



Method Development and Validation of Velpatasvir and Sofosbuvir by RP-HPLC

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ABSTRACT

Aim: The given RP-HPLC technique was found to be clear-cut, specific, exact, and economical for the estimation of Velpatasvir and Sofosbuvir in bulk and tablet dosage form.

Methodology: Stationary phase Hypersil C18 column (4.6×150, 5µm) was utilized as chromatographic conditions. Water: Acetonitrile in the ratio of 30:70 was used as a fluid phase. The flow rate was maintained at 1.0ml/min

Results: The wavelength of detection was 230 nm. Velpatasvir linearity was found to be 10-50 ppm and Sofosbuvir linearity was found to be 20-100 ppm with $r^2=0.999$. Assessment of the formulated market was carried out by using the above method and the percentage of Velpatasvir and Sofosbuvir was found to be 99.6 % and 99.8% respectively.

Conclusion: Hence the method can be successfully applied for routine analysis of Velpatasvir and Sofosbuvir in bulk and tablet formulation.

Keywords: Velpatasvir, Sofosbuvir, RP-HPLC, Validation parameters.

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INTRODUCTION

Sofosbuvir-velpatasvir is a pan-genotypic NS5A-NS5B inhibitor single-pill combination regimen that has potent activity against hepatitis C virus (HCV) genotypes 1, 2, 3, 4, 5, and 6. It provides a much-needed option for patients with HCV genotype 3 infection, including those with compensated cirrhosis who lack the Y93H resistance-associated variant¹⁻³. Sofosbuvir-velpatasvir can, in contrast to HCV protease-inhibitor-containing regimens, be used safely in persons with decompensated cirrhosis. Levels of sofosbuvir-velpatasvir can be significantly reduced with concurrent use of acid-reducing agents, particularly proton-pump inhibitors⁴. The most common adverse effects, observed in at least 10% of phase 3 trial participants, were headache and fatigue. Sofosbuvir-velpatasvir is an oral fixed-dose combination of sofosbuvir, a nucleotide analog NS5B polymerase inhibitor, and velpatasvir, an NS5A replication complex inhibitor. Sofosbuvir is currently approved in the United States for the treatment of HCV genotypes 1-6 HCV. Velpatasvir (formerly GS-5816) is a novel NS5A inhibitor that has potent in vitro anti-HCV activity across all genotypes at the picomolar level.⁵⁻⁶ The combination of sofosbuvir-

velpatasvir is the first once-daily single-tablet regimen with pan-genotypic activity.

Description of Sofosbuvir

Drug category: Treatment of hepatitis C.

Structure: Structure of Sofosbuvir shown in figure 1.

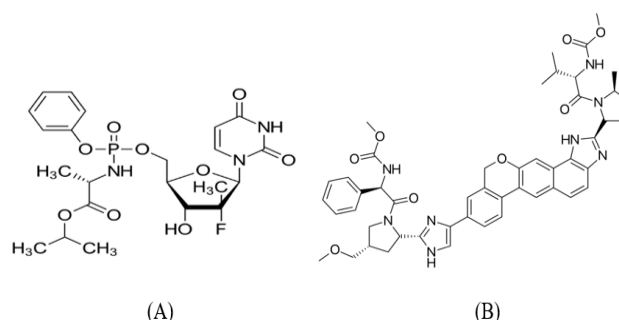


Figure 1: (A) Structure of Sofosbuvir and (B) Structure of Velpatasvir

Chemical name/ Nomenclature / IUPAC Name:

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 3 O 9 P

Molecular Weight: 529.453gm/mole.

pH: 2-7.7

pKa: 9.3



Description of Velpatasvir

Drug category: Velpatasvir is an inhibitor of NS5A in the treatment of hepatitis C

Structure: Structure of Velpatasvir shown in figure 1.

Chemical name/ Nomenclature / IUPAC Name:

Methyl $\{(2S)-1-[(2S,5S)-2-(9-\{2-[(2S,4S)-1-\{(2R)-2-[(methoxycarbonyl)amino]-2-$

phenylacetyl)-4-(methoxymethyl)-2-pyrrolidinyl]-1H-imidazol-4-yl)-1,11-dihydroisochromeno[4,3-b:6,7]naphtho[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl}carbamate.

Molecular Formula: C 49 H 54 N 8 O 8

Molecular Weight: 883.02gm/mole.⁷⁻¹⁰

MATERIALS AND METHODS

Instruments and Glassware:

HPLCWATERS Alliance 2695 separation module, pH meter, weighing machine, volumetric flasks, pipettes, burettes, Digital ultra Sonicator, and Beakers were used for the experiment.

Chemicals and reagents:

Standard Velpatasvir and standard Sofosbuvir were given as gift samples by Sura labs Water, Methanol, and Acetonitrile used were of HPLC grade. VELASOF tablet formulations (Velpatasvir and Sofosbuvir) were purchased from the local pharmacy store

Lambda max Determination

The detection wavelength was selected by dissolving the drug in the mobile phase to get a concentration of 10 μ g/ml for individual and mixed standards. The resulting solution was scanned in the U.V. range from 200-400nm. The overlay spectrum of Velpatasvir and Sofosbuvir was obtained and the isobestic point of Velpatasvir and Sofosbuvir showed absorbance's maxima at 230nm.

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Velpatasvir and Sofosbuvir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Preparation of working standard for U.V Spectroscopy:

Further, pipet out 0.1 ml of Trifluridine and Tipiracil from the above stock solutions into a 10ml volumetric flask separately and dilute up to the mark with methanol it resulting in 10 μ g/ml solution.

Procedure: Scan the prepared working standard in U.V spectrophotometer using blank as methanol. Spectrum showing overlapping of SOF and VEL is shown in figure 2. Optimized chromatogram is shown in figure 3. Table 1 shows the optimised chromatographic conditions. Results of chromatogram are shown in table 2.

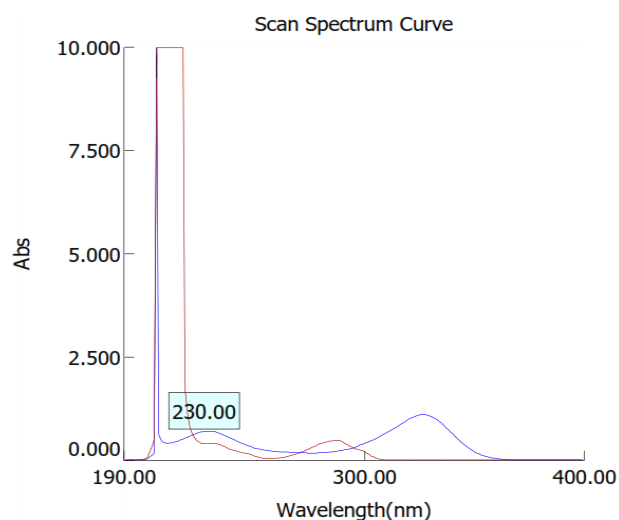


Figure 2: Spectrum showing overlapping of SOF and VEL

Table 1: Optimized Chromatographic Conditions

Parameters	Conditions
Column	Hypersil C18 (4.6 x 150mm, 5 μ m, Make:Waters)
Mobile phase	Water and Acetonitrile (70:30 % v/v)
Flow rate	1 ml per min
Wavelength	230 nm
Injection volume	10 μ l
Run time	7 min

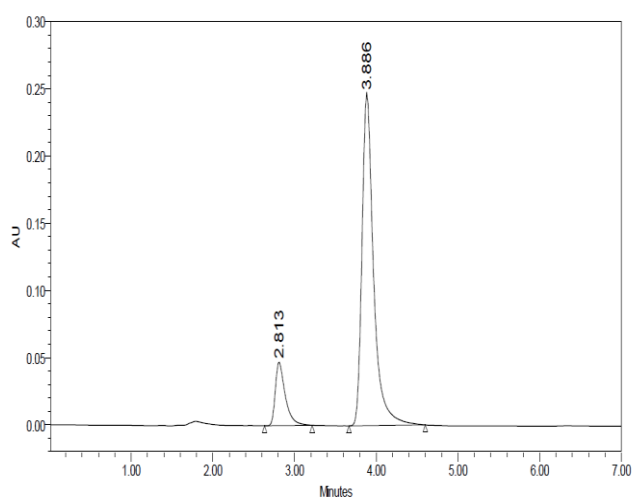


Figure 3: Optimized Chromatogram

Table 2: Peak results for optimized chromatogram

S. No	Peak name	R _t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Velpatasvir	2.813	399693	47206		1.6	2667.2
2	Sofosbuvir	3.886	2431064	247830	4.5	1.5	4142.6

VALIDATION METHODOLOGY

Preparation of mobile phase:

Accurately measured 700 ml (70%) of Acetonitrile and 300 ml of Water (30%) were mixed and degassed in digital ultrasonication for 10 minutes and then filtered through a 0.45 μ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Preparation of standard solution:

Accurately weighed and transferred 10 mg of Velpatasvir and 10mg of Sofosbuvir working standard into 10 ml of clean dry volumetric flasks individually and added about 7ml of Diluents to each volumetric flasks and sonicated to dissolve it completely and volume is made up to the mark with the same solvent. (Stock solution).

Further pipetted 0.3ml and 1.98ml of the above Velpatasvir, and Sofosbuvir stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent and sonicate for 10 minutes.

SPECIFICITY STUDY OF DRUG

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\text{Assay} = \frac{\text{Area of sample solution} \times \text{Wt of std} \times (100 - \text{Std WC})}{\text{Area of standard solution} \times \text{Wt of sample} \times (100 - \text{Sample WC})} \times \text{Std assay}$$

Linearity

Preparation of stock solution:

Accurately weighed 10 combination tablets crushed in mortar and pestle and transferred equivalent to 10 mg of velpatasvir, and Sofosbuvir sample into a 10ml clean dry volumetric flask and added about 7ml of Diluents and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

0.1ml of velpatesvir and 0.66ml of sofosbuvir stock solution were taken in 10ml of volumetric flask diluted up to the mark with diluent to get 10ppm of Velpatasvir & 66.6ppm of Sofosbuvir (10:66.6) In the same manner other concentrations like 20:132, 30: 198, 40:264, 50:330 were prepared.

Procedure: Injected each level into the chromatographic system and measured the peak area. Plot a graph of peak area versus concentration (on the X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

REPEATABILITY:

The standard and sample solutions of 30μg/ml of Velpatasvir, and 19.8μg/ml of Sofosbuvir were injected for five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

INTERMEDIATE PRECISION/ RUGGEDNESS:

The standard and sample solutions containing concentrations were 30μg/ml of Velpatasvir and 19.8μg/ml of Sofosbuvir.

Procedure:

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.

ACCURACY

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Velpatasvir, and Sofosbuvir calculated the individual recovery and mean recovery values. These solutions were filtered through a 0.45μ membrane and then each concentration; three replicate injections were made under the optimized conditions. Recorded the chromatograms and measured the peak responses.

LIMIT OF DETECTION (LOD)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

Where

σ - Standard deviation (SD)

S - Slope



LIMIT OF QUANTIFICATION (LOQ)

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$LOD = 10 \times \frac{\sigma}{S}$$

Where

σ - Standard deviation

S - Slope

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

RESULTS AND DISCUSSION

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantities sofosbuvir and Velpatasvir in drug product.

Assay (Standard):

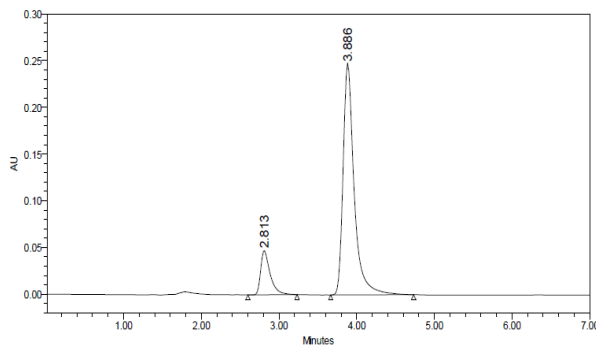


Figure 4: Chromatogram showing assay of standard injection

Assay (Sample):

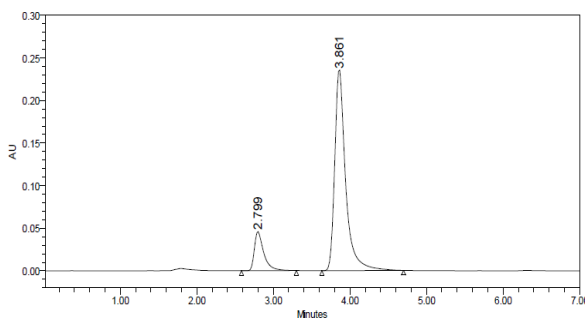


Figure 5: Chromatogram showing assay of sample injection

$$\text{Assay} = \frac{\text{Area of sample solution}}{\text{Area of standard solution}} \times \frac{\text{Wt of std}}{\text{Wt of sample}} \times \frac{(100\text{-Std WC})}{(100\text{-Sample WC})} \times \text{Std assay}$$

Vepatasvir

$$= \frac{386148}{405090} \times \frac{10}{30} \times \frac{630}{0.0284} \times \frac{99.7}{100} \times 1.4201/500 \times 100 = 99.6\%$$

Sofosbuvir

$$= \frac{235771}{239894} \times \frac{10}{19.8} \times \frac{19.8}{0.0284} \times \frac{99.7}{100} \times 1.4201/500 \times 100 = 99.8\%$$

The retention time of Velpatasvir and Sofosbuvir was found to be 2.799mins and 3.863mins respectively. The % purity of Velpatasvir and Sofosbuvir in pharmaceutical dosage form was found to be 99.7%.

Linearity:

The linearity range was found to lie from 10-50 µg/ml of Velpatasvir, 20µg/ml to 100µg/ml of Sofosbuvir and chromatograms are shown below.

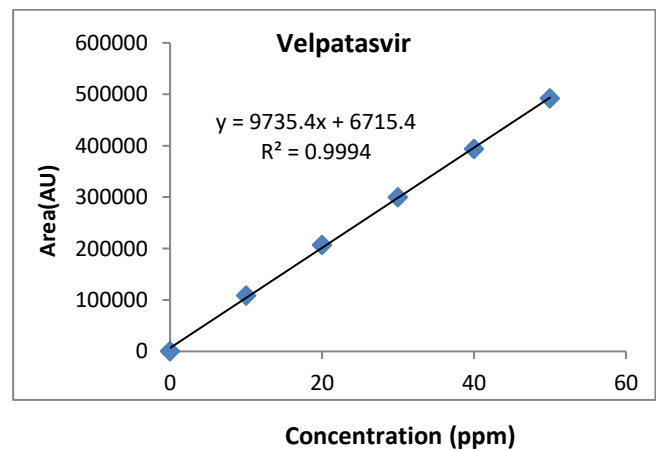


Figure 6: Calibration graph for Velpatasvir

Acceptance Criteria: Correlation coefficient should be not less than 0.999

Linearity Results: (for Sofosbuvir)

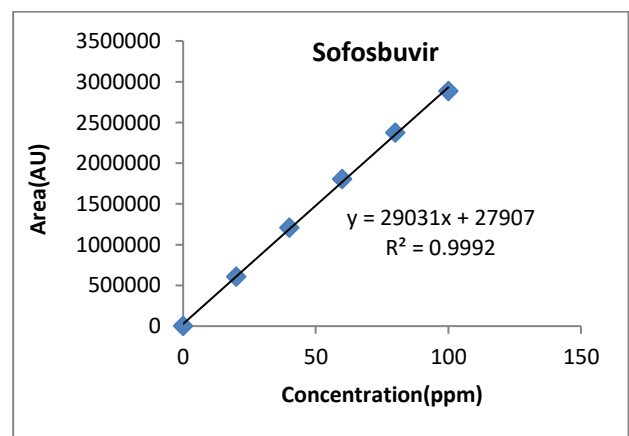


Figure 7: Calibration graph for Sofosbuvir



Acceptance Criteria: Correlation coefficient should be not less than 0.99.

PRECISION

Precision of the method was carried out for both sample and standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Acceptance criteria:

- %RSD for sample should be NMT 2

Intermediate precision/Ruggedness:

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Acceptance Criteria:

The percentage recovery was found to be within the limit (97-103%). The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Velpatasvir:

Result:

$$= 3.3 \times 1921.9 / 9735 = 0.6 \mu\text{g/ml}$$

Sofosbuvir:

Result:

$$= 3.3 \times 32927 / 29031 = 3.7 \mu\text{g/ml}$$

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Velpatasvir:

Result:

$$= 10 \times 1921.9 / 9735 = 1.9 \mu\text{g/ml}$$

Sofosbuvir:

Result:

$$= 10 \times 32927 / 29031 = 11.3 \mu\text{g/ml}$$

Table 3: Summary of validation parameters of Velpatasvir and sofosbuvir

Parameter	Velpatasvir	Sofosbuvir
Retention time	2.813	3.886
Linearity	10-50 $\mu\text{g/ml}$	20-100 $\mu\text{g/ml}$
Correlation coefficient	0.999	0.999
Precision (% RSD)	0.9%	0.42%
Intermediate precision	0.8%	0.7%
LOD	0.6 $\mu\text{g/ml}$	3.7 $\mu\text{g/ml}$
LOQ	1.9 $\mu\text{g/ml}$	11.3 $\mu\text{g/ml}$
Accuracy (% recovery)	99.8%	99.4%
%Assay	99.6%	99.8%

CONCLUSION

High-performance liquid chromatography is at present one of the most sophisticated tools of analysis. The estimation of Velpatasvir and Sofosbuvir was done by RP-HPLC. The mobile phase was optimized with consists of Acetonitrile: Water. Acetonitrile and water mixed in the ratio of 70:30 % v/ v. A Hypersil C 18 column (4.6 x150mm, 5 μm , make: Waters) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1ml/min. the linearity range of Velpatasvir and Sofosbuvir was found to be from 10-50ppm, 20-100ppm respectively. The linear regression coefficient was not more than 0.999, 0.999. The values of % RSD are less than 2% indicating the accuracy and precision of the method. The percentage recovery of Velpatasvir is 99.6 and Sofosbuvir is 99.8%. For routine analytical purposes, it is always necessary to establish methods capable of analysing a huge number of samples in a short time period. RP- The HPLC method is more specific than other analytical techniques. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise, and linear. The method was found to behave in suitable application in the routine determination of Velpatasvir and Sofosbuvir in bulk drug and pharmaceutical dosage forms. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise, and linear.



The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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