Research Article

Stability indicating RP-HPLC method development and validation for the simultaneous determination of Sofosbuvir and Velpatasvir in tablet dosage forms

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ABSTRACT

Stability indicating RP-HPLC method was developed for the simultaneous quantitation of Sofosbuvir and Velpatasvir in its pharmaceutical dosage form and validated. The drugs were separated on Discovery C18 (150mm x 4.6mm, 5μ) column using 0.01N potassium dihydrogen phosphate buffer and acetonitrile (50:50%v/v) as mobile phase on isocratic mode. The mobile phase is pump into the column at flow rate of 1.0ml/min and column oven temperature is maintained at 30ºC. The drugs were detected at a wavelength 240nm. The retention time for Sofosbuvir and Velpatasvir were found to be 2.32min and 3.34min respectively. The developed method is validated in accordance with ICH guidelines. The method was found to be accurate, precise, specific and robust. The method obeys Beer's law at a concentration range of 100μg/ml – 600μg/ml of Sofosbuvir and 25μg/ml – 150μg/ml of Velpatasvir, with correlation coefficient of 0.999 for both the drugs. The drugs were found to be stable and less prone to degradation when they are subjected to various stress conditions.

Introduction

Sofosbuvir (Figure 1A) chemically is 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. It is white to off-white crystalline solid, slightly soluble in water with a pKa value of 9.38. It is an antiviral drug used in the treatment of Hepatitis C [1,2]. Velpatasvir (Figure 1B) chemically is 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. It is white to tan or yellow hygroscopic solid, soluble in water, methanol and acetonitrile with pKa values of 3.72 and 5.98. It is an antiviral drug used to treat chronic hepatitis C [3,4]. The literature survey reveals that there is only one method i.e., RP-HPLC method [5] was developed for the simultaneous estimation of Sofosbuvir and Velpatasvir in pharmaceutical dosage from. The present study aimed to develop and validate a stability indicating method for the simultaneous determination of Sofosbuvir and Velpatasvir in the pharmaceutical dosage form using RP-HPLC.
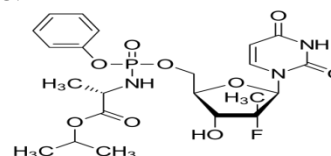
Figure 1A :Sofosbuvir

Figure 1B: Velpatasvir

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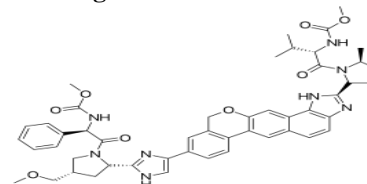
Material and Methods

Chemicals and reagents
Sofosbuvir standard drug and Velpatasvir standard drug were supplied as gift samples by Spectrum labs, Hyderabad. The Sofosbuvir and Velpatasvir tablets (Velpanat) were purchased from local pharmacy. The chemicals used for development of the method were of AR grade and purchased from Sigma Aldrich. The solvents used were of HPLC grade and purchased from Merck.

Instrument and chromatographic conditions
Waters HPLC system with Discovery C18 (150mm x 4.6mm, 5µ) column, autosampler and PDA detection mode running on empower 2 software was used. An isocratic mode with 0.01N potassium dihydrogen phosphate buffer and acetonitrile in 1:1 as mobile phase at 1.0ml/min flow rate was used for separation of drugs. The detection of drugs was done at 240nm with column oven temperature maintained at 30ºC. The other instruments used were pH meter (EI), Digital Balance (Infra Instruments), Ultrasonic Bath (Wadegati), Hot air oven (Cisco).

Preparation of diluent: Water and acetonitrile in the ratio 1:1 were mixed and used as diluent.

Preparation of standard and sample solution: Dissolve an accurately weighed 40mg of Sofosbuvir working standard and 10mg of Velpatasvir working standard in 10ml of diluent. Dilute 1ml of above solution with 10ml of diluent.
Average weight was calculated for 20 tablets (Velpanat) and an amount equivalent to 40mg of Sofosbuvir was taken into 10mL volumetric flask. The sample was dissolved in 10mL of diluent. The above solution was filtered using HPLC filters. Pipette out 1mL of the above solution into 10mL volumetric flask and made up with diluent.

Method Validation
The developed method was validated in accordance with ICH guidelines [6].

System suitability: Inject standard solution into the chromatographic system and calculate the parameters such as % relative standard deviation (RSD), tailing factor, plate count and resolution.

Linearity: Serial dilutions of standard Sofosbuvir and Velpatasvir in the range of 100µg/mL - 600µg/mL and 25µg/mL - 150µg/mL respectively were prepared and injected into the HPLC. A linearity graph was plotted between concentration and peak areas.

Accuracy: The solutions were prepared in three different concentration levels of 50%, 100% and 150%, injected into HPLC and % recoveries were calculated.

Precision: The precision of the method was determined by injecting the standard solution six times into HPLC and the % RSD was calculated.

Specificity: The specificity of the method was determined by injecting the placebo solution and comparing with standard solution for the interference with Sofosbuvir andVelpatasvir peak.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ are determined by standard deviation (SD) and slope of the calibration curve. The limiting values are calculated as per the following equations: LOD = $(3.3 \times$ SD)/ Slope and LOQ = $(10 \times$ SD)/ Slope.

Robustness: Robustness of the method was determined by varying the optimum chromatographic conditions such as mobile phase ratio ($\pm$10%), flow rate ($\pm$0.2mL/min) and column oven temperature ($\pm$5ºC). The system suitability parameters were calculated and recorded.

Forced degradation studies: The drugs solution was subjected to the various stress conditions such as acidic (2N Hydrochloric acid, 60 ºC for 30 mins), basic (2N sodium hydroxide, 60 ºC for 30 mins), oxidative (20% hydrogen peroxide, 60 ºC for 30 mins), neutral (refluxingthedrugsinwaterfor6hrsatatemperature of 60ºC), photolytic (exposing the drugs solution to UV light by keeping the beaker in UV Chamber for 7 days or 200Watt hours/m^2 in photo stability chamber) and thermal (drugs solution was placed in an oven at 105ºC for 6 hours) conditions.

Results and Discussion
Initially various mobile phases and columns were tried to elute the drugs. Discovery C18 (150mm x 4.6mm, 5µ) column, mobile phase consisting of 0.01N Potassium dihydrogen phosphate and Acetonitrile (50:50) on isocratic mode at flow rate 1.0ml/min was used to separate the drugs. The detection wavelength was selected from the overlay UV spectrum and was found to be 240nm as shown in Figure 2.
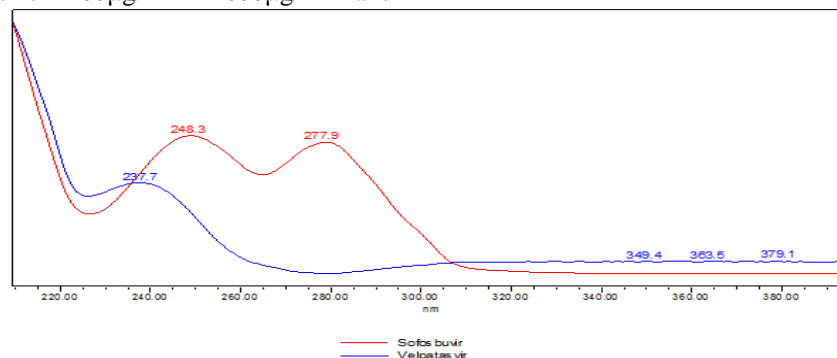

Fig.2: Overlay UV spectrum of Sofosbuvir and Velpatasvir

The standard solution, sample solution and blank solution were prepared and injected into the HPLC system. The standard, sample and blank chromatograms were shown in Figures 3, 4 and 5 respectively.
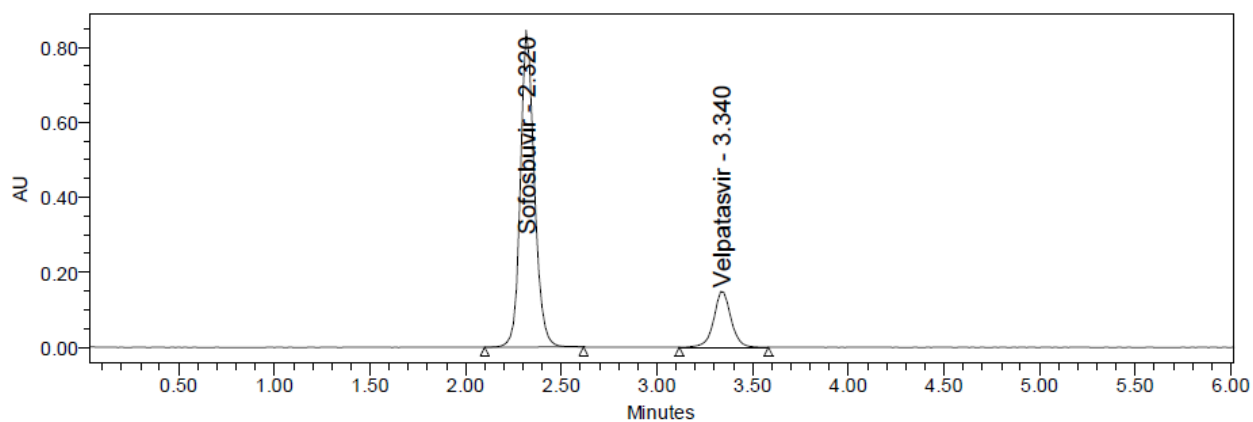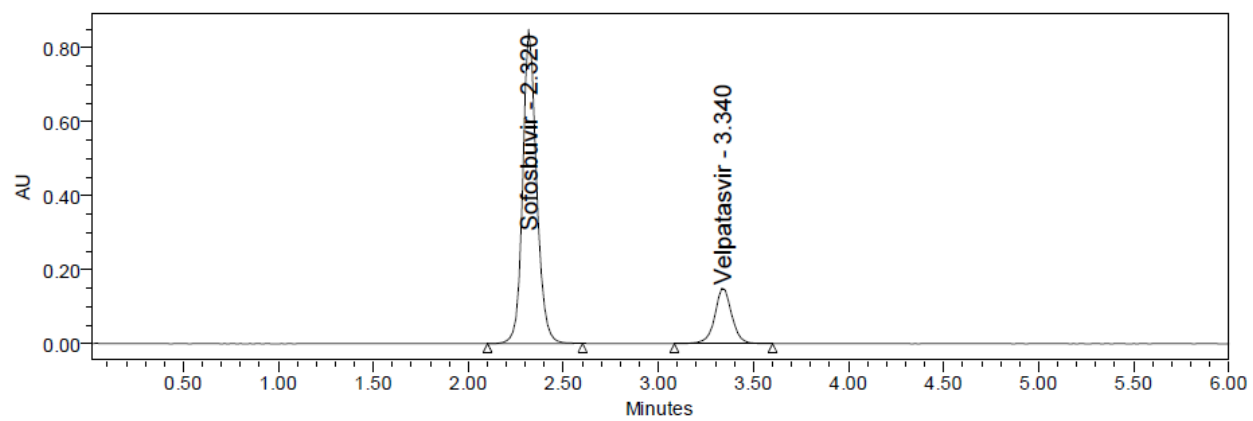
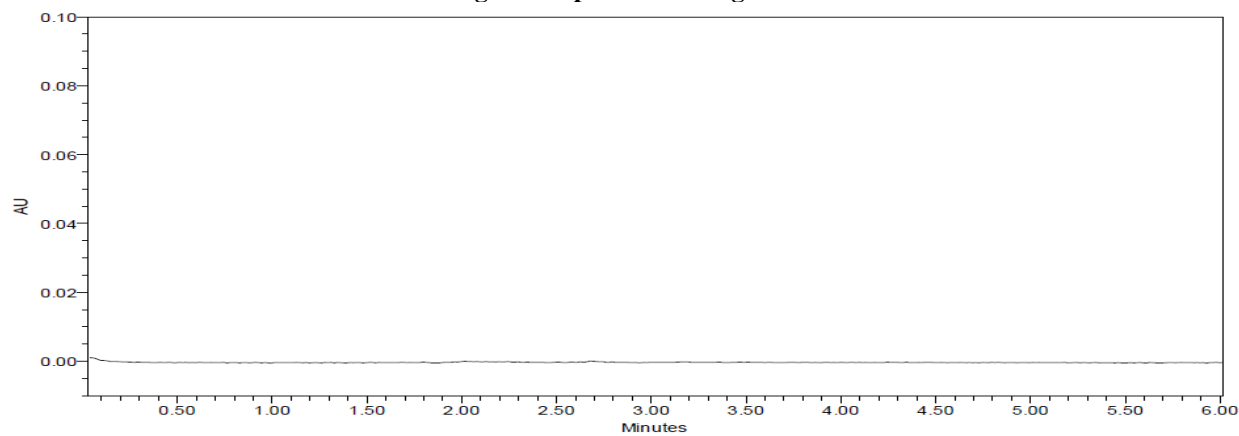**Fig.3: Standard Chromatogram**

Fig.4: Sample Chromatogram

Fig.5: Blank Chromatogram

The linearity of the method was determined by preparing serial dilutions of Sofosbuvir and Velpatasvir in the concentration range of 100µg/ml – 600µg/ml and 25µg/ml – 150µg/ml respectively. A linear response was observed in the above concentration ranges with a correlation coefficient of 0.999. The linearity plots were shown in Figure 6A and 6B.
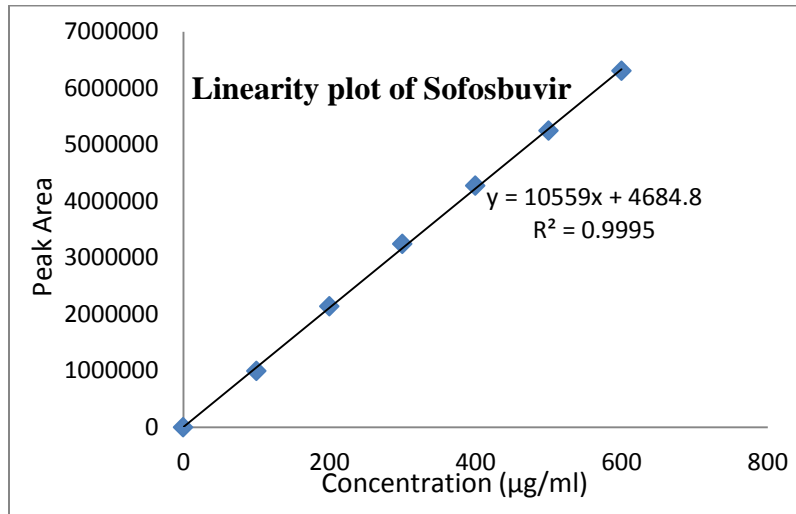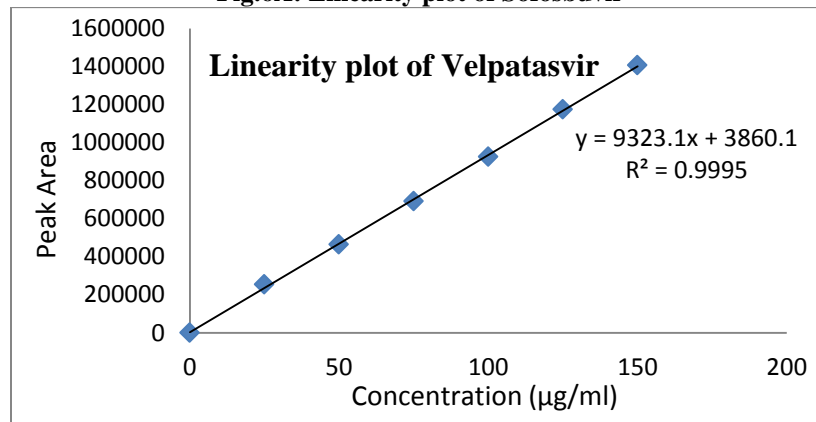
Fig.6A: Linearity plot of Sofosbuvir

y = 10559x + 4684.8
R² = 0.9995

Fig.6B: Linearity plot of Velpatasvir

y = 9323.1x + 3860.1
R² = 0.9995

The % RSD was found to be 0.3 for Sofosbuvir and 0.8 for Velpatasvir and % recovery was found to be 99.58% - 100.13% for Sofosbuvir and 99.80% - 100.45% for Velpatasvir, indicating the method to be accurate and precise. The method was found to berugged, robust and stable up to 24 hours. The developed method was found to be specific for the drugs, as there is no interference of placebo peaks with the retention time of drugs. The placebo chromatogram was shown in figure 7.The system suitability parameters and validation parameters results are summarized in table 1.
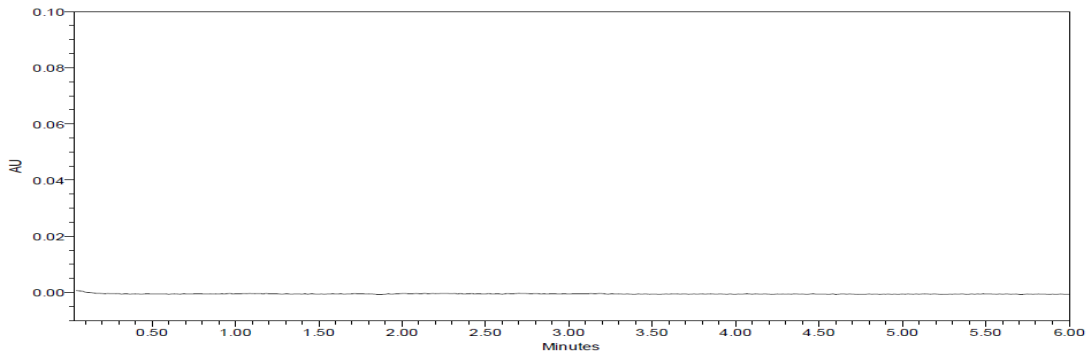

Fig.7: Placebo chromatogram

Table No. 1 : System suitability and validation parameters

Parameter	Sofosbuvir	Velpatasvir
Specificity	Specific, No interference	Specific, No interference
Precision (% RSD)	0.3	0.8
Accuracy (% Recovery)	99.58% - 100.13%	99.80% - 100.45%
Linearity range (µg/mL)	100 – 600	25 – 150
Correlation coefficient, r	0.9997	0.9997
Limit of Detection (µg/mL)	0.26	0.17
Limit of Quantitation (µg/mL)	0.78	0.50
Ruggedness (% RSD)	0.6	0.5
Robustness	Robust	Robust
Stability	Stable	Stable
USP Plate Count	4913	7145
USP Tailing factor	1.13	1.01
USP Resolution	6.8	

The forced degradation studies were conducted by exposing the standard solution to the various stress conditions. The net degradation was found to be within the limits, indicates that the drugs are stable at various stress conditions. The forced degradation studies results were summarized in table 2 and chromatograms were shown in figure 8.

Table 2: Forced degradation studies results

| S.No.	Stress condition	Sofosbuvir			Velpatasvir			% area of degradation peak
		% Assay	Peak purity Angle	Peak purity threshold	% Assay	Peak purity angle	Peak purity threshold	
1	2N HCL for 30mins at 60°C	95.21	0.185	0.285	95.03	0.286	0.291	-
2	2N NaOH for 30mins at 60°C	97.21	0.172	0.237	97.34	0.242	0.283	-
3	20% H_2O_2 for 30mins at 60^0C	98.04	0.310	0.402	98.33	0.209	0.308	-
4	Water for 6hrs at60^0C	99.19	0.130	0.290	99.04	0.142	0.308	-
5	UV light 200wts/hr or 7 days	99.41	0.126	0.288	99.24	0.129	0.302	-
6	105^0C for 6hrs	99.16	0.211	0.287	99.49	0.100	0.297	-
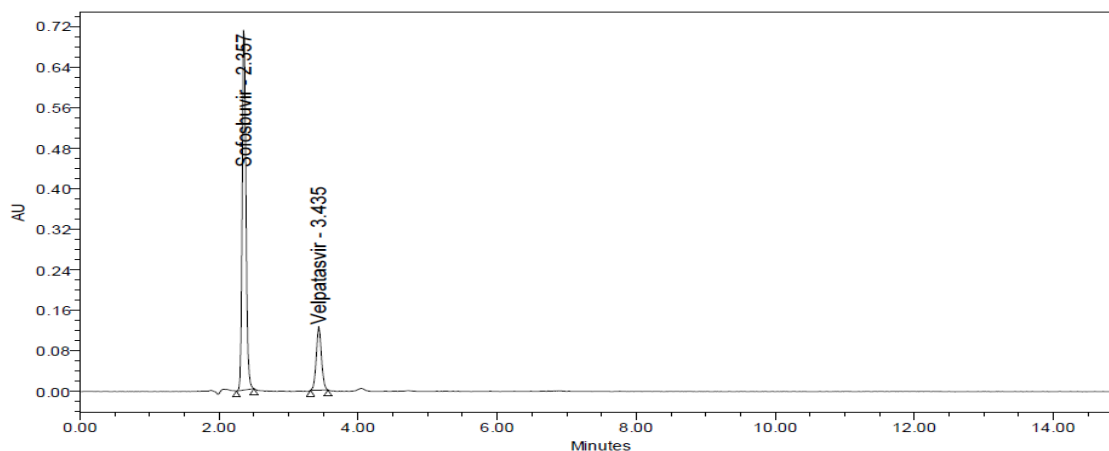
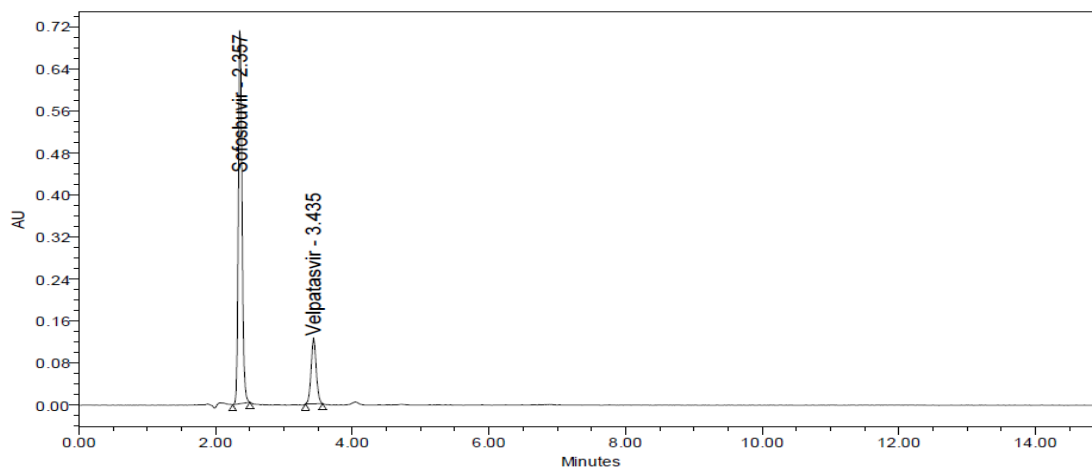**Fig.8A: Acid degradation chromatogram**
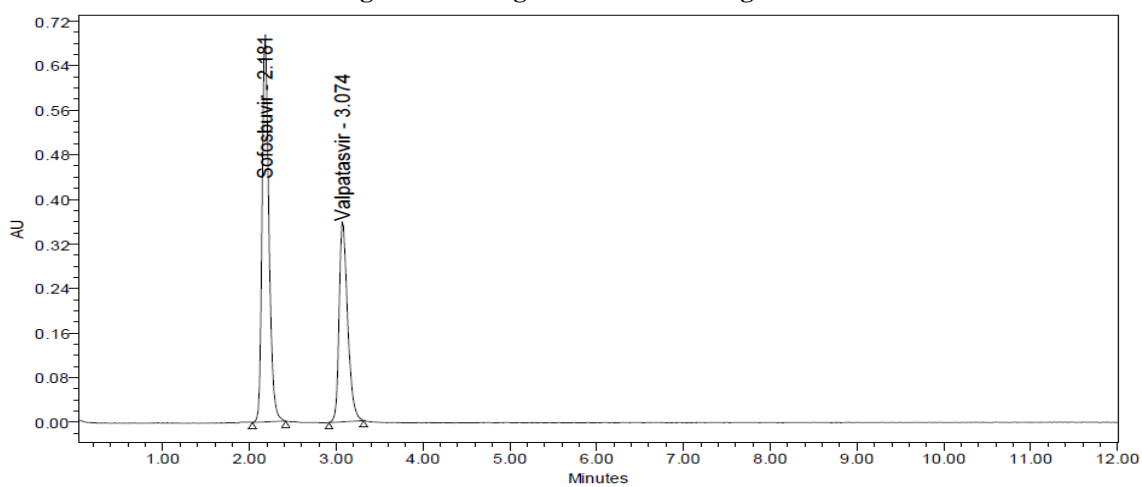
Fig.8B: Base degradation chromatogram

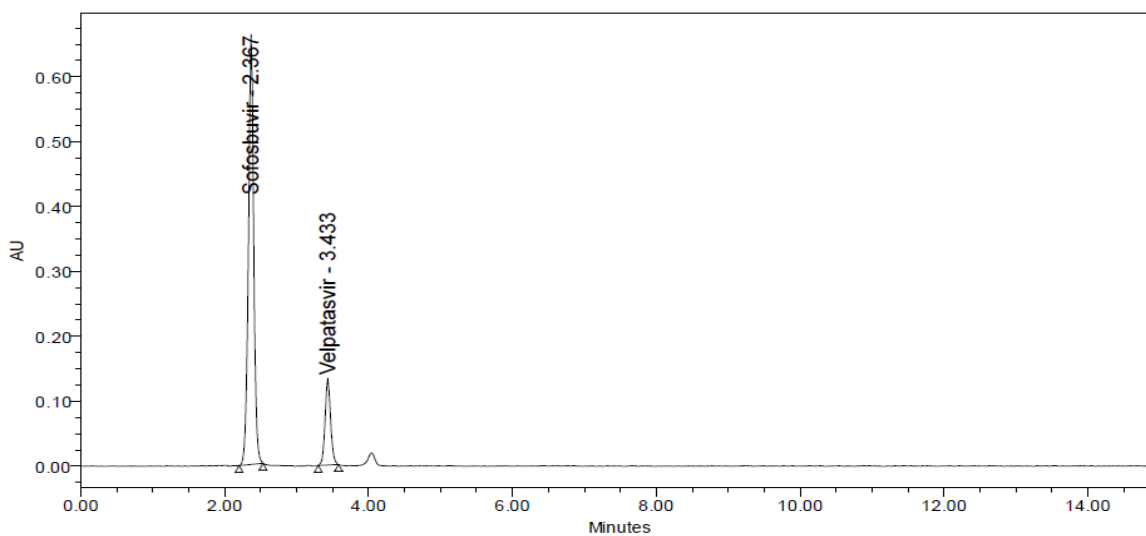**Fig.8C: Peroxide degradation chromatogram**

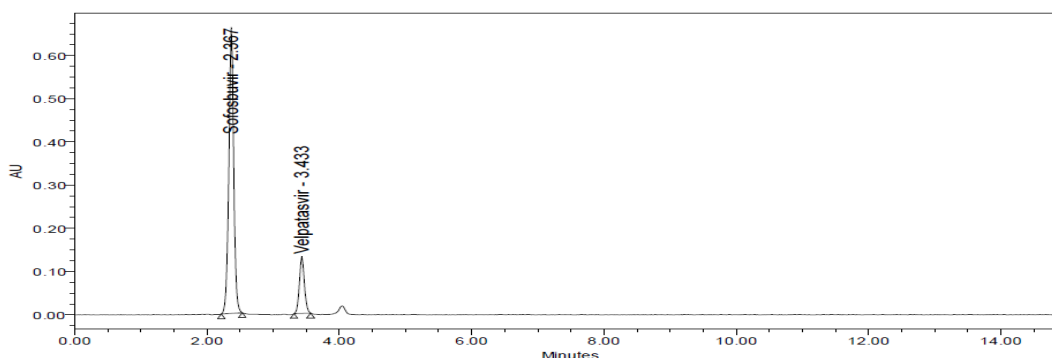**Fig.8D: Water stress study chromatogram**

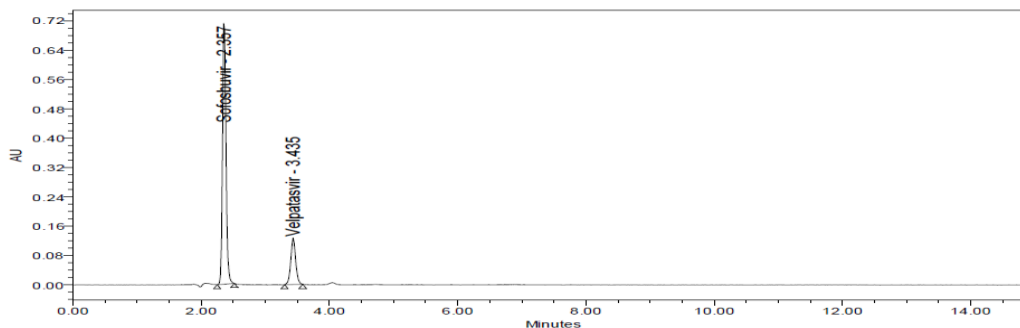**Fig.8E: Photo stability degradation chromatogram**

Fig.8F: Dry heat study chromatogram

Conclusion

Stability indicating RP-HPLC method was developed for the simultaneous estimation of Sofosbuvir and Velpatasvir in pharmaceutical dosage form. The developed method was validated and found to be specific, accurate, precise, linear and robust. The drugs, Sofosbuvir and Velpatasvir were stable under different forced degradation conditions. The developed method can be used for the rapid quantification of Sofosbuvir and Velpatasvir in its pharmaceutical dosage form.

Aknowledgement

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