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STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR IN DRUG PRODUCT

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ABSTRACT
The new Analytical method was developed for the estimation of Sofosbuvir and Velpatasvir in drug product by RP-HPLC. The chromatographic separation was achieved by ECLIPSE XDB Phenyl Column 250 mm*4.6,5um at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Formic acid :Acetonitrile: Methanol(30:40:30) The flow rate was 1.2ml/ minute and UV-detection was at 267nm. The average retention time for Sofosbuvir and Velpatasvir was found to be 2.671 min and 3.425 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 40-120µg/ml for Sofosbuvir and 10-30µg/ml of Velpatasvir.

KEY WORDS: Sofosbuvir and Velpatasvir, HPLC, Eclipse XDB phenyl Formic acid, Acetonitrile, Methanol, and validation.

SOFOSBUVIR
Sofosbuvir(Isopropyl(2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluro-3-hydroxy-4methyltetrahydrofuran2yl]methoxyphenoxyphosphoryl] aminopropanoate. It is slightly soluble in water. **Sofosbuvir** having the pKa value is 9.38 **Sofosbuvir** is an inhibitor of the HCV NS5B RNA polymerase, which undergoes intracellular metabolism to form uridine analogue triphosphate and inhibits the viral replication by incorporating into HCV RNA and acts as a chain terminator.

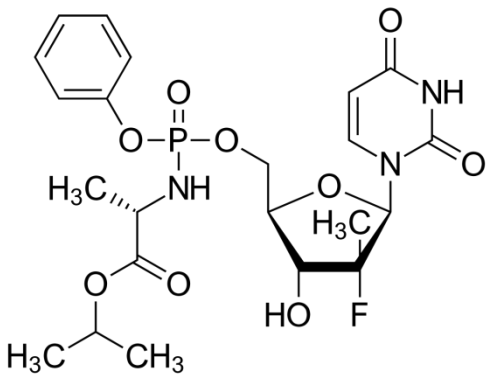

Fig 1: Chemical structure of sofosbuvir.

VELPATASVIR
Velpatasvir(Methyl**{(2**S)-1-[(2S,5S)-2-(9-{2-{(2S,4S)-1-{(2R)-2-[(methoxymethyl)-2-pyrrolidinyl]-1H-imidazol-4-yl}-1,11-dihydroisochromeno[4',3'.6,7]naphtha[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl}carbamate. It is soluble in water, methanol and Acetonitrile**. Velpatasvir** is an inhibitor of HCV NS5A protein, which blocks the action of the protein and inhibits the viral replication.

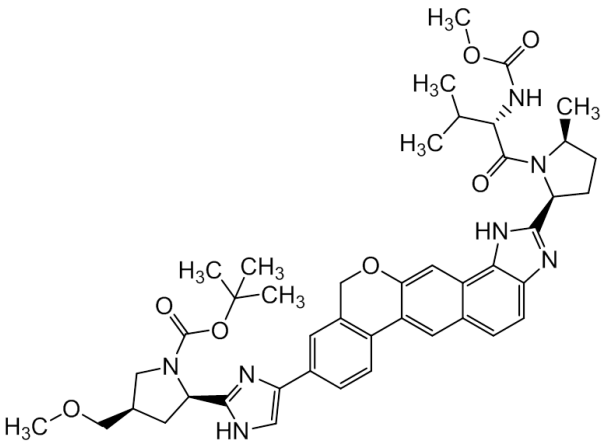

Fig 2: Chemical structure of velpatasvir.

Sofosbuvir and **Velpatasvir** is a fixed-dose combination for the treatment of **Hepatitis C.** It is more than 90% effective for **Hepatitis C** genotypes one through six. It also works for **Hepatitis C** in those who also have **Cirrhosis or HIV/AIDS.**

Sofosbuvir/Velpatasvir was approved for medical use in the united states in 2016. It is on the **World Health Organization's list of essential medicines,** the most effective and safe medicines needed in a **health system.** As of 2017, in the United States, a course of treatment costs about 74,800 USD while in the developing world it costs about 900 USD.

The combination of drug is available in tablet dosage form.Common side effects include Headache, fatigue, low blood iron, nausea, insomnia, diarrhea, weakness, rash, depression A very few analytical methods were reported in the literature for the estimation of Sofosbuvir and Velpatasvir in plasma and bioequivalence studies in presence of its metabolites in single and in combination with other drugs. Hence the aim of this research works to develop a simple, rapid & robust stability indicating a method for the estimation of **Sofosbuvir and velpatasvir** in pharmaceutical formulations by RP-HPLC.

EXPERIMENTAL

2. MATERIALS AND METHODS

Equipment: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase Eclipse XDB Phenyl column 250mm*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. Formic acid (AR grade) was from sd fine chem. Sodium Hydroxide is from SDFCL and HCl is from QUALIGENS.

Chromatographic conditions The sample separation was achieved on **a (5 µ, 250 mm X 4.6** mm i.d.)Eclipse XDB Phenyl Column, aided by mobile phase mixture of 0.1%v/v Formic acid Acetonitrile: Methanol (30:40:30)The flow rate was 1.2ml/ minute and ultraviolet detector at 269nm, that was filtered and degassed prior to use, Injection volume is 10 µl and ambient temperatures.

Preparation of mobile phase

Buffer Preparation: Taken accurately 1ml of formic acid in 1000mL of water.

Mobile phase: 0.1% Formic acid 300ml and 400 ml of Acetonitrile and 300ml of Methanol are taken accurately, stirred well and sonicated.

Diluents: Water: Acetonitrile: 50:50 v\v.

Preparation of standard stock solution: A 40 mg of pure Sofosbuvir and 10mg of Velpatasvir were weighed and transferred to 50 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. From the above solution 1ml of solution is pipetted out into a 10 ml volumetric flask and volume was made up to mark with water to give a solution containing 80ug/ml of Sofosbuvir and 20µg/ml of Velpatasvir.

Preparation of sample solution: Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 40mg of Sofosbuvir and 10mg Velpatasvir sample was weighed and transferred to 50 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. From the above solution, 1 ml of solution is pipetted out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 80 µg/ml of Sofosbuvir and 20ug/ml of Velpatasvir.

Forced degradation studies

The study was intended to ensure the effective separation of Sofosbuvir and Velpatasvir and its degradation peaks of formulation ingredients at the retention time of Sofosbuvir and Velpatasvir. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the methods.

Acid degradation Forced degradation in acidic media was performed by keeping the standard solution in contact with 0.1 N HCl for 3h at room temperature. After 3h the solution was neutralized with 0.1 N NaOH and solution was diluted up to 10 ml with mobile phase. Dilution was done to achieve the appropriate concentration 80 µg/ml of Sofosbuvir and 20 µg/ml Velpatasvir**.**

Alkaline degradation Forced degradation in basic media was performed by keeping the standard solution in contact with 0.1 N NaOH for 3h at room temperature. After 3h the solution was neutralized with 0.1 N HCl and solution was diluted up to 10 ml with mobile phase. Dilution was done to achieve the appropriate concentration 80 µg/ml of Sofosbuvir and 20 µg/ml Velpatasvir**.**

Oxidation degradation Forced degradation in 5% H2O2 media was performed by keeping the standard solution in contact with 5% H2O2 for 3h at room temperature. After 3h, the solution was diluted with mobile phase up to 10 ml to achieve the appropriate concentration 80 µg/ml of Sofosbuvir and 20 µg/ml Velpatasvir.

Thermal degradation Sample solution was exposed to a temperature of 105°c for 24h in an oven. After 24h, the solution was diluted with mobile phase up to 10 ml. From this solution, dilution was done to achieve the

appropriate concentration 80 µg/ml of Sofosbuvir and 20 µg/ml Velpatasvir**.**

Photolytic degradation Sample solution was exposed to the sunlight for 24h. After 24h. The solution was diluted with mobile phase up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration 80 µg/ml of Sofosbuvir and 20 µg/ml Velpatasvir.

RESULTS AND DISCUSSIONS

In order to validate an effective method for analysis of drug (Sofosbuvir and Velpatasvir) in a pharmaceutical product, preliminary tests were performed with the target to pick out adequate and optimum conditions. Parameters, like an ideal mobile phase and their proportions, wavelength detection, standard concentration solutions & optimized pH were thoroughly studied and therefore the flow rate of 1.2 mL/min was chosen after preliminary tests. Developed a stability indicating assay method was optimized for the quantitative determination of Sofosbuvir and Velpatasvir in pharmaceutical formulations. Pure drug solution of **Epclusa** and also the degraded products were injected into the HPLC system and run in several solvent systems at first Formic acid Acetonitrile and Methanol in varying ratios were tried on Eclipse XDB phenyl (250mm length*4.6,5um)column. Finally, the mobile phase made up of 0.1%Formic acid: Acetonitrile: methanol in the ratio of 30:40:30 (v/v/v) at a rate of flow of 1.2 mL/min. UV detection at a wavelength of 269nm provided the sharp peaks of sofosbuvir and Velpatasvir at an Rt of 2.671 min and 3.425 min.The chromatogram was shown in the figure 1.

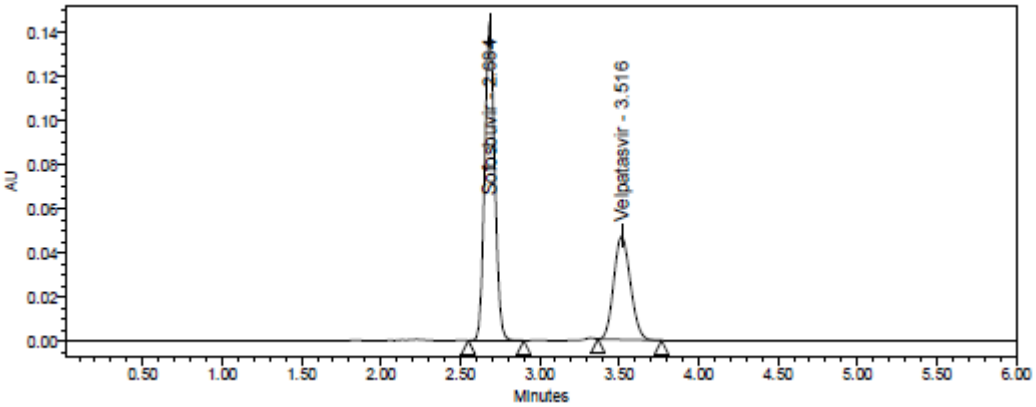

Figure: 3 Chromatogram of Sofosbuvir and Velpatasvir.

METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 80-120ug/ml of Sofosbuvir and 10-30ug/ml of Velpatasvir. From the primary stock solution 0.5ml,0.75ml,1.0ml,1.25ml,1.5 ml of aliquots are pipette into 10 ml volumetric flasks and made mark with the water to give a concentrations of 40ug/ml, 60ug/ml, 80ug/ml, 100ug/ml, 120ug/ml of Sofosbuvir and 10ug/ml, 15ug/ml, 20ug/ml, 25ug/ml, 30ug/ml of Velpatasvir respectively.

A calibration curve with concentration verses 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 of Sofosbuvir.

Level	Concentration of Sofosbuvir(mg/mL)	Sofosbuvir Peak area
50%	0.04	311618
75%	0.06	464902
100%	0.08	613993
125%	0.10	765378
150%	0.12	924747

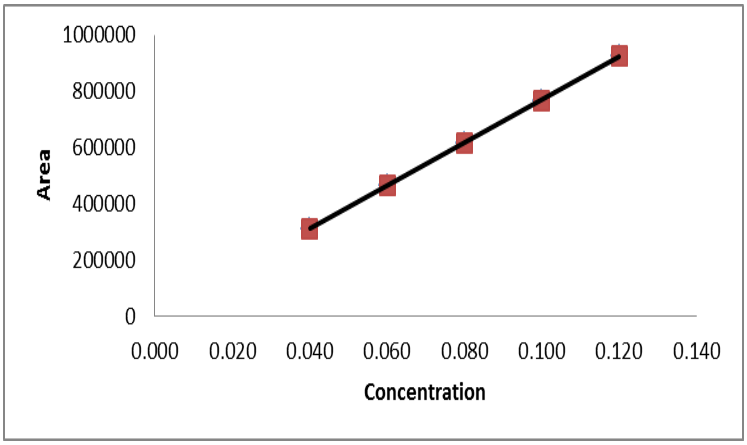

Figure 4: Linearity (calibration) curve of Sofosbuvir.

Table No: 2 : Linearity data of Velpatasvir.

Level	Concentration of Velpatasvir (mg/mL)	Peak area of Velpatasvir
50%	0.01	163036
75%	0.015	250909
100%	0.02	329186
125%	0.025	410352
150%	0.3	494035

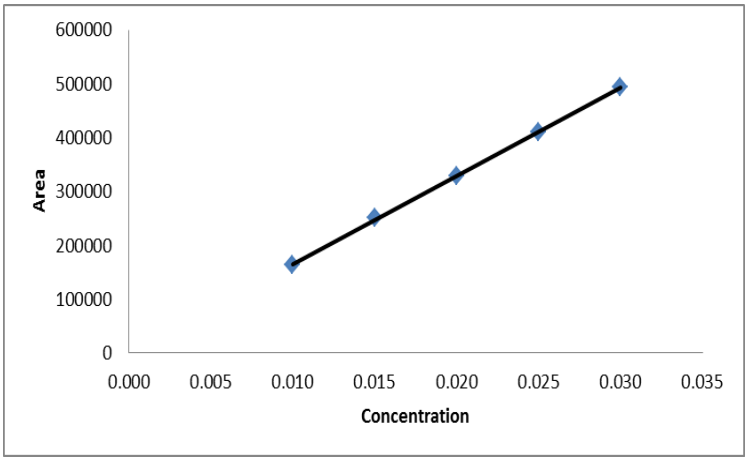

Figure 5: Linearity (calibration) curve of Velpatasvir.

RESULT

A linear relationship between peak areas versus concentrations was observed for Sofosbuvir and Velpatasvir in the range of 50% to 150% of nominal concentration. The correlation coefficient was 0.9999 and 0.9999 for both Sofosbuvir and Velpatasvir which prove that the method is linear in the range of 50% to 150%.

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.

$$LOD = 3.3\ \sigma / S \quad \text{..... (1)}$$

$$LOQ = 10\ \sigma / S \quad \text{..... (2)}$$

Where,

σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve

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

Table no.3: LOD and LOQ values Calculated from calibration curve.

	Sofosbuvir mg	Velpatasvir mg
LOD	0.0014	0.0005
LOQ	0.0041	0.0015

Method precision (repeatability)

The precision of the method was checked by repeated preparation(n=6) of 80ug/ml of Sofosbuvir and 20 µg/ml of Velpatasvir without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times.

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

Sample No	Retention time	Peak area	% Assay
1	2.668	671224	100.2
2	2.667	683844	101.2
3	2.668	675399	100.3
4	2.668	674476	99.8
5	2.669	681241	101.1
6	2.668	668058	99.0
Mean	2.668	675707	100.3
%RSD	0.001	5951	0.82

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

Sample No	Retention time	Peak area	% Assay
1	3.680	380790	99.5
2	3.679	383429	99.3
3	3.679	383236	99.6
4	3.680	380376	99.6
5	3.682	388261	100.9
6	3.681	382551	100.5
Mean	3.680	383107	99.9
%RSD	0.001	2822	0.64

RESULT

Results of variability were summarized in the above table. Percentage relative standard deviation (%RSD) was found to be less than 2.0% which proves that method is precise.

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Valsartan and Sacubitril by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Valsartan and Sacubitril. The percentage recovery results obtained are listed in Table 6 &7.

Table No.6: Recovery data of Sofosbuvir.

LEVEL	S.No	%Recovery of Sofosbuvir	Average
50	1	101.0	100.20%
	2	98.4	
	3	98.7	
100	1	100.2	100.50%
	2	101.2	
	3	100.3	
150	1	101.2	101.10%
	2	101.1	
	3	101.1	

Table No.7: Recovery data of Velpatasvir.

LEVEL	S.No	%Recovery of Velpatasvir	Average
50	1	101.0	99.40%
	2	98.4	
	3	98.7	
100	1	99.5	99.45%
	2	99.3	
	3	99.6	
150	1	101.1	100.60%
	2	99.8	
	3	100.8	

RESULT

Results of accuracy study are presented in the above table. All the results indicate that the method is highly accurate**.**

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&9).

Table No.8: Results of Sofosbuvir.

parameter	Rt of Sofosbuvir	Area of Sofosbuvir
Decreased flow rate (1.0ml/min)	2.916	673028
Increased flow rate (1.4ml/min)	2.479	559606
Wave Length 267nm	2.683	603908
Wave Length 271nm	2.684	605852

Table No.9: Results of of Velpatasvir.

parameter	Rt of Velpatasvir	Area of Velpatasvir
Decreased flow rate (1.0ml/min)	3.824	370055
Increased flow rate (1.4ml/min)	3.243	305934
Wave Length 267nm	3.471	335782
Wave Length 271nm	3.473	336413

RESULT

The results of Robustness of the present method had shown that changes are not significant we can say that the method is Robust.

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table no.10&11.

Table No.10: Results of Sofosbuvir.

		%Assay	%RSD
Analyst-1	**SOFOSBUVIR**	**100.2**	0.69
Analyst-2		**101.2**	

Table No.11: Results of Velpatasvir.

		%Assay	%RSD
Analyst-1	**VELPATASVIR**	**99.5**	0.13
Analyst-2		**99.3**	

RESULT

The %RSD assay values between two analysts were calculated, this indicates the method was rugged.

Table 12: Summary of Degradation data for Sofosbuvir and Velpatasvir.

S.No	Degradation Condition	Sofosbuvir		Velpatasvir	
		Degradation (%)	Active drug present after degradation (%)	Degradation (%)	Active drug present after degradation (%)
1	**Acid degradation 0.1N HCl 3h**	1.62	98.38	5.38	94.62
2	**Base degradation 0.1N NaOH 3h**	1.84	98.16	7.86	92.14
3	**Peroxide degradation**	0.99	99.01	12.65	87.35

	5% H2O2 3h				
4	**Thermal degradation 24 h**	3.62	96.38	1.29	98.71
5	**Photolytic degradation 24h**	1.04	98.96	1.64	98.36

Table No.13: Summary of Sofosbuvir.

S.No	Parameter	Result	Acceptence Criteria
1	System suitability		
	Theoretical plates	8532	Not less than 3000
	Asymmetry	1.09	Not more than 2.0
	Retention time	2.684	
	%RSD	1.08	Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.82	Not more than 2.0%
4	Linearity Range(mcg/ml)	40.0- 120.0	
	Correlation coefficient(r^2)	0.9999	Not less than 0.9990
5	Accuracy		
	(Mean % recovery)		97 - 103%
	50%	100.2	
	100%	100.5	
	150%	101.1	
6	Robustness	All the system suitability parameters are within the limits.	

Table No.14: Summary of Velpatasvir.

S.No	Parameter	Result	Acceptence Criteria
1	System suitability		
	Theoretical plates	5228	Not less than 3000
	Asymmetry	1.09	Not more than 2.0
	Retention time	3.476	
	%RSD	1.01	Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.65	Not more than 2.0%
4	Linearity Range(mcg/ml)	10.0- 30.0	
	Correlation coefficient(r^2)	0.9999	Not less than 0.9990
5	Accuracy		
	(Mean % recovery)		97 - 103%
	50%	99.4	
	100%	99.5	
	150%	100.6	
6	Robustness	All the system suitability parameters are within the limits.	

*RSD = Relative standard deviation

CONCLUSION

From the above experimental results it was concluded that, 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 in industries, approved testing laboratories.

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