Research Article

Validated Stability Indicating Method for Simultaneous Estimation of Velpatasvir and Sofosbuvir RP-UPLC

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Received: 07-08-2018; **Revised:** 25-08-2018; **Accepted:** 07-09-2018.

ABSTRACT

The aim of this work was focused on to develop and validate accurate, simple reverse phase ultra-performance liquid chromatography method for simultaneous estimation of velpatasvir and sofosbuvir in pharmaceutical dosage form. The chromatographic separation was performed on HSS (100 X 2.1mm, 1.8m) column, with a mobile phase comprising of a mixture Buffer: Acetonitrile (50:50)at flow rate of 0.3 mL/min with detection at 260 nm. Retention times of velpatasvir and sofosbuvir were found to be 0.97 minutes and 1.33 minutes respectively. The developed method was validated according to ICH guidelines, linearity of sofosbuvir was found to be in the range of 20-120 mg/mL and that of velpatasvir was found to be in the range of 5-30 µg/mL. The percentage recoveries for both drugs were found in the range of 100-101%. The limit of detection and the limit of quantification values for velpatasvir were found to be 3.3 and 10.0 and that for sofosbuvir were found to be 3.3 and 10.0 respectively. The proposed UPLC method is simple, rapid, specific, accurate, isocratic and precise and can used for route analysis of velpatasvir and sofosbuvir. The run time was about only 3 minutes which shows the consumption of solvents will be less.

Keywords: Velpatasvir, Sofosbuvir, RP-UPLC,Validation, Stability, Degradation.

INTRODUCTION

Velpatasvir is a Hepatitis C virus NS5A inhibitor. Chemically it is methyl {(2S)-1-[(2S,5S)-2-(9-{2-[(2S,4S)-1-{(2R)-2-[(methoxy carbonyl) amino]-2-phenyl acetyl}-4-methoxy methyl) pyrrolidin -2 -yl] -1H -imidazol-4-yl}-1,11-dihydro[2]benzopyrano[4',3':6,7] naphtho[1,2-d] imidazol -2-yl)-5-methylpyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl}carbamate. The mechanism of action of velpatasvir is as a Breast Cancer Resistance Protein Inhibitor, and P-Glycoprotein inhibitor, and Organic Anion Transporting Polypeptide 1B1 Inhibitor, and Organic Anion Transporting Polypeptide 1B3 Inhibitor, and Organic Anion Transporting Polypeptide 2B1 Inhibitor. [1, 2] The chemical structure of Velpatasvir is shown in Fig.1.

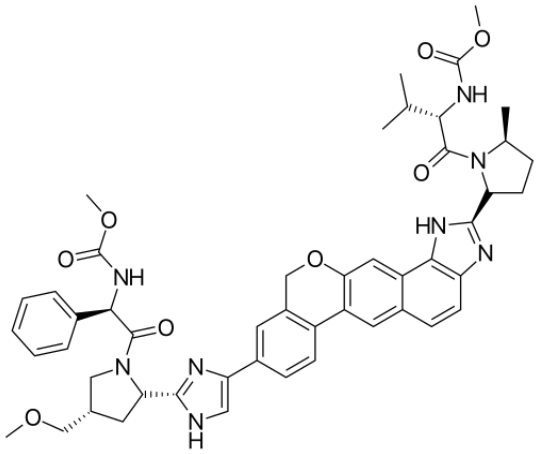

Figure 1: Structure of Velpatasvir

Sofosbuvir is an orally available nucleotide prodrug and a hepatitis C virus (HCV) NS5B polymerase inhibitor with potential HCV inhibiting activity. Chemically it is S)-Isopropyl 2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyl tetrahydrofuran-2-yl) methoxy)- (phenoxy) phosphoryl amino) propanoate Upon oral administration, sofosbuvir is metabolized to 2'-deoxy-2'-alpha-fluoro-beta-C-methyluridine-5'-monophosphate, which is then converted into the active triphosphate nucleotide that inhibits the NS5B polymerase, thereby preventing viral replication. The HCV NS5B protein, an RNA-dependent RNA polymerase, is essential for the replication of the viral HCV RNA genome.[3, 4] The chemical structure of sofosbuvir is shown in Fig.2.

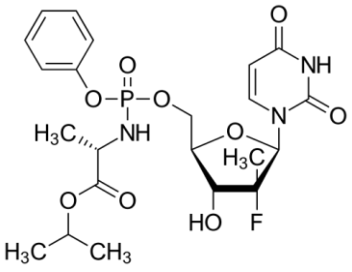

Figure 2: Structure of Sofosbuvir

Literature survey revealed few methods for simultaneous estimation of Velpatasvir and Sofosbuvir by RP-HPLC but many methods haven't reported about stability indicating studies.In the present study stability indicating UPLC method have been developed with very less retention

time where no other method has reported this less retention time.[5-9]

MATERIALS AND METHODS

Reagents used were acetonitrile of HPLC grade, water of HPLC grade, Potassium dihydrogen phosphate: AR grade. Marketed formulation containing 100 mg of Velpatasvir, 400 mg of Sofosbuvir was procured from local pharmacy.

Instrumentation and Chromatographic Conditions: UPLC system with photodiode detector was used and data was acquired and processed by using Empower software. The separation was carried out at ambient temperature by using a HSS (100 X 2.1mm, 1.8µm) column. The mobile phase consisting of Buffer: Acetonitrile (50:50v/v) was by maintain the flow rate at 0.3 ml/min. The injection volume was 3 µL and detection was carried out at 260 nm. Fig. 3

Mobile Phase

Buffer (0.1%OPA)

Ortho phosphoric acid 1 mL of was taken in a 1000 mL volumetric flask and adds about 100 ml of water and final volume make up to 1000 ml.

Above prepared buffer and HPLC grade Acetonitrile were taken in 50:50 ratios and degased in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration and used as mobile phase. The same mobile phase is used as diluents throughout the method.

Standard Solution Preparation

Accurately weigh and transfer 5 mg of Velpatasvir and 20 mg of Sofosbuvir working standards into a 25 ml clean dry volumetric flask, add 10 ml of diluent, sonicate for 5 minutes and made to the final volume with diluents. 1ml from the above stock solutions was taken into a 10 ml volumetric flask and made up to the volume.

Linearity of pure standard solution

The linearity of the samples of Velpatasvir and Sofosbuvir was prepared by suitably diluting working solution and found to be linear response of drug over a range of 5-30 µg/ml for Velpatasvir and 20-120 µg/ml for the Sofosbuvir respectively. The three such linearities of Velpatasvir and Sofosbuvir were taken for correlation co-efficient and standard deviation calculation. (Table 1 & Fig 3& 4)

Table 1: Linearity of Velpatasvir and Sofosbuvir

S.No	Velpatasvir (µg/mL)	Area (mV.s)	Sofosbuvir (µg/mL)	Area (mV.s)
1	5	35666	20	114008
2	10	69400	40	218477
3	15	106484	60	335722
4	20	139381	80	448785
5	25	175470	100	560690
6	30	208230	120	662011

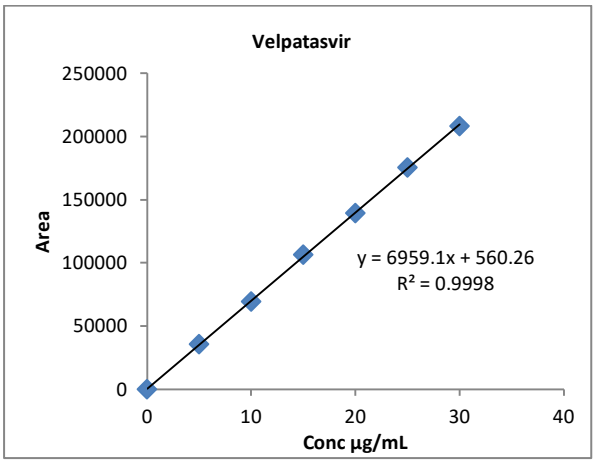

Figure 3: Linearity of Velpatasvir

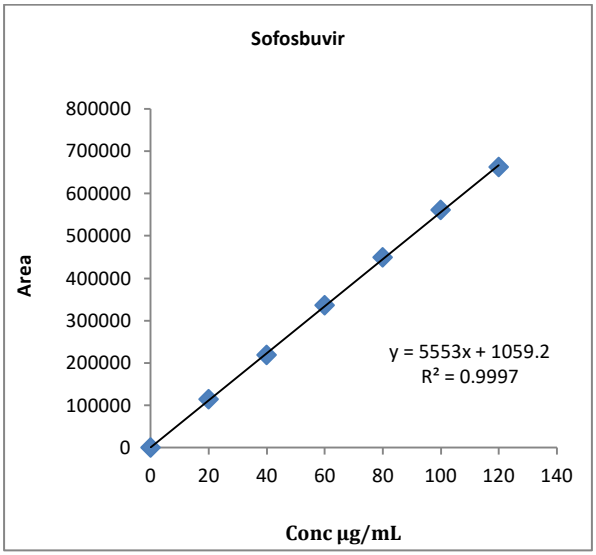

Figure 4: Linearity of Sofosbuvir

Analysis of Formulation

The developed procedure was extended to formulation of Velpatasvir and Sofosbuvir, the combination was available in the market of strength 100 mg Velpatasvir and 400 mg Sofosbuvir respectively. Average weight of twenty tablets was taken and crushed to make powder, powder equivalent to 100 mg of Velpatasvir was transferred to 100 mL volumetric flask and volume was made up to the mark with diluent and filtered through whatmann and transferred to 100 ml volumetric flask and made upto the mark with diluent. The same procedure as mentioned for the pure drug was followed for the formulation and the assay results were tabulated in Table 2.

Method Validation [10]

Preparation of stock solution

Accurately weigh and transfer 10 mg and 40 mg of Velpatasvir and Sofosbuvir working standard into a 10ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent and this is used as stock solution.

Precision

From the above stock solutions different dilutions were made and the test solution was injected for six times and measured the area for six replicate injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. Results are summarized in Table 3.

Table 2: Assay Results

Sl. No.	Drug	Labeled Amount (mg)	Amount Found mg/tab	% Recovery	%RSD (n=3)
1	Velpatasvir	100 mg	100.88	100.88	0.10
2	Sofosbuvir	400 mg	402.32	100.58	0.70

Table 3: Precision results of Velpatasvir and Sofosbuvir

S.No.	Velpatasvir		Sofosbuvir	
	Method Precision	Day Day Precision	Method Precision	Day Day Precision
1	141230	137255	452250	435777
2	140256	138156	451916	439502
3	139486	140050	448442	438382
4	138832	138352	455720	440569
5	141308	138818	447335	440772
6	140120	137613	452224	439810
Average	140205	138374	451315	439135
SD	967.4	988.5	3018.6	1853.1
%RSD	0.7	0.7	0.7	0.4

% RSD should not be more than 2%.

Accuracy

Preparation Sample solutions

From the above stock solution further dilutions were done to obtain the solution of 50%, 100% and 150% concentrations and these were injected in triplicate and the recovery studies was done for Velpatasvir and Sofosbuvir. Results are summarized in Table 4&5

Table 4: Accuracy results for Velpatasvir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	208775	10	10.00	100.01	99.72
100%	277723	20	19.91	99.54	
150%	347136	30	29.88	99.61	

Table 5: Accuracy results for Sofosbuvir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	666550	40	40.04	100.1	100.39
100%	891179	80	80.49	100.61	
150%	1113759	120	121.76	100.69	

% Recovery should be between 98.0 to 102.0%

Linearity

From the above stock solution further dilutions were made to get the concentrations equivalent to 5, 10, 15, 20, 25, 30 ppm and 20,40,60,80,100,120 ppm of Velpatasvir and Sofosbuvir respectively. Inject each level into the chromatographic system and measure the peak areas. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Correlation coefficient should be not less than 0.999.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) parameters were calculated using the following equations; LOD=3.3σ/s and LOQ=10σ/s, where σ is standard deviation of y intercept of calibration curve and s is slope of regression equation. The results are summarized in **Table 6**

Table 6: LOD and LOQ Results

Drug	LOD	LOQ
Velpatasvir	0.09	0.28
Sofosbuvir	0.18	0.54

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

Standard solution 15 ppm & 60 ppm of Velpatasvir and Sofosbuvir were prepared and analysed using the varied flow rate along with method flow rate. The results are summarized in Table 7.

Table 7: Robustness evaluation of method

Sl.No	Change in the Chromatographic Conditions	%RSD of Velpatasvir	%RSD of Sofosbuvir
1	Flow rate (-) 0.9ml/min	1.77	1.8
2	Flow rate (+) 1.1ml/min	1.4	1.1
3	Mobile phase (-) 60B:40A	1.36	1.4
4	Mobile phase (+) 50B:50A	1.3	1.7
5	Temperature (-) 25°C	1.1	1.2
6	Temperature (+) 35°C	1.09	0.9

Degradation Studies [11]

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on Velpatasvir and Sofosbuvir using the proposed method. The results are summarized in Table 8

Preparation of Stock solution

Accurately weigh and transfer 10mg and 40mg of Sofosbuvir and Velpatasvir working standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent used as stock solution.

Oxidation

To 1 ml of stock solution of Sofosbuvir and Velpatasvir 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min. For HPLC study, the resultant solution was diluted to obtain (80ppm&20ppm) solution and 3 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of stock solution Sofosbuvir and Velpatasvir 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins.The resultant solution was diluted to obtain (400ppm&100ppm) solution and 3 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution Sofosbuvir and Velpatasvir 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins. The resultant solution was diluted to obtain (80ppm&20ppm) solution and 3 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

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 (80ppm&20ppm) solution and 3µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the (800ppm&200ppm) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (80ppm&20ppm) solutions and 3 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to (80ppm&20ppm) solution and 3 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Table 8: Results of Degradation studies

	Velpatasvir	%degraded	Sofosbuvir	%degraded
Standard	139857		449206	
Acid	135309	3.35	429132	4.56
Base	135838	2.97	430670	4.22
Peroxide	134696	3.79	436065	3.02
Thermal	137542	1.75	440853	1.96
UV	137624	1.69	443418	1.39
Water	138696	0.93	446065	0.80

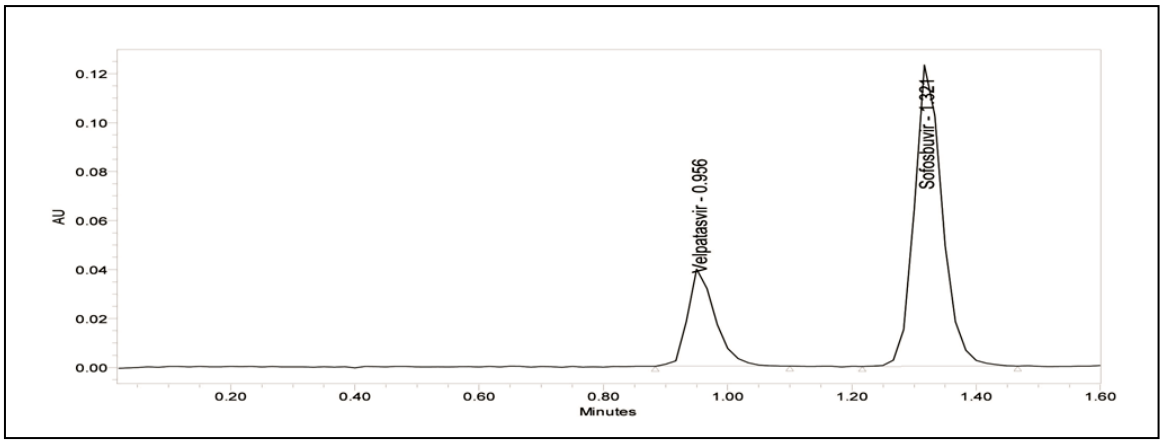

Figure 5: Typical Chromatogram of Velpatasvir and Sofosbuvir

CONCLUSION

The present study is an attempt to report new validated stability indication UPLC method for simultaneous estimation of Velpatasvir and Sofosbuvir. The run retention time was found less than 3 minutes and so far no authentication method has been reported about the degradation data.

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Source of Support: Nil, **Conflict of Interest:** None.