September - October 2018; 7(5): 3092-3099

International Journal of Research and Development in Pharmacy & Life Science
An International open access peer reviewed journal
ISSN (P): 2393-932X, ISSN (E): 2278-0238
Journal homepage: http://ijrdpl.com

Original Article

Simultaneous Estimation of Sofosbuvir and Velpatasvir Tablets by RP- HPLC Method

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Keywords: Sofosbuvir, Velpatasvir, Retention time, Method development, Method Validation

Article Information:

Received: August 10, 2018;
Revised: August 25, 2018;
Accepted: September 15, 2018
Available online on:
01.11.2018@http://ijrdpl.com

http://dx.doi.org/10.21276/IJRDPL.2278-0238.2018.7(5).3092-3099

ABSTRACT: OBJECTIVES: To develop a simple, accurate, precise method was developed for the simultaneous estimation of the Sofosbuvir and Velpatasvir in tablet dosage form by Reverse Phase–High Performance Liquid Chromatographic Method (RP-HPLC).
METHODS: Chromatogram was run through Standard Discovery C_{18} 250 x 4.6 mm, 5µ. Mobile phase containing 0.1% Orthophosphoric acid (Buffer with pH 3.2): Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 240 nm.
RESULTS: Retention time (RT) of Sofosbuvir and Velpatasvir were found to be 2.836 min and 3.678. %Relative Standard deviation (RSD) of the Sofosbuvir and Velpatasvir were found to be 0.4 and 0.2 respectively. %Recovery was obtained as 99.10% and 100.01% for Sofosbuvir and Velpatasvir respectively. Limit of detection (LOD), Limit of quantification (LOQ) values obtained from regression equations of Sofosbuvir and Velpatasvir were 0.10, 0.31 and 0.15, 0.46 respectively.
CONCLUSIONS: Retention times were decreased and run time was decreased, hence, the suggested RP-HPLC method can be used for routine analysis of Sofosbuvir and Velpatasvir in Pharmaceutical dosage form.

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INTRODUCTION

Sofosbuvir is a Human immune deficiency Virus (HIV) protease inhibitor which prevents HIV replication by binding to the enzyme's active site, thereby preventing the dimerization and the catalytic activity of the HIV-1 protease. Sofosbuvir selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteinsin virus-infected cells, which prevents the formation of mature infectious virus particles. It is chemically propan-2-yl (2S)-2-[[[(2R,3R,4R,5R)-5-2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxyphenoxyphophoryl] amino}propanoate (Figure 1). It is white powder and is soluble in chloroform and methanol. Its molecular formula is$C_{22}H_{29}FN_3O_9P$ and molecular weight is 529.458 g/mol.

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). 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.

It is chemically (2S)-2-{[hydroxy(methoxy)methylidene]amino}-1-[(2S,5S)-2-(17-{2-[(2S,4S)-1-[(2R)-2 {[hydroxyl (methoxy) methylidene]amino}- 2-phenylacetyl]-4-(methoxymethyl) pyrrolidin-2-yl]-1H-imidazol-5-yl}-21-oxa-5,7-diazapenta cyclo [11.8.0.0^{3,11}.0^{4,8}.0^{14,19}]henicosa1(13),2,4(8),6,9,11 ,14(19),15,17-nonaen-6-yl)-5-methylpyrrolidin-1-yl]-3-methyl butan-1-one (Figure 2).

It is white powder and is soluble in water, methanol and acetonitrile. Its molecular formula is$C_{49}H_{54}N_8O_8$ and molecular weight is 883.019 g/mol. A detailed survey of the literature for Sofosbuvir and Velpatasvir reveals that the available analytical methods are costly and with more retention time. Kalpana Nekkala *et al* [1-4] developed a rapid and sensitive RP-HPLC method with Ultraviolet(UV) detection 269 nm for routine analysis of Sofosbuvir and Velpatasvir in a pharmaceutical formulation (Epclusa). Literature review revealed few methods on method development and validation of Sofosbuvir and Velpatasvir by RP-HPLC. So, now the main aim is to develop an economical method with less run time and retention time compared to those methods.

MATERIALS AND METHODS

Chemicals and Reagents:

Sofosbuvir pure drug (API) obtained from Spectrum Research laboratory, Hyderabad. Velpatasvir pure drug (API) obtained from Spectrum Research laboratory, Hyderabad. Combination Sofosbuvir and Velpatasvir tablets (Epclusa) obtained from Apollo Pharmacy. Milli-Q Water, Acetonitrile, Phosphate buffer, Methanol, Ortho-phosphoric acid (OPA) were obtained from RANKEM**.**

Instruments:

Precision balance from Sartorius, pH Meter from Thermo Orion, UV-Spectrophotometer from Shimadzu with model No. UV-1800, Sonicator from Spectralab with model No. UCB 70, HPLC from Waters with model No. HPLC 2965 system with Empower 2 software.

Methods:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 40 mg of Sofosbuvir, 10 mg of Velpatasvir and transferred to 10ml volumetric flasks and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labelled as Standard stock solution [5,6]. (4000µg/ml of Sofosbuvir and 1000µg/ml Velpatasvir)

Preparation of Standard working solutions (100% solution):1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluents. (400µg/ml of Sofosbuvir and 100µg/ml of Velpatasvir)

Preparation of Sample stock solutions:5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (4000µg/ml of Sofosbuvir and 1000µg/ml of Velpatasvir).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (400µg/ml of Sofosbuvir and 100µg/ml of Velpatasvir)

Preparation of 0.1%Orthophosphoric acidbuffer:1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

RESULTS:

Method Development:

Many trials were done by changing columns and Mobile phases and were reported below.

Trials	Column Used	Mobile phase	Flow rate	Wave length	Temperature	Injection Volume
Trial: 1	Discovery 250 x 4.6 mm, 5µ.	Water: Methanol (50:50)	1ml/min	240nm	30°C	10µl
Trial: 2	Discovery 250 x 4.6 mm, 5µ.	Water: Acetonitrile (40:60)	1ml/min	240nm	30°C	10µl
Trial: 3	Discovery 250 x 4.6 mm, 5µ.	0.1N KH_2PO_4(4.8PH): Acetonitrile (45:55)	1ml/min	240nm	30°C	10µl
Trial: 4	Discovery 250 x 4.6 mm, 5µ.	buffer: Acetonitrile (45:55)	1ml/min	240nm	30°C	10µl
Optimized Method	Discovery 250 x 4.6 mm, 5µ.	Buffer: Acetonitrile (60:40)	1ml/min	240nm	30°C	10µl

Trials	Observation
Trial: 1	Sofosbuvir were eluted but not eluted velpatasvir so, further trial is carried out. (Figure-3)
Trial: 2	Both peaks were eluted but peak shape not good so further Trial is carried out. (Figure-4)
Trial: 3	Both Peaks shapes & tailing factor were not good so, further trial is carried out. (Figure-5)
Trial: 4	Both peaks were good but retention time & resolution were less so further trial is carried out. (Figure-6)
Optimized Method	Both peaks have good resolution, tailing factor, theoretical plate count and resolution. (Figure-7)

Method Validation

The present study was carried and the method was validated based on ICH (Q2B) parameters [7].The following parameters were validated for the proposed method.

System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines [8]. According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were within the limits (Table 1).

Specificity: Retention times of Sofosbuvir and Velpatasvir were 2.836 min and 3.678 min respectively. We did not find any interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Linearity: Six Linear concentrations of Sofosbuvir (0-600µg/mL) and Velpatasvir (0-150µg/mL) were prepared and injected. Regression equation of the Sofosbuvir and Velpatasvir were found to be, y = 4186.x+702.0, and y = 6838.x + 1450and regression co-efficient was 0.999 (Table 2 Figure 11 & 12).

Precision:

System precision: System precision was performed and % RSD for Sofosbuvir and Velpatasvir was found to be 0.5% and 0.3% respectively (Table 3).

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Sofosbuvir and Velpatasvir were found to be 0.4% and 0.2% respectively (Table 4).

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD obtained for Sofosbuvir and Velpatasvir were 0.7% and 0.5% (Table 5).

Accuracy: Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 6.

Limit of detection: Limit of detection was calculated by standard deviation method[9] and LOD for Sofosbuvir and Velpatasvir were found to be 2.852 and 3.673 respectively (Figure 13).

Limit of Quantification: Limit of Quantification was calculated by standard deviation method and LOQ for Sofosbuvir and Velpatasvir were found to be 2.847and 3.672 respectively (Figure 14).

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65:35), mobile phase plus (55:45), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table 7)

Assay: Epclusa, bearing the label claim Sofosbuvir 400mg, Velpatasvir 100mg. Assay was performed with the above formulation. Average % Assay for Sofosbuvir and Velpatasvir obtained was 98.91and 99.66 % respectively (Table 8).

Degradation studies: Standards and degraded samples are injected and calculated the percentage of drug degraded in solution by applying different conditions like acid, alkali, and oxidative, photolytic, thermal and neutral analysis (Table 9, Figure 15-20).

DISCUSSION:

A new method was established for simultaneous estimation of Sofosbuvir and Velpatasvir by RP-HPLC method. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Sofosbuvir and Velpatasvir in pharmaceutical dosage form.

The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. Hence the suggested RP-HPLC method can be used for routine analysis of Sofosbuvir and Velpatasvir in Pharmaceutical dosage form.

TABLE 1: SYSTEM SUITABILITY STUDIES OF SOFOSBUVIR AND VELPATASVIR

S. no.	Sofosbuvir			Velpatasvir			
Injection	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.835	6390	1.57	3.675	7430	1.46	5.2
2	2.836	6335	1.57	3.677	7794	1.48	5.2
3	2.836	6607	1.56	3.678	7448	1.46	5.2
4	2.836	6488	1.56	3.678	7423	1.47	5.2
5	2.836	6747	1.56	3.678	7510	1.46	5.3
6	2.838	6734	1.55	3.678	7701	1.48	5.2

RT: Retention time, USP- United States Pharmacopeia

TABLE 2: CALIBRATION DATA OF SOFOSBUVIR AND VELPATASVIR

S.No.	Concentration Sofosbuvir(µg/ml)	Response	Concentration Velpatasvir(µg/ml)	Response
1	0	0	0	0
2	100	393036	25	169023
3	200	856939	50	353441
4	300	1278408	75	514582
5	400	1673311	100	677667
6	500	2092329	125	856157
7	600	2502312	150	1029478

TABLE 3: SYSTEM PRECISION TABLE OF SOFOSBUVIR AND VELPATASVIR

S. No	Area of Sofosbuvir	Area of Velpatasvir
1.	1667706	676694
2.	1655795	674565
3.	1665632	674109
4.	1677295	672945
5.	1653231	670129
6.	1666146	671547
Mean	1664301	673332
S. D.	8723.7	2324.7
%RSD	0.5	0.3

*Average of six determinations, S.D- Standard Deviation, RSD- Relative Standard deviation.

TABLE 4: REPEATABILITY RESULTS FOR SOFOSBUVIR AND VELPATASVIR

S. No	Area ofSofosbuvir	Area ofVelpatasvir
1.	1642562	670745
2.	1658958	671011
3.	1650545	670516
4.	1642369	672047
5.	1647341	673787
6.	1645484	672180
Mean	1647877	671714
S.D.	6237.1	1224.2
%RSD	0.4	0.2

*Average of six determinations, S.D- Standard Deviation, RSD- Relative Standard deviation.

TABLE 5: INTER DAY PRECISION RESULTS FOR SOFOSBUVIR AND VELPATASVIR

S. No	Area of Sofosbuvir	Area of Velpatasvir
1.	1642562	670745
2.	1658958	671011
3.	1650545	670516
4.	1642369	672047
5.	1647341	673787
6.	1645484	672180
Mean	1647877	671714
S.D	6237.1	1224.2
%RSD	0.4	0.2

*Average of six determinations, S.D- Standard Deviation, RSD- Relative Standard deviation.

TABLE 6: TABLE OF ACCURACY

Sample	Concentration (%) (µg/ml)	Recovery (%)	Mean % Recovery
Sofosbuvir	50	99.42	99.10%
	100	98.76	
	150	99.11	
Velpatasvir	50	100.8	100.01%
	100	99.63	
	150	98.64	

TABLE 7: ROBUSTNESS DATA OF SOFOSBUVIR AND VELPATASVIR

S.no.	Condition	%RSD of Sofosbuvir	%RSD of Velpatasvir
1	Flow rate (-) 1.1ml/min	0.4	1.3
2	Flow rate (+) 1.3ml/min	0.5	0.1
3	Mobile phase (-) 65:35	0.8	0.9
4	Mobile phase (+) 55:45	0.4	0.3
5	Temperature (-) 25°C	0.7	0.6
6	Temperature (+) 35°C	0.3	0.2

RSD- Relative Standard deviation

TABLE 8: ASSAY OF TABLET

S. No.	Sofosbuvir %Assay	Velpatasvir%Assay
1	98.60	99.52
2	99.58	99.56
3	99.07	99.48
4	98.58	99.71
5	98.88	99.97
6	98.77	99.73
AVG	98.91	99.66
S.D.	0.37	0.1816
%RSD	0.38	0.2

AVG-Average, S.D- Standard Deviation, RSD- Relative Standard deviation.

TABLE 9: DIFFERENT TYPES OF DEGRADATION STUDIES

Types of Degradation	SOFOSBUVIR		
	Purity Angle	Purity Threshold	%Degraded
Acid	0.218	0.328	4.62
Base	0.134	0.278	2.62
Peroxide	0.218	0.328	1.96
Thermal	0.134	0.278	0.93
UV	0.129	0.277	0.65
Water	0.040	0.284	0.86
VELPATASVIR			
Acid	0.120	0.306	4.64
Base	0.115	0.292	2.67
Peroxide	0.120	0.306	1.84
Thermal	0.115	0.292	0.71
UV	0.123	0.293	0.75
Water	0.117	0.294	0.83

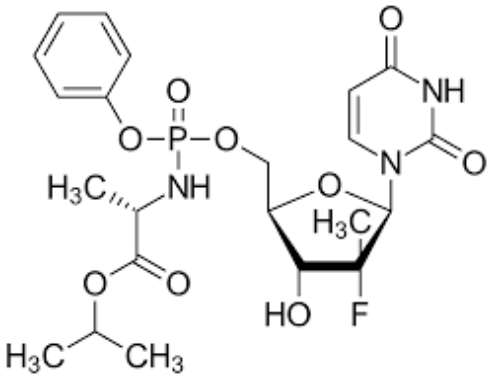

Figure 1: Structure of Sofosbuvir

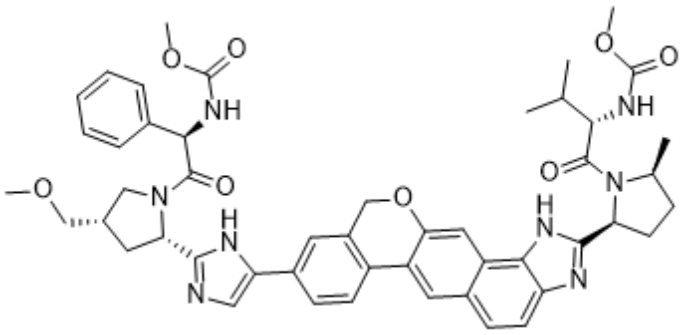

Figure 2: Structure of Velpatasvir

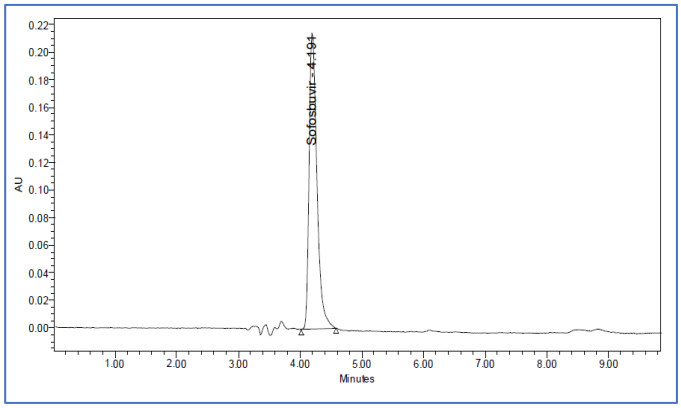

Figure 3: Trial chromatogram 1

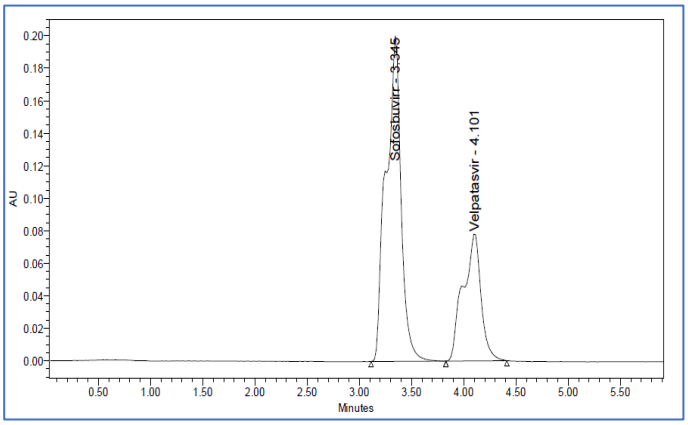

Figure 4: Trial chromatogram 2

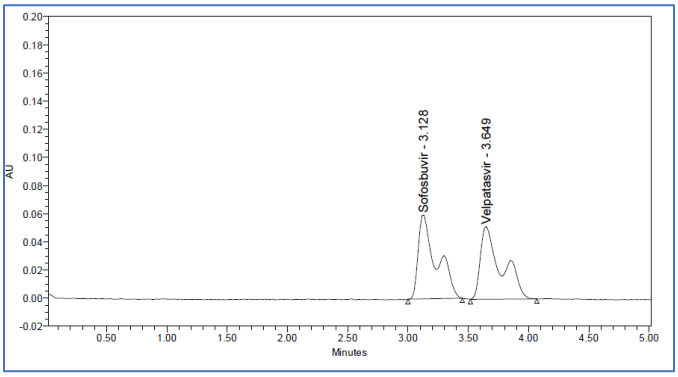

Figure 5: Trial chromatogram 3

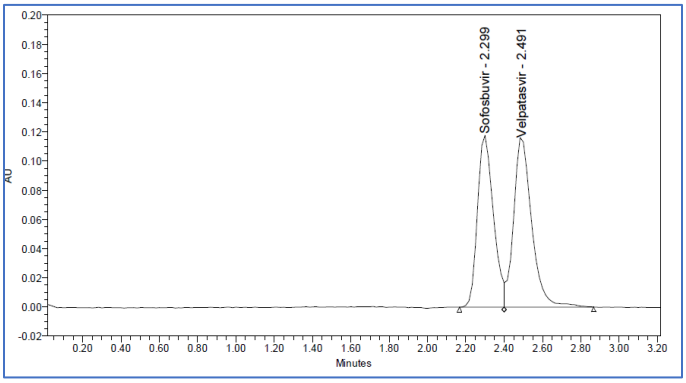

Figure 6: Trial chromatogram 4

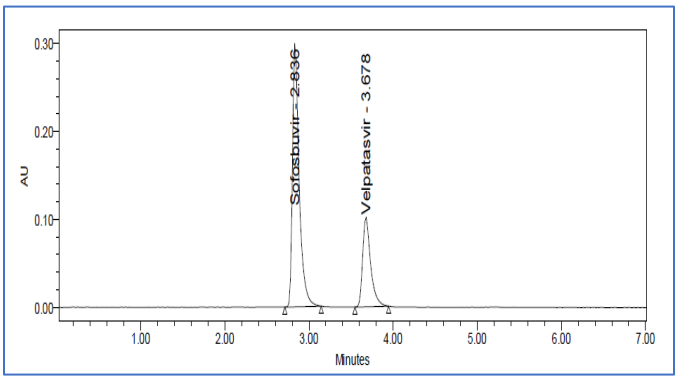

Figure 7: Optimized chromatogram of Sofosbuvir and Velpatasvir

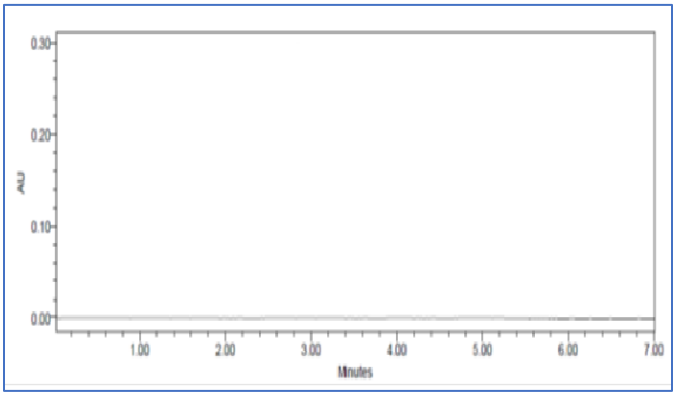

Figure 8: Chromatogram of blank.

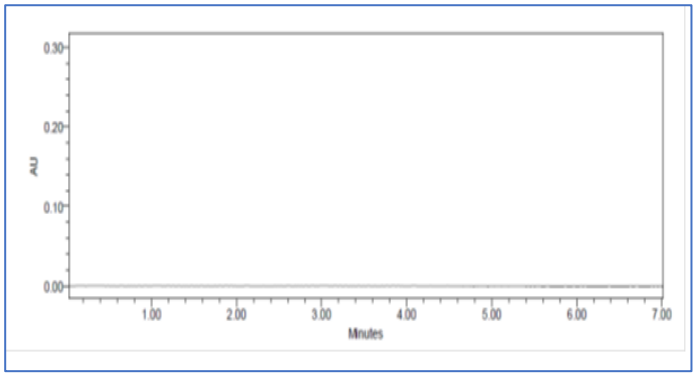

Figure 9: Chromatogram of placebo.

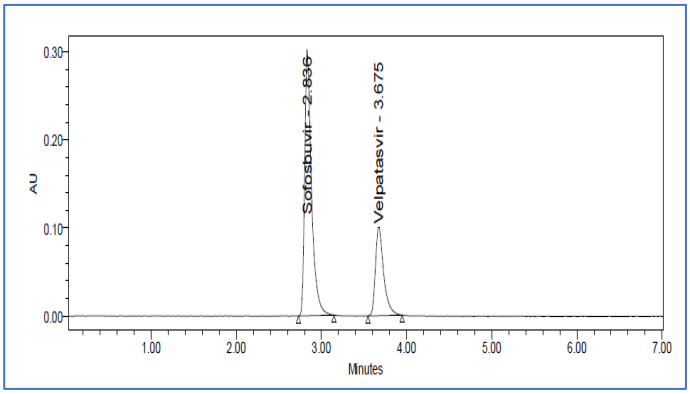

Figure 10: Typical chromatogram of Sofosbuvir and Velpatasvir

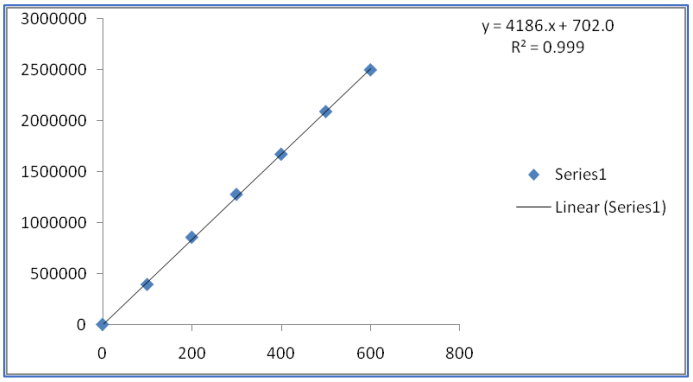

Figure 11: Calibration curve of Sofosbuvir

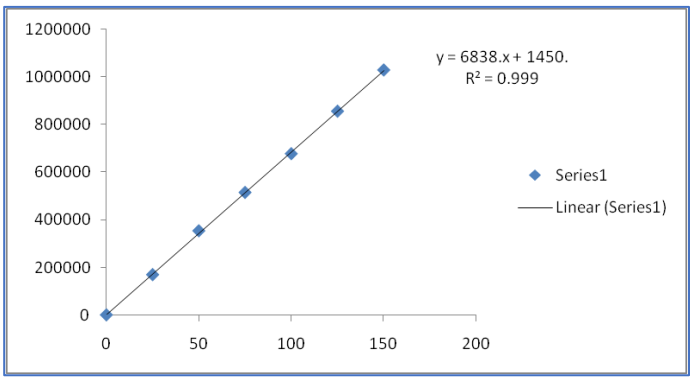

Figure 12: Calibration curve of Velpatasvir

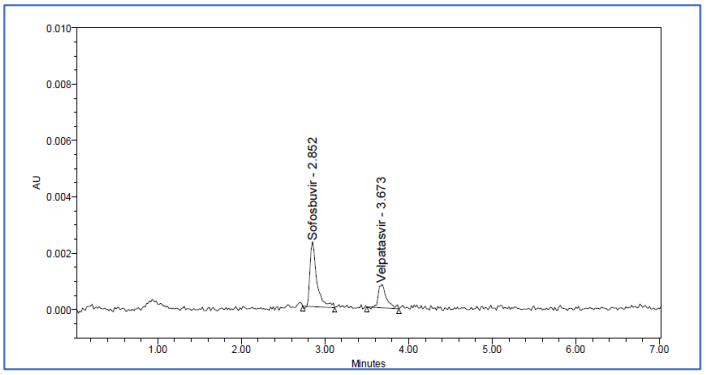

Figure 13: Limit of Detection Chromatogram of Sofosbuvir and Velpatasvir

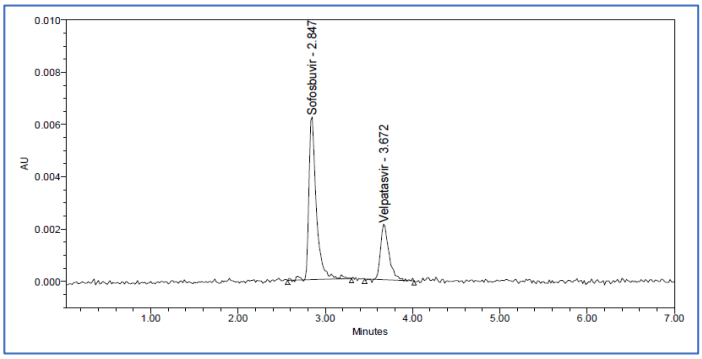

Figure 14: Limit of Quantification Chromatogram of Sofosbuvir and Velpatasvir

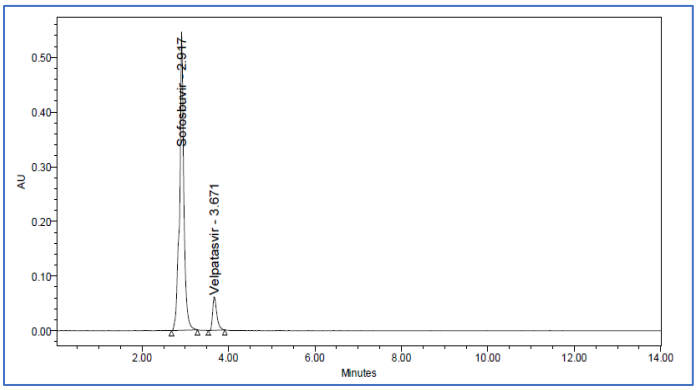

Figure 15: Chromatogram showing Acid degradation

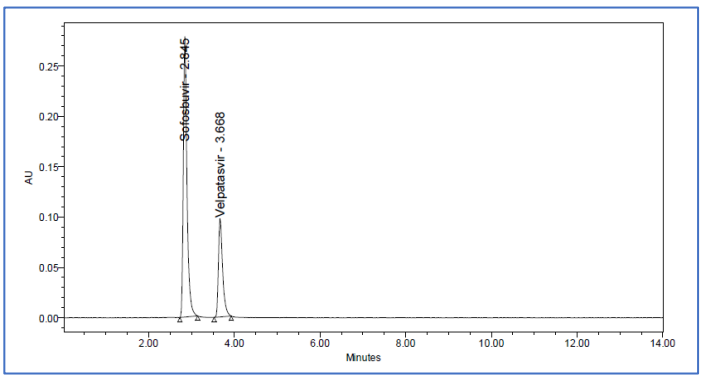

Figure 16: Chromatogram showing Base degradation

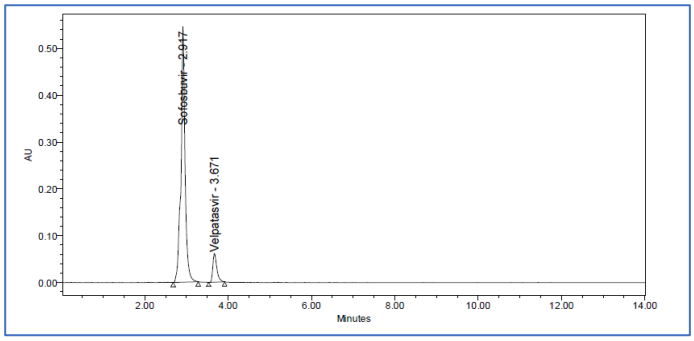

Figure 17: Chromatogram showing Peroxide degradation

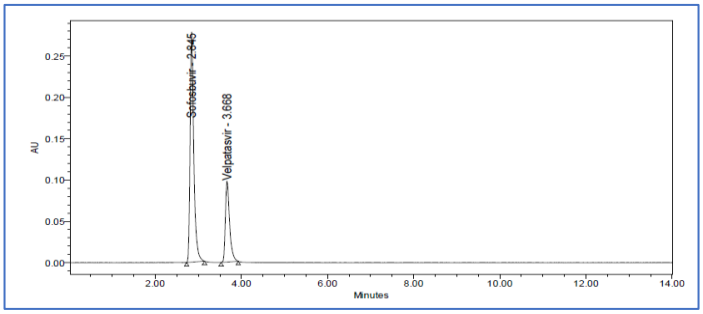

Figure 18: Chromatogram showing Thermal degradation

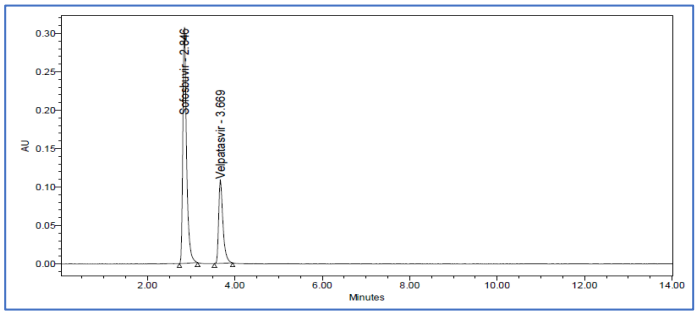

Figure 19: Chromatogram showing UV degradation

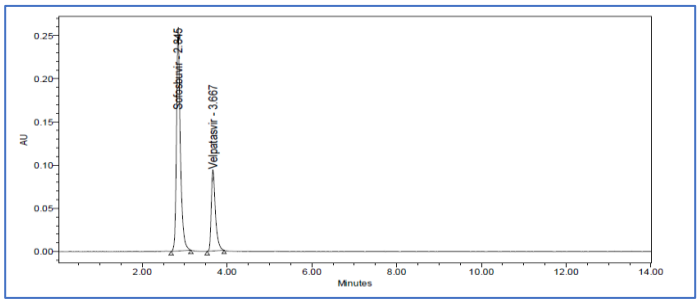

Figure 20: Chromatogram showing Water Degradation

ACKNOWLEDGEMENT: I am very much thankful to the management of Seven Hills College of Pharmacy for their encouragement and support.

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How to cite this article:
Sreehitha V, Sireesha R, Sivagami B, Kumar VP, Chandrasekar R, Babu MN. Simultaneous Estimation of Sofosbuvir and Velpatasvir Tablets by RP- HPLC Method Simultaneous Estimation of Sofosbuvir and Velpatasvir Tablets by RP- HPLC Method. *Int. J. Res. Dev. Pharm. L. Sci.* 2018; 7(5): 3092-3099. doi: 10.13040/IJRDPL.2278-0238.7(5).3092-3099.