RSD		0.1	0.15	%RSD	0.8	0.17

Table 6: Intermediate precision results of Sofosbuvir and Velpatasvir

Sofosbuvir				Velpatsvir		
S. No	**Concentration (µg/ml)**	**Peak Area**	**% Assay**	**Concentration (µg/ml)**	**Peak Area**	**% Assay**
1	600	744533	99.51	150	162345	100.25
2	600	747232	99.87	150	162432	100.30
3	600	744531	99.51	150	162971	100.64
4	600	744399	99.50	150	162899	100.59
5	600	744018	99.42	150	162898	100.59
6	600	744689	99.53	150	162333	100.24
Average		744900	99.55	Average	162646	100.43
SD		1164.7	0.15	SD	305.8	0.19
%RSD		0.2	0.15	%RSD	0.2	0.18

Assay:

Standard and Sample solution containing tablet powder equivalent to 100mg of velpatasvir and 400mg of sofosbuvir was injected and % purity was found to be within limits

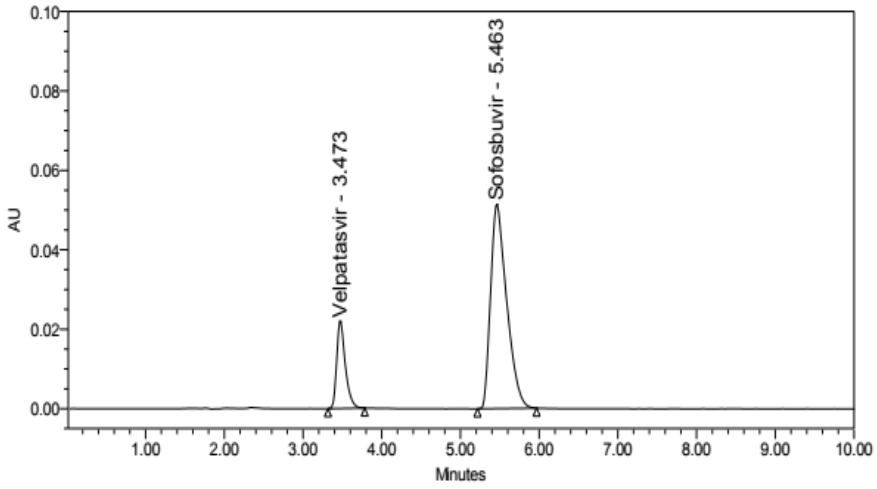

Figure 6: Standard chromatogram of velpatasvir and sofosbuvir

Table 7: Assay results of Velpatasvir and Sofosbuvir

Drug	Label Claim (Velsof) (mg)	% Assay
Velpatasvir	100	100.08
Sofosbuvir	400	99.97

Robustness:

Robustness of the proposed analytical method is a measure of its capacity to remain unaffected, and it reflects the reliability of the analysis with respect to deliberate changes in the parameters such as flowrate (1mL ± 0.1 mL), mobile phase ratio. The parameters chosen for the study of robustness is the change in flow rate and mobile phase composition. From the results obtained there were no significant changes observed at the end of the study.

Table 8: Results for variation in flow for Velpatasvir:

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4353.29	1.30
2	1	4822.40	1.36
3	1.1	4543.29	1.40

Table 9: Results for variation in flow for Sofosbuvir:

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3433.94	1.42
2	1	4722.40	1.26
3	1.1	3863.94	1.44

Table 10: Results for variation in mobile phase composition for Velpatasvir

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4543.29	1.40
2	*Actual	4822.40	1.36
3	10% more	4543.29	1.40

Table 11: Results for variation in mobile phase composition for Sofosbuvir:

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	3863.94	1.44	9.50
2	*Actual	3115.92	1.12	9.50
3	10% more	3863.94	1.44	9.50

Degradation studies:

Degradation studies were performed and compared with undegraded samples to demonstrate the stability of the drug substances. Forced degradation studies of sofosbuvir and velpatasvir were conducted under various stress conditions like acid degradation, base, thermal, photo and oxidative

degradation, both the drugs showed very less degradation. In all the degradation studies, the purity angle was found to be less than the purity of threshold. The degradation results are given in Table

Table 12: Force degradation results for Velpatasvir

Sample Name	% Degraded	Purity Angle	Purity Threshold
Standard	-----	0.152	1.263
Acid	5.17	0.178	1.362
Base	5.00	0.253	1.275
Peroxide	5.80	0.117	1.217
Thermal	2.51	0.092	0.842
Photo	3.19	0.126	0.978

Table 13: Force degradation results for Sofosbuvir

Sample Name	% Degraded	Purity Angle	Purity Threshold
Standard	-------	0.478	2.191
Acid	3.78	0.124	1.967
Base	3.77	0.325	1.535
Peroxide	5.69	0.246	1.752
Thermal	4.37	0.310	1.252
Photo	4.38	0.263	1.278

SUMMARY AND CONCLUSION

The assay of Velpatasvir and Sofosbuvir was performed with tablets and the % assay was found to be 100.08 and 99.97 which shows that the method is useful for routine analysis. The linearity of Velpatasvir and Sofosbuvir was found to be linear with a correlation coefficient of 0.999, which shows that the method is capable of producing good sensitivity. %RSD of precision study was found to be 0.8 and 0.1 for velpatasvir and sofosbuvir which is with in acceptance criteria. %RSD of intermediate precision was found to be 0.2 for both velpatasvir and sofosbuvir which is with in acceptance criteria. The percentage recovery was found to be 97.0% - 103.0%. %RSD of recovery study was found to be 100.08% and 100.02% for Velpatasvir and Sofosbuvir respectively. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The LOD and LOQ for Velpatasvir was found to be 0.24 µg/ml and 0.16 µg/ml and for Sofosbuvir was found to be 0.14 µg/ml and 0.38 µg/ml respectively. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions. The forced degradation studies were carried out, and the reported method effectively separates the drug substances without interference of excipients and degradation products. Hence, it can be concluded that the developed method can be used for the routine analysis of sofosbuvir and velpatasvir in the pharmaceutical dosage form.

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