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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR BY HPLC

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ABSTRACT

A specific, accurate, precise and reproducible HPLC method has been developed and subsequently validated for Sofosbuvir and Velpatasvir in pure API and tablet dosage forms. The proposed HPLC method utilizes X-Terra C18 column (150 mm - 4.6 mm) and mobile phase consisting of acetonitrile and water in the ratio of (75:25 v/v) , pH 5.00 at a flow rate of 1.0 ml/min. The method was validated in terms of accuracy (% recovery 99.6% and99.5), precision (%RSD 0.04 and 0.05), linearity, limits of detection (3 ng/ml), limits of quantitation (10ng/ml), assay (100.5%), and robustness. This method has been successively applied to Pharmaceutical formulation.

INTRODUCTION

Sofosbuvir (tradename 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). Velpatasvir is a complex organic heteropentacyclic compound that is a hepatitis C virus nonstructural protein 5A inhibitor used in combination with Sofosbuvir (under the brand name Epclusa) for treatment of patients with chronic hepatitis C of all six major genotypes. It has a role as an antiviral drug and a hepatitis C virus nonstructural protein 5A inhibitor.

MATERIALS AND METHOD

Chemicals and Reagents: Reference standard of were Sofosbuvir and Velpatasvir gifted by Hetero laboratory. The formulation used for assay is Sofosbuvir and Velpatasvir and the Solvents used in this method were acetonitrile, methanol, sodium dihydrogen phosphate and water of HPLC grade.

Instrumentation: Simultaneous estimation for method development and validation was carried out by HPLC with PDA detector module with auto-sampler. Column used was X-Terra C18 column (150*4.6 * 5µm), and data recorded using LC Solutions software.

Mobile Phase: ACN: Water (75:25 v/v) pH 5.00 at a flow rate of 1.0 ml/min.

Diluent: Mobile phase is used as a diluent.

Preparation of stock solution: 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard was taken into a 10 ml clean

volumetric flask to this add 7 ml of mobile phase, dissolve it completely and make upto the mark with mobile phase. Take 1.5 ml from the stock solutions into a 10ml volumetric flask and dilute up to the mark with mobile phase.

Sample Solution Preparation: Take an equivalent tablet powder to 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard was taken into a 10 ml clean volumetric flask to this add 7 ml of mobile phase, dissolve it completely and make upto the mark with mobile phase. Take 1.5 ml from the stock solutions into a 10ml volumetric flask and dilute up to the mark with mobile phase.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration): 50 mg of Velpatasvir and 200 mg of Sofosbuvir working standard was taken into a 10 ml clean volumetric flask to this add 7 ml of mobile phase, dissolve it completely and make upto the mark with mobile phase. Take 1.5 ml from the stock solutions into a 10ml volumetric flask and dilute up to the mark with mobile phase.

For preparation of 100% solution (With respect to target Assay concentration): 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard was taken into a 10 ml clean volumetric flask to this add 7 ml of mobile phase, dissolve it completely and make upto the mark with mobile phase. Take 1.5 ml from the stock solutions into a 10ml volumetric flask and dilute up to the mark with mobile phase.

For preparation of 150% solution (With respect to target Assay concentration): 150 mg of Velpatasvir and 600 mg of Sofosbuvir working standard was taken into a 10 ml clean volumetric flask to this add 7 ml of mobile phase, dissolve it completely and make upto the mark with mobile phase. Take 1.5 ml from the stock solutions into a 10ml volumetric flask and dilute up to the mark with mobile phase.

DEGRADATION STUDIES

Preparation of stock: Accurately weigh and transfer 100 mg of Velpatasvir working standard into a 10 ml clean dry 100 mg of Velpatasvir and 400 mg of Sofosbuvir working standard was taken into a 10 ml clean volumetric flask to this add 7 ml of mobile phase, dissolve it completely and make upto the mark with mobile phase.

Hydrolytic degradation under acidic condition: Take 1.5 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 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent.

Hydrolytic degradation under alkaline condition: Take 1.5 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluents.

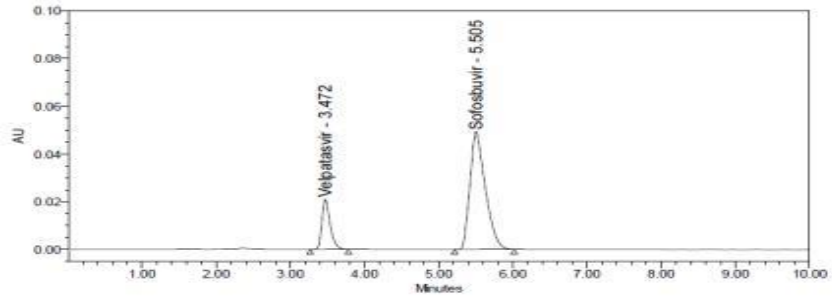
Thermal induced degradation: Velpatasvir and Sofosbuvir sample was taken in petri dish and kept in hot air oven at 110^0 C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

Oxidative degradation: Take 1.5 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.

Photo degradation: Pipette 0.3 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.

SUMMARY AND CONCLUSION

The estimation of Velpatasvir and Sofosbuvir was done by RP-HPLC. The assay of Velpatasvir and Sofosbuvir was performed with tablets and the percentage assay was found to be 100.08 and 99.97 which shows that the method is useful for routine analysis. The linearity of Velpatasvir and Sofosbuvir was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.1 for Velpatasvir and Sofosbuvir which shows that the method is precise.

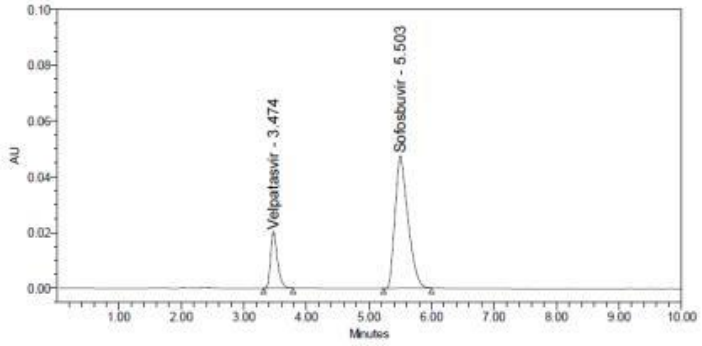


Results of system suitability parameters

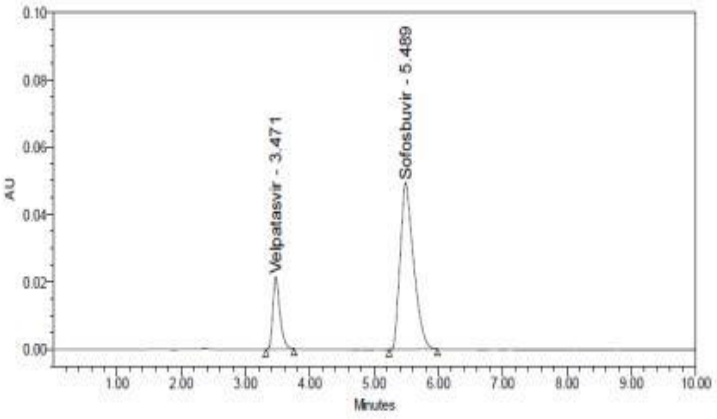
S. No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Velpatasvir	3.472	161234	21536	-	1.36	4822.40
2	Sofosbuvir	5.505	747339	36534	2.81	1.26	4722.40

VALIDATION PARAMETERS

Chromatogram for Standard



Chromatogram for Sample



Results of Assay for Velpatasvir and Sofosbuvir

	Label Claim (mg)	**% Assay**
Velpatasvir	100	100.08
Sofosbuvir	400	99.97

LINEARITY

Linearity Results: (for Velpatasvir)

S.No	Linearity level	Concentration	Area
1	I	50	53953
2	II	100	110011
3	III	150	166601
4	IV	200	227887
5	V	250	285840
Correlation Coefficient			0.999

Linearity Results: (for Sofosbuvir)

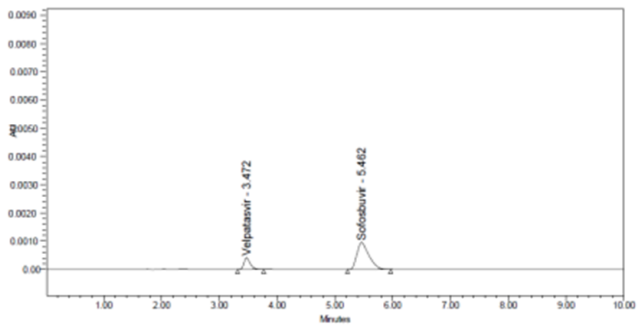
S.No	Linearity level	Concentration	Area
1	I	200	243401
2	II	400	488042
3	III	600	744612
4	IV	800	1013904
5	V	1000	1300811
Correlation Coefficient			0.999

PRECISION

Results showing values of Velpatasvir and Sofosbuvir

Injection	Area for Velpatasvir	Area for Sofosbuvir
Injection-1	161345	747339
Injection-2	161232	746432
Injection-3	161671	747131
Injection-4	161999	747399
Injection-5	162898	747018
Injection-6	164679	747649
Average	162304.0	747161.3
Standard Deviation	1308.1	419.3
%RSD	0.8	0.1

LIMIT OF DETECTION FOR VELPATASVIR AND SOFOSBUVIR



Chromatogram of Velpatasvir, Sofosbuvir showing LOD

Results of LOD

Drug name	**Baseline noise(µV)**	**Signal obtained (µV)**	**S/N ratio**
Velpatasvir	58	174	3.00
Sofosbuvir	58	173	2.98

Results

Sample Name	Velpatasvir		Sofosbuvir	
	Area	% Degraded	Area	% Degraded
Standard	161608.8		747503	
Acid	153252	5.17	719259	3.78
Base	153532	5.00	719321	3.77
Peroxide	152239	5.80	704978	5.69
Thermal	157552	2.51	714851	4.37
Photo degradation	156452	3.19	714789	4.38

The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.2 and 0.2 for Velpatasvir and Sofosbuvir which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.08% and 100.02% for Velpatasvir and Sofosbuvir. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ are 3 and 10. The LOD and LOQ for Velpatasvir were found to be 3.0 and 9.98 and LOD and LOQ for Sofosbuvir was found to be 2.98 and 10.00. The method which is developed is used for routine analysis of drugs and it is validated according to the ICH.

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