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NOVEL RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF SOFOSBUVIR AND VELPATASVIR IN BULK AND COMBINED TABLET DOSAGE FORMS

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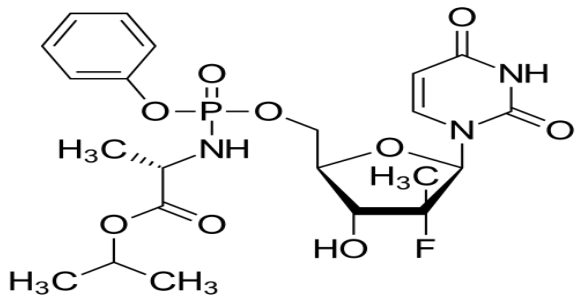
ABSTRACT
A novel reversed phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of Sofosbuvir and Velpatasvir drug product by liquid chromatography. The chromatographic separation was achieved on Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5um) at ambient temperature.The separation achieved employing a mobile phase consists of 0.1%v/v Trifluoroacetic acid in water: Methanol (17:83). The flow rate was 1.5 ml/ minute and ultraviolet detector at 269nm. The average retention time for Sofosbuvir and Velpatasvir found to be 2.169 and 2.799 min. The proposed method was validated for selectivity, precision, linearity, and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 40-120 µg/ml for Sofosbuvir and 10-30µg/ml for Velpatasvir.

KEYWORDS: Sofosbuvir and Velpatasvir, Isocratic, HPLC, Phenyl, Trifluoroacetic acid, Methanol, and Validation.

SOFOSBUVIR

Sofosbuvir 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.



Structure of Sofosbuvir

IUPAC Name: Isopropyl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2, 4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl] methoxy-phenoxy-phosphoryl] amino] propanoate
Molecular formula : C22H29FN3O9P
Molecular Weight : 529.453 g/mol

Solubility: Soluble in Methanol, Acetonitrile, and water.

Pka: 9.3.

Mechanism of action

Sofosbuvir inhibits the hepatitis C NS5B protein. Sofosbuvir appears to have a high barrier to the development of resistance.

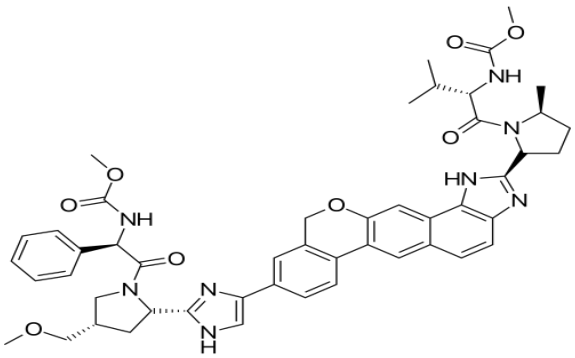
Sofosbuvir is a prodrug. It is metabolized to the active antiviral agent GS-461203 (2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate). GS-461203 serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis. Although sofosbuvir has a 3' hydroxyl group to act as a nucleophile for an incoming NTP, a similar nucleotide analog, 2'-deoxy-2'-α-fluoro-β-C-methylcytidine, is proposed to act as a chain terminator because the 2' methyl group of the nucleotide analog causes a steric clash with an incoming NTP. Sofosbuvir would act in a similar way.

Half-Life

Sofosbuvir has a terminal half-life of 0.4 hours. Route of elimination: Sofosbuvir, as a single agent, has very mild toxicity. The most common adverse reactions are headache and fatigue.

Velpatasvir

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



Structure of Velpatasvir

IUPACName

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':6,7]naphtho[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl}carbamate

Molecular formula: C49H54N8O8
Molecular Weight: 883.02 g·mol−1
Solubility: Soluble in Water, Methanol, and Acetonitrile.
Pka: 3.74

Indication

Used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.

Mechanism of action

The substance blocks NS5A, a protein necessary for hepatitis C virus replication and assembly

Experimental

Equipment

The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5um) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance, Vaccum microfiltration unit with 0.45µ membrane filter was used in the study.

Materials

HPLC-grade Methanol was from qualigens reagents Pvt Ltd. Trifluoroacetic acid (AR grade) was from sd fine chem.

Chromatographic conditions The sample separation was achieved on a Phenyl (Eclipse XDB-Phenyl 250*4.6, 5um) column, aided by mobile phase mixture of 0.1%v/v Trifluoroacetic acid in water: Methanol (17:83). The flow rate was 1.5 ml/ minute and ultraviolet detector at 269nm, that was filtered and degassed prior to use, Injection volume is 20 µl and ambient temperatures.

Preparation of mobile phase

Buffer Preparation

Take accurately 1ml of Trifluoroacetic acid in 1000mL of water.

Mobile phase

Then add 17 volumes of buffer and 83 volumes of Methanol mixed well and sonicated for 10 min.
Diluents: water: Acetonitrile: 50:50 v\v

Preparation of standard stock solution

A 400mg of pure Sofosbuvir and 100mg Velpatasvir were weighed and transferred to 100 ml of volumetric flask and dissolved in the diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution containing 800µg/ml of Sofosbuvir and 200 µg/ml of Velpatasvir. From the above solution 1ml of solution is pipetted out into a 50 ml volumetric flask and volume was made up to mark with methanol to give a solution containing 80µg/ml of Sofosbuvir and 20 µg/ml of Velpatasvir.

Preparation of sample solution

Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 400 mg of Sofosbuvir and 100mg of Velpatasvir sample was weighed and transferred to 100 ml of volumetric flask and dissolved in diluents. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution, 1 ml of solution is pipetted out into a 50 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 80µg/ml of Sofosbuvir and 20 µg/ml of Velpatasvir.

RESULTS AND DISCUSSIONS

Determination Of Working Wavelength (λ max)

10 mg of the Sofosbuvir and Velpatasvir standard drug is taken in a 10 ml volumetric flask and dissolved in Acetonitrile and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made up to the mark with the Acetonitrile to give a concentration of 10 µg/ml. The above-prepared solution is scanned in UV between 200-400 nm using Acetonitrile as blank. The λmax was found to be 269nm After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Trifluoroacetic acid in water: Methanol (17:83). The flow rate was 1.5 ml/ minute brought sharp peaks. The chromatogram was shown in Figure-1.

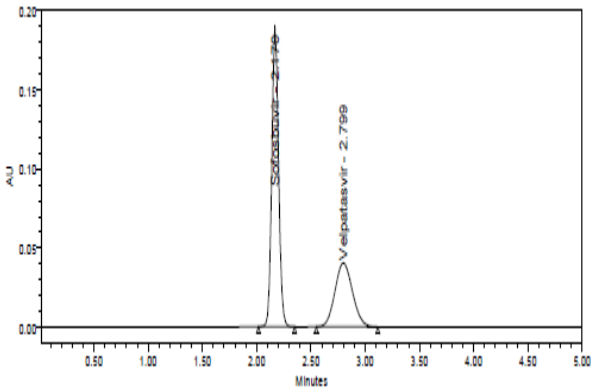


Figure. 1: Chromatogram of Sofosbuvir and Velpatasvir.

Method Validation

Linearity: Linearity was studied by analyzing five standard solutions covering the range of 40-120 µg/ml for Sofosbuvir and 10-30µg/ml for Velpatasvir. From the primary stock solution 0.5ml,0.75ml,1.0ml,1.25ml,1.5 ml of aliquots are pipetted into 50 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 40µg/mL , 60µg/mL ,80µg/mL ,100µg/mL and 120 µg/mL of Sofosbuvir and 10µg/mL , 15µg/mL ,20µg/mL ,25µg/mL and 30 µg/mL Velpatasvir. A calibration curve with concentration versus peak areas was plotted by injecting the above-prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Table No. 1: Linearity data for Sofosbuvir

S.NO	Level	Peak area
1.	50%	**441524**
2.	75%	**655044**
3.	100%	**845542**
4.	125%	**1086603**
5.	150%	**1315728**
Correlation coefficient	Correlation coefficient	**0.9992**

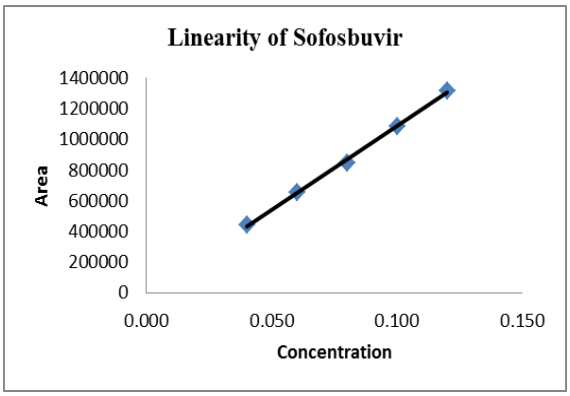


Figure. 2: Linearity plot of Sofosbuvir.

Table No. 2: Linearity data for Velpatasvir.

S. No	level	Area
1.	**50**	**245066**
2.	**75**	**366340**
3.	**100**	**471625**
4.	**125**	**613440**
5.	**150**	**725533**
Correlation coefficient		**0.9992**

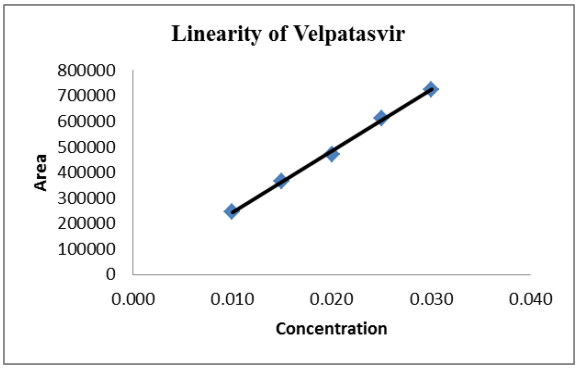


Figure. 3: Linearity plot of Velpatasvir.

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

LOD = 3.3 δ/S ……. (1)
LOQ =10 δ/S …….. (2)

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Table. 3: LOD and LOQ value Calculated from calibration curve.

	Sofosbuvir (mg)	Velpatasvir
LOD	**0.005**	**0.001**
LOQ	**0.015**	**0.004**

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 100 µg/ml of SOFOSBUVIR AND VELPATASVIR without changing the parameter of the proposed chromatographic method.

Table. 4: Summary of peak areas for method precision for Sofosbuvir.

Sample No	Retention time	Peak area	% Assay
1	2.168	841201	99.8
2	2.168	840880	99.2
3	2.169	843664	99.9
4	2.168	841637	99.5
5	2.168	840746	99.4
6	2.168	841520	99.7
Mean			99.6
%RSD			0.27

Table. 5*:* Summary of peak areas for method precision for Velpatasvir.

Sample No	Retention time	Peak area	% Assay
1	2.808	469505	99.0
2	2.808	471353	99.6
3	2.807	471128	99.5
4	2.808	469056	99.9
5	2.809	469051	99.3
6	2.812	469672	99.6
Mean			99.5
%RSD			0.30

Accuracy (recovery study): The accuracy of the method was determined by calculating the recoveries of Sofosbuvir and Velpatasvir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Sofosbuvir and Velpatasvir. The percentage recovery results obtained are listed in Table 4.

Table No.6: Recovery data for Sofosbuvir

S.NO	Accuracy level	injection	Sample area
1	50%	1	99.2
		2	99.3
		3	99.6
2	100%	1	99.8
		2	99.2
		3	99.9
3	150%	1	99.8
		2	99.6
		3	99.7

Table No.7: Recovery data for Velpatasvir

S.NO	Accuracy level	injection	Sample area
1	50%	1	99.9
		2	99.3
		3	99.3
2	100%	1	99.0
		2	99.6
		3	99.5
3	150%	1	99.4
		2	99.3
		3	99.9

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table no.8).

Table No. 8: Results of Robustness data for Sofosbuvir.

Parameter	RT	Area
Decreased flow rate(1.4ml/min)	**2.322**	**905222**
Increased flow rate(1.6ml/min)	**2.037**	**787794**

Table No. 9: Results of Robustness data for Velpatasvir.

Parameter	RT	Area
Decreased flow rate (1.4ml/min)	**3.023**	**505438**
Increased flow rate (1.6ml/min)	**2.643**	**440158**

Table No.10: Validation parameters of evaluated method Sofosbuvir.

S. No	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 5793 1.08 2.169 1.55	 Not less than 2000 Not more than 2 Not more than 2
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.13	Not more than 2.0%
4	Linearity parameter Slope Intercept Correlation coefficient(r^2)	40-120 mcg/ml 0.9992	 Not less than 0.999
5	Accuracy (Mean % recovery) 50% 100% 150%	 99.4 99.7 99.7	 97 - 103%
6	Robustness	All the system suitability parameters are within the limits.	

*RSD = Relative standard deviation

Table No.11: Validation Parameters of the evaluated method of VELPATASVIR:

S. No	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	 3650 1.10 2.799 1.67	 Not less than 3000 Not more than 2 Not more than 2
2	Specificity	Specific	Specific
3	Method precision (%RSD)	0.22	Not more than 2.0%
4	Linearity parameter Slope Intercept Correlation coefficient(r^2)	10-30 mcg/ml 0.9992	 Not less than 0.999
5	Accuracy (Mean % recovery) 50% 100% 150%	 99.5 99.4 99.5	 97 - 103%
6	Robustness	All the system suitability parameters are within the limits.	

CONCLUSION

From the experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of SOFOSBUVIR AND VELPATASVIR was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost-effective and it can be effectively applied for routine analysis in

research institutions, quality control department and approved testing laboratories.

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