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## RESEARCH ARTICLE

### Stability Indicating RP-HPLC Method for Simultaneous Estimation of Sofosbuvir and Velpatasvir in Bulk and Pharmaceutical Dosage Form

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#### ABSTRACT

A simple, precise, accurate rp-hplc method has been developed and validated for the sofosbuvir and velpatasvir in bulk and pharmaceutical dosage form. This method was carried out by using Inertsil ODS-3(4.6 x 150mm, 5µm). Mobile phase containing phosphate buffer (ph4.8): Acetonitrile (35:65) at a flow rate 0.6ml/min. Optimized wavelength 244nm. Retention time of sofosbuvir and velpatasvir were found to be 2.22 min and 5.80 min. The precision study was precise, robust, repeatable. LOD and LOQ were both drugs found to be 3.0, 10.02 and 3.00, 10.07 respectively. The %RSD values were found to be <2. A Forced degradation study of sofosbuvir and velpatasvir under the condition of hydrolysis, thermal, oxidative, photolysis. The developed method is validated in accordance with ich guideline. The result of the study showed that the proposed method is simple, rapid, and accurate.

**Key words:** Sofosbuvir, Velpatasvir, RP-HPLC, Validation

#### ARTICLE INFO

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#### 1. Introduction

Sofosbuvir is chemically known as propan-2-yl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2,4-dioxypyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxyphenoxy phosphoryl] amino] propanoate. Sofosbuvir, is a NS5B

RNA Polymerase Inhibitor direct acting antiviral drug used in the treatment of chronic Hepatitis C.<sup>[1-2]</sup> Velpatasvir chemically known as Carbamic acid N-[(1R)-2-[(2S,4S)-2-[5-[1,11-dihydro-2-[(2S,5S)-1-[(2S)-2 [(methoxycarbonyl)

amino]-3-methyl-1-(2-oxobutyl)-methypyrrolidinyl] [2-benzopyrano [4',3':6,7] naphth[1,2-d]imidazol-9-yl]-1H-imidazol-2-yl]-4-(methoxymethyl)-1-pyrrolidinyl]-2-oxo-1-phenylethyl]-, methyl ester. Velpatasvir act as an NS5A RNA Polymerase inhibitor, it is used in the combination therapy to treat chronic Hepatitis C.<sup>[3-4]</sup>

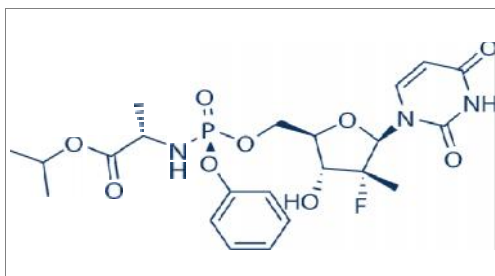


Fig 1: Structure of Sofosbuvir

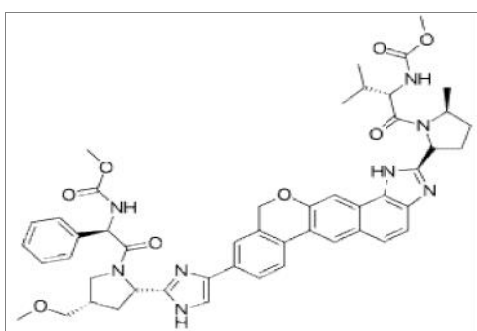


Fig 2: Structure of Velpatasvir

The literature review shows few methods for sofosbuvir and velpatasvir by simultaneous estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC for compounds either individually or in combination with other dosage form. (5-7) Hence, it was felt that, there is a need of new, precise and much efficient analytical method development for the simultaneous estimation of Sofosbuvir and velpatasvir in pharmaceutical dosage form.<sup>[8-9]</sup> The main objective of this study is to develop a new, simple, fast, rapid, accurate, and reproducible RP-HPLC method for the simultaneous analysis of sofosbuvir and velpatasvir. The developed method will be validated according to ICH guidelines. Degradation studies were also performed.<sup>[10-12]</sup>

## 2. Materials and Methods

Table 1: Instruments used

Instrument	Model
HPLC	WATERS, software: Empower, 2695 separation module, uv detector.
UV/VIS spectrophotometer	Labindia Uv 3000 <sup>+</sup>
pH meter	Adwa – AD 1020
Weighing machine	Afcoset ER-200A
Pipettes and Burettes	Borosil
Beakers	Borosil

Table 2: Sample and standard details

Sample	Sofosbuvir & Velpatasvir Tablets 400 mg & 100 mg
Standard	Sofosbuvir & Velpatasvir

Table 3: Chemicals used

Chemical	Company Name
Sofosbuvir	Pharmatrain
Velpatasvir	Pharmatrain
KH <sub>2</sub> PO <sub>4</sub>	Finer Chemical Ltd
Water and Methanol for HPLC	Lichrosolv (Merck)
Acetonitrile for HPLC	Molychem
Ortho phosphoric Acid	Merck

Table 4: Results of % Assay for Sofosbuvir and Velpatasvir

Drug	Label Claim (mg)	% Assay
Sofosbuvir	400	100.1
Velpatasvir	100	100.6

### Wave length selection:

UV spectrum of 10 µg/ml Sofosbuvir and 10 µg/ml Velpatasvir in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 244 nm. At this wavelength both the drugs show good absorbance. The chromatographic method development for the simultaneous estimation of sofosbuvir and velpatasvir were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of sofosbuvir and velpatasvir in bulk and pharmaceutical dosage form by RP-HPLC method.

### Optimized Chromatographic Conditions:

Instrument used : Waters HPLC with auto sampler and UV detector.

Temperature : Ambient (250C)

Mode of separation : Isocratic mode

Column : Inertsil ODS-3(4.6 x 150mm, 5µm)

Mobile phase: Phosphate buffer 4.8 pH and Acetonitrile (35: 65)

Flow rate : 0.6 ml per min

Wavelength : 244 nm

Injection volume : 10 µl

Run time : 15 min.

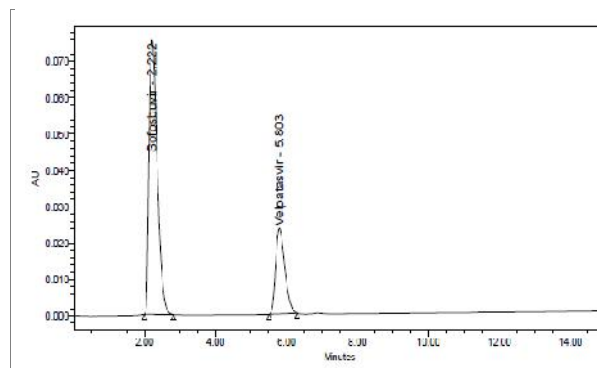


Fig 1: Optimized chromatogram of sofosbuvir and velpatasvir

**Preparation of phosphate buffer:**

Take 6.8gms of Potassium dihydrogen ortho phosphate in 1000ml of water and adjust the pH-4.8 with orthophosphoric acid and degassed in an ultrasonic water bath for 10 minutes. The solution was filtered through 0.45  $\mu$  filter under vacuum filtration.

**Preparation of mobile phase:**

Mix a mixture of above buffer 350 ml (35%) of Phospahte Buffer and 650 ml (65%) of Acetonitrile were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 $\mu$  filter under vacuum filtration.

**Diluent Preparation:**

Used the mobilephsae as diluent.

**Standard Solution Preparation:** Accurately weigh and transfer 100 mg of Sofosbuvir and 25 mg of Velpatasvir working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate. volume made up to the diluents with the same solvent. (Stock solution) then 3.0 ml of the above stock solutions was transfer into a 10ml volumetric flask and dilute up to the mark with diluent.

**Sample Solution Preparation:**

100 mg of Sofosbuvir and 25 mg of Velpatasvir sample into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate. made up with a volume up to the mark with the same solvent. (Stock solution). Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and and made up to the markwith diluent.

**3. Results and Discussions****Method Development**

The typical values for evaluating the system suitability parameters i.e. resolution between peaks, tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Sofosbuvir at 3.222 min and velpatasvir at 5.803 min. The total run time is 15 minutes with all system suitability parameters as ideal for the mixture of standard solutions.

**System suitability acceptance criteria:**

- Tailing factor for the peaks due to Sofosbuvir and Velpatasvir in Standard solution should not be more than 2.0
- Theoretical plates for the Sofosbuvir and Velpatasvir peaks in Standard solution should not be less than 2000.
- Resolution for the Sofosbuvir and Velpatasvir peaks in standard solution should not be less than 2

**Method Validation**

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. The RP-HPLC method developed was validated according to International Conference on Harmonization10 guidelines for validation of analytical procedures. The method was validated for the parameters in terms of system suitability, selectivity, linearity, accuracy, precision, ruggedness, robustness, limit of detection(LOD) and limit of quantitation(LOQ).

**Specificity:**

Interference with the diluent was evaluated by injecting the diluent into HPLC as per proposed method. No peaks were found except for the solvent front peaks. Specificity was also evaluated by forced degradation studies as per the ICH guideline.

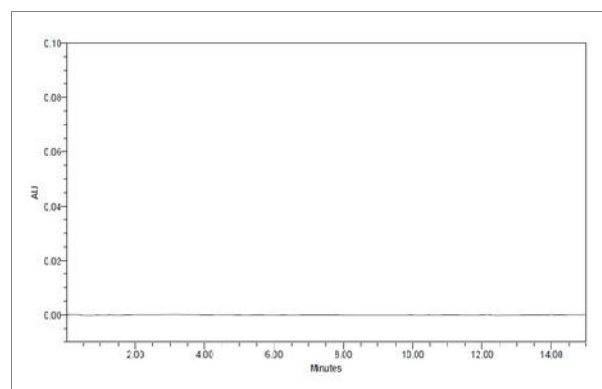


Fig 2: Chromatogram of blank

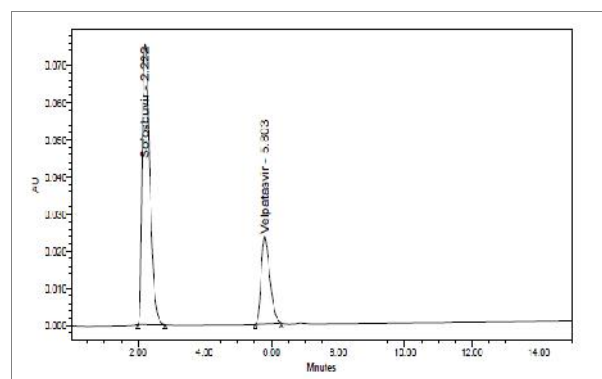


Fig 3: Chromatogram for Standard

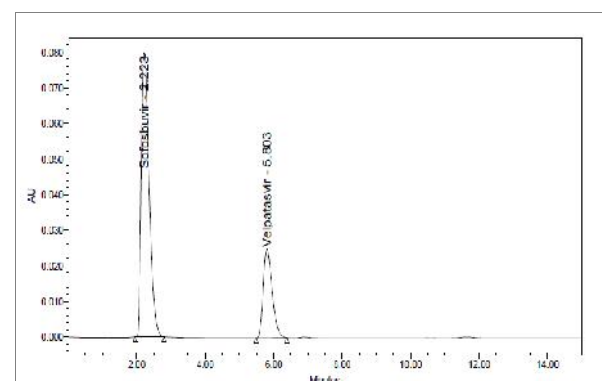


Fig 4: Chromatogram for Sample

For Specificity Blank and Standard are injected into system. There is no any inteferece of any peak in blank with the retion time of the analytical peaks.

**Linearity:**

The linearity study was performed for the concentration of 100 ppm to 500ppm Sofosbuvir and 25 ppm to 125ppm velpatasvir level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in Table 6.

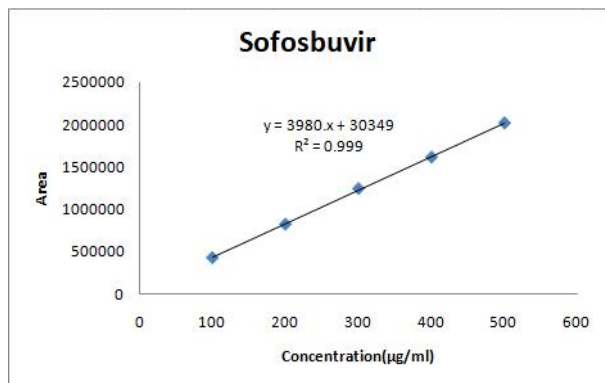


Fig 5: Linearity graph of sofosbuvir

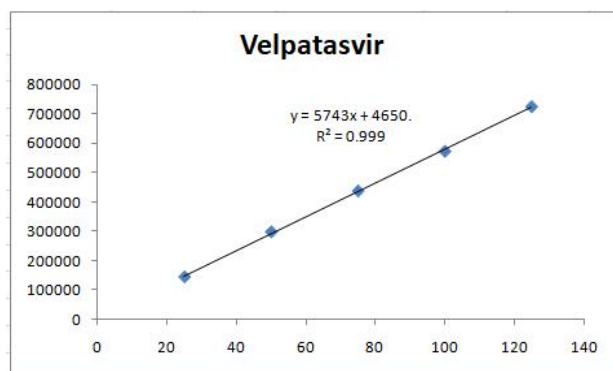


Fig 6: Linearity graph of velpatasvir

The linearity study was performed for concentration range of 100µg -500µg sofosbuvir and 25µg - 125 µg velpatasvir and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999)respectively.

#### Accuracy:

The accuracy study was performed for 50%, 100% and 150 % for sofosbuvir and velpatasvir Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. The results are tabulated in Table.No.7&8.

#### Precision:

- Repeatability
- Intermediate Precision/ Ruggedness

#### Repeatability:

The precision study was performed for five injections of sofosbuvir and velpatasvir. Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of % RSD. The results are tabulated in Table.9& 10.

#### Intermediate Precision/Ruggedness:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

#### Limit of Detection:(Sofosbuvir)

The detection limit of sofosbuvir was found to be 3.04

#### Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution

#### Limit of Quantification:

The quantification limit of sofosbuvir was found to be 10.02

#### Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution

#### Limit of Detection: (For Velpatasvir)

The detection limit of Velpatasvir was found to be 3.00

#### Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution

#### Limit of Quantification:

The quantification limit of velpatasvir was found to be 10.07

#### Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution

#### 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.

**A.** The flow rate was varied at 0.45 ml/min to 0.55ml/min. Standard solution 300 ppm of Sofosbuvir & 75 ppm of Velpatasvir was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate  $\pm 10\%$ .

**B.** The Organic composition in the Mobile phase was varied from  $\pm 10\%$ . Standard solution 300 ppm of Sofosbuvir & 75 ppm of Velpatasvir was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%. Organic composition in the mobile phase affected the method significantly. Hence it Indicates that the method is robust even by change in the Mobile phase  $\pm 10$ .

#### Degradation results for sofosbuvir and velpatasvir

##### Preparation of stock:

Accurately weigh and transfer 100 mg of Sofosbuvir and 25 mg of Velpatasvir 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. (Stock solution)

##### Hydrolytic degradation under acidic condition:

Pipette 3 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60°C for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

##### Hydrolytic degradation under alkaline condition:

Pipette 3 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60°C for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

##### Thermal induced degradation:

Sofosbuvir and Velpatasvire sample was taken in petridish and kept in Hot air oven at 1100 C fo 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

##### Oxidative degradation:

Pipette 3 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

**Photo degradation:**

Pipette 3 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

**Table 5:**System suitability results of Sofosbuvir and Velpatasvir

S.No	Peak name	Retention time	Area	USP Resolution	USP Tailing	USP Plate Count
1	Sofosbuvir	2.222	1216123	7.59	1.56	3399.02
2	Velpatasvir	5.803	423289		1.36	5167.98

**Table 6:**Linearity Results of Sofosbuvir& Velpatasvir

Linearity Level	Sofosbuvir		Velpatasvir	
	Concentration	Area	Concentration	Area
I	100	424986	25	144310
II	200	821489	50	297966
III	300	1243214	75	437053
IV	400	1614178	100	572746
V	500	2019024	125	724791
Correlation Coefficient			0.999	

**Table 7:**Accuracy results for Sofosbuvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	609631.3	50	50.08	100.17	100.42
100%	1222083	100	100.40	100.40	
150%	1838335.7	150	100.68	100.68	

**Table 8:**Accuracy results for velpatasvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	214986.7	12.5	12.58	100.62	100.62
100%	430906	25	25.21	100.84	
150%	643576	37.5	37.65	100.40	

**Table 9:**Summarized precision results for Sofosbuvir and Velpatasvir

Injection	RT (Sofosbuvir)	Area for Sofosbuvir	RT (Velpatasvir)	Area for Velpatasvir
Injection-1	2.216	1235278	5.811	436704
Injection-2	2.223	1220850	5.831	435672
Injection-3	2.217	1239231	5.816	439902
Injection-4	2.214	1212072	5.813	435887
Injection-5	2.228	1237137	5.840	442806
Injection-6	2.223	1228702	5.832	444747
<b>Average</b>		1228878.3		439286.3
<b>Standard Deviation</b>		10613.9		3843.8
<b>%RSD</b>		0.9		0.9

**Acceptance Criteria:** The % RSD for the area of six standard injections results should not be more than 2.

**Table 10:** Summarized ID precision results for Sofosbuvir and Velpatasvir

Injection	RT (Sofosbuvir)	Area for Sofosbuvir	RT (Velpatasvir)	Area for Velpatasvir
Injection-1	2.222	1235386	5.833	434628
Injection-2	2.221	1223334	5.833	434399



Injection-3	2.224	1222572	5.830	434591
Injection-4	2.226	1236362	5.822	439920
Injection-5	2.225	1232244	5.830	432316
Injection-6	2.223	1221786	5.830	440024
<b>Average</b>		1228614.0		435979.7
<b>Standard Deviation</b>		6783.4		3211.2
<b>%RSD</b>		0.6		0.7

**Table 11:** Results for Ruggedness

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results				
		Sofosbuvir		Velpatasvir		
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing	USP Resolution
1	10% less	1.55	3445.74	5082.74	1.43	8.34
2	Actual	1.56	3399.02	5167.98	1.36	7.59
3	10% more	1.52	3427.53	5667.09	1.43	5.70

**Table 12:** Degradation Results of sofosbuvir and velpatasvir

Sample Name	Sofosbuvir		Velpatasvir	
	Area	% Degraded	Area	% Degraded
<b>Standard</b>	1214803		426473	
<b>Acid</b>	1196736	1.49	416562	2.32
<b>Base</b>	1175633	3.22	410776	3.68
<b>Peroxide</b>	1097863	9.63	407623	4.42
<b>Thermal</b>	1167563	3.89	403572	5.37
<b>Photo</b>	1165552	4.05	398772	6.50

#### 4. Conclusion

The proposed reverse phase high performance liquid chromatography method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the linear concentration range. The analytical method validation of sofosbuvir and velpatasvir by RP-HPLC was found satisfactory and could be used for the routine pharmaceutical analysis of sofosbuvir and velpatasvir. Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specify, forced degradation studies, ruggedness, robustness. Therefore, this hplc method can be used as a routine analysis of these drugs in bulk, pharmaceutical formulation and also stability studies.

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