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Research Article

Analytical Method Development and Validation for Velpatasvir and Sofosbuvir in Combined Dosage Form by RP-HPLC

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Abstract
High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Velpatasvir and Sofosbuvirwas done by RP-HPLC. The Phosphate buffer was p^{H}3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5μm) or equivalent chemically bonded to porous silica particles was used as stationary phase.The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Velpatasvir and Sofosbuvir were found to be from 100-500 μg/mlof Velpatasvirand 1-5μg/ml ofSofosbuvir. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Velpatasvirand Sofosbuvir. LOD and LOQ were found to be within limit.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.

Keywords: Methanol: Phosphate buffer, Inertsil C18 column, Velpatasvir and Sofosbuvir, RP-HPLC.

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1. Introduction

Velpatasvir is a Direct-Acting Antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients[8] . Velpatasvir

acts as a defective substrate for NS5A (Non-Structural Protein 5A), a non-enzymatic viral protein that plays a key role in Hepatitis C Virus replication, assembly, and modulation of host immune responses. Treatment options for chronic Hepatitis C have advanced significantly since 2011, with the development of Direct Acting Antivirals (DAAs) such as velpatasvir.

Sofosbuvir (trade name Sovaldi) is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients. Treatment options for chronic Hepatitis C have advanced significantly since 2011, with the development of Direct Acting Antivirals (DAAs) such as sofosbuvir. As a prodrug nucleotide analog, Sofosbuvir is metabolized into its active form as the antiviral agent 2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate (also known as GS-461203), which acts as a defective substrate for NS5B (non-structural protein 5B) .

NS5B, an RNA-dependent RNA polymerase, is essential for the transcription of Hepatitis C viral RNA and for its high replicative rate and genetic diversity. Sofosbuvir and other direct acting antivirals are therefore very potent options for the treatment of Hepatitis C, as they exhibit a high barrier to the development of resistance. This is an important advantage relative to HCV drugs that target other viral enzymes such as the protease, for which rapid development of resistance has proven to be an important cause of therapeutic failure.

2. Methodology

Instrumentation

TheinstrumentusedwasHPLCAlliance Waters model No. 2695 separation module.2487 UV detector,Software-EMpower. The stationaryphase used was Agilent C18 column (4.6×150mm)5µ. Semi micro balance–ModelnumberSartorius ME235P,Sonicator(Enertech)-SE60US, pHmeterLab India, UV/VIS spectrophotometer UV3000 Lab India Software-UVWin5

Materials and reagents

Velpatasvir and Sofosbuvirwere gift samples provided by Dr. Reddy's Laboratories Hyderabad, Potassium dihydrogenorthophosphate was supplied by finer chemicals, Methanol, Water were supplied by Merck Acetonitrile for HPLC was supplied by Molychem.

Method development

Five trials were made by changing the mobile phase ratios and solventsWater: Methanol (50:50%v/v), Phosphate buffer (0.05m) pH 4.0: Methanol (40:60%v/v), Phosphate buffer (0.05m) pH 4.0: Methanol (40:60%v/v), Phosphate buffer (0.05M) pH 3.6: ACN (40:60%v/v), Phosphate buffer pH 3.0: Methanol (30:70%v/v) . Finally, the mobile phase optimized mobile phase ratio was 30% buffer 70% Methanol.

Chromatographic conditions

The Phosphate buffer was p^H3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase.The detection was carried out using UV detector at 260 nm.

3. Results and Discussion

Validation Parameters:

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

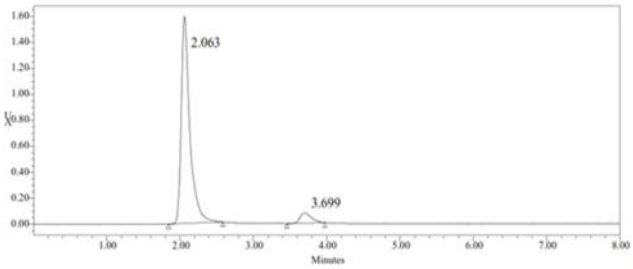

Figure 1 chromatogram for standard injection

Table 1 Results of method precession for Velpatasvir

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 2 Results of method precession for Sofosbuvir

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate Precession (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.

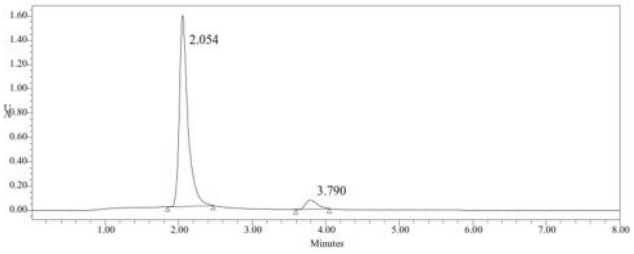

Figure 2: chromatogram for sample injectiocn

Table 3 Results of Intermediate precision for Velpatasvir

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 4 Results of Intermediate precision for Sofosbuvir

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Acceptance criteria:

- %RSD of five 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.

Table 5 accuracy (recovery) data for Velpatasvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 6 accuracy (recovery) data for Sofosbuvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.
- 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

Linearity: The linearity range was found to lie from 100µg/ml to 500µg/ml of Velpatasvir, 5µg/ml to 25µg/ml of Sofosbuvirand chromatograms are shown below.

Table 7 Area of different concentration of Velpatasvir

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.997

Table 8 Area of different concentration of Sofosbuvir

S.No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

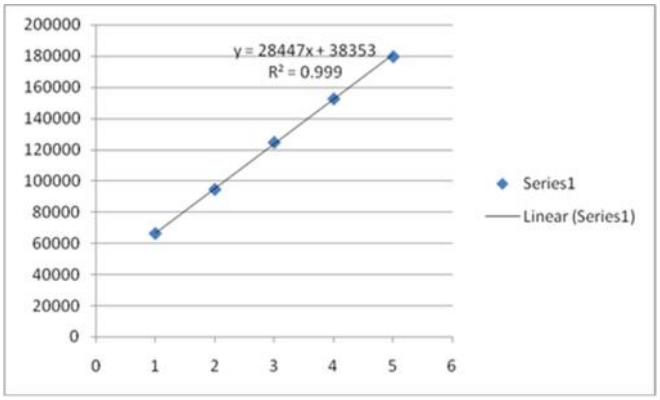

Figure 3 calibration graph for Velpatasvir at 225 nm

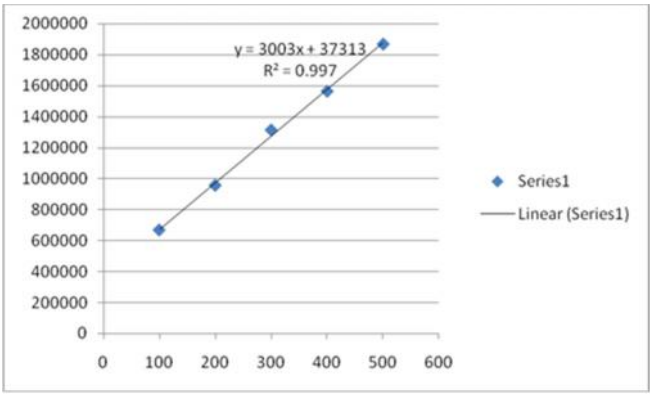

Figure 4 calibration graph for Sofosbuvir at 225 nm

Table 9 Analytical performance parameters of Velpatasvir and Sofosbuvir

Parameters	Velpatasvir	Sofosbuvir
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria:

- Correlation coefficient (R^2) should not be less than 0.999
- The correlation coefficient obtained was 0.999 which is in the acceptance limit. The linearity was established in the range of 10% to 50% ofVelpatasvirand 5% to 25% of Sofosbuvir.

Limit of Detection forVelpatasvir and Sofosbuvir: The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Table 10 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Velpatasvir	52	152	2.9
Sofosbuvir	52	156	3

- Signal to noise ratio shall be 3 for LOD solution
- The result obtained is within the limit.

Limit of Quantification (LOQ): The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Table 11 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Velpatasvir	52	522	10.03
Sofosbuvir	52	524	10.1

- Signal to noise ratio shall be 10 for LOQ solution
- The result obtained is within the limit.

Robustness: The standard and samples of Velpatasvir and Sofosbuvir were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 12 Flow Rate (ml/min) data forVelpatasvir

S.No		System Suitability Results	
	Flow Rate (ml/min)	USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 13 flow rate (ml/min) data for Sofosbuvir

S.No	Flow Rate (ml/min)	System Suitability Results USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 14 Change in Organic Composition in the Mobile Phase for Velpatasvir

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 15 Change in Organic Composition in the Mobile Phase for Sofosbuvir

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

Acceptance criteria:

- Percentage RSD should be below 2.
- The %RSD obtained for change of flow rate, variation in mobile phase was found to be below 1, which is within the acceptance criteria. Hence the method is robust

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Velpatasvir and Sofosbuvir was done by RP-HPLC. The Phosphate buffer was p^H3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Velpatasvir and Sofosbuvir were found to be from 100-500 µg/ml of Velpatasvir and 1-5µg/ml of Sofosbuvir. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Velpatasvir and Sofosbuvir. LOD and LOQ were found to be within limit.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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