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Development and Validation of Stability Indicating Rp-Hplc Method for the Simultaneous Estimation of Sofosbuvir and Daclatasvir Dihydrochloride in Bulk Drug and Pharmaceutical Dosage Form

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Original Research Article

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Abstract: A new stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Sofosbuvir and Daclatasvir in pharmaceutical dosage form and bulk drug. The optimized conditions for the simultaneous estimation of drug includes Discovery C18 (150mm x 4.6 mm, 5µ) column, 0.01N potassium dihydrogen phosphate and Acetonitrile (50:50%v/v) as mobile phase run on isocratic mode at a flow rate 1.0ml/min. The column is maintained at 30ºC temperature and the drugs are detected at a wavelength of 254nm. The retention time for Sofosbuvir and for Daclatasvir were found to be 2.47 min and 3.31 min respectively. The %RSD for Sofosbuvir and Daclatasvir were found 0.8 and 0.5 respectively. The % Recovery for Sofosbuvir was found to be 99.90% - 100.03% and % recovery for Daclatasvir was found to be 99.90% - 99.93%. A linear response was found in the concentration range of 100µg/ml – 600µg/ml for Sofosbuvir and 15µg/ml – 90µg/ml for Daclatasvir, with correlation coefficient of 0.999 for both the drugs. The method was found to specific, accurate, precise, robust, rugged and stable in solution for 24 hours. The forced degradation studies indicated that the drugs are stable in various stress conditions as the net degradation was found to be within the limits. The developed method can be used for the quality control for simultaneous estimation of Sofosbuvir and Daclatasvir in pharmaceutical dosage form.

Keywords: Sofosbuvir, Daclatasvir, Stability indicating, Method development, Validation, RP-HPLC.

INTRODUCTION

Sofosbuvir Isopropyl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl] methoxy-phenoxy-phosphoryl] amino]propanoate (Figure-1A), is a white crystalline solid, slightly soluble in water and has a pKa value of 9.38 [1]. It is an antiviral drug acts by inhibiting NS5B polymerase, used in the treatment of Hepatitis C [2]. Daclatasvir dihydrochloride , Methyl N-[(2S)-1-[(2S)-2-[5-[4-[4-[2-[(2S)-1-[(2S)-2-(methoxycarbonylamino)-3-methylbutanoyl]pyrrolidin-2-yl]-1H-imidazol-5-yl]phenyl]phenyl]-1H imidazol-2-yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate;dihydrochloride (Figure-1B), is a white to yellow crystalline solid, freely soluble in water [3, 4]. It has pKa values of 6.09 and 11.15. It is an antiviral drug acts by inhibiting HCV viral RNA replication and protein translation by directly inhibiting HCV protein NS5A which is critical for HCV viral transcription and translation. It is used in the treatment of chronic Hepatitis C. According to literature survey, only one spectrophotometric method was developed for the simultaneous estimation of Sofosbuvir and Daclatasvir in pharmaceutical dosage form [5]. As there is no RP-HPLC method for the simultaneous estimation of both the drugs, the aim of the present study was to develop and validate stability indicating RP-HPLC method for the simultaneous estimation of Sofosbuvir and Daclatasvir in bulk and pharmaceutical dosage form.

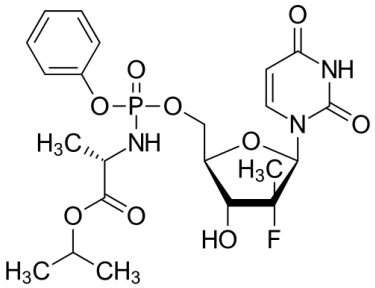

Fig-1A: Chemical structure of Sofosbuvir

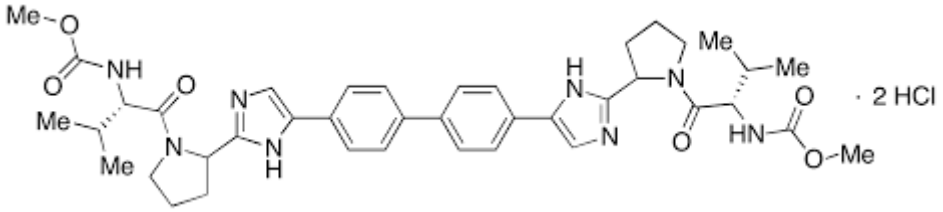

Fig-1B: Chemical structure of Daclatasvir dihydrochloride

MATERIALS AND METHODS:

Chemicals and reagents

Sofosbuvir standard drug and Daclatasvir standard drug were supplied as gift samples by Spectrum labs, Hyderabad. The Sofosbuvir and Daclatasvir tablets (Sovodak) 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 (50:50%v/v) as mobile phase at 1.0ml/min flow rate was used for separation of drugs. The detection of drugs was done at 254nm 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

Mixture of water and acetonitrile in the ratio 50:50%v/v was used as diluent.

Preparation of mobile phase

Dissolve 1.36g of potassium dihydrogen orthophosphate in 1000ml of distilled water. pH of the solution was adjusted to 3 using dilute ortho-phosphoric acid. This gives 0.01N potassium dihydrogen orthophosphate buffer.

Mixture of above buffer solution with acetonitrile in the ratio of 50:50%v/v was used as mobile phase.

Preparation of standard and sample solution. Dissolve an accurately weighed 40mg of Sofosbuvir working standard and 6mg of Daclatasvir working standard in 10ml of diluent. Dilute 1ml of above solution with 10ml of diluent.

Average weight was calculated for 20 tablets (Sovodak) 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 and pipetted out 1mL of the above solution into 10mL volumetric flask and made up with diluent.

Method Validation

The developed method was validated in compliance with ICH guidelines [6].

System suitability

Injected standard solution into the chromatographic system and calculated the parameters such as % relative standard deviation (RSD), tailing factor, plate count and resolution.

Linearity

Serial dilutions of standard Sofosbuvir and Daclatasvir in the range of 100µg/mL - 600µg/mL and 15µg/mL - 90µg/mL respectively were prepared and injected into the HPLC. A linearity graph was plotted between peak areas and concentration.

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 six solutions of standard 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 and Daclatasvir peaks.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ are determined from the values of standard deviation (SD) and slope of the calibration curve. The limiting values were calculated as per the following equations: LOD = (3.3 × SD)/ Slope and LOQ = (10 × SD)/ Slope.

Robustness

Robustness of the method was determined by varying the optimum chromatographic conditions such as mobile phase ratio (±10%), flow rate (±0.2mL/min) and column oven temperature (±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 min), basic (2N sodium hydroxide, 60 ºC for 30 min), oxidative (20% hydrogen peroxide, 60 ºC for 30 min), neutral (refluxing the drugs in water for 6hrs at a temperature 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 254nm as shown in Figure-2.

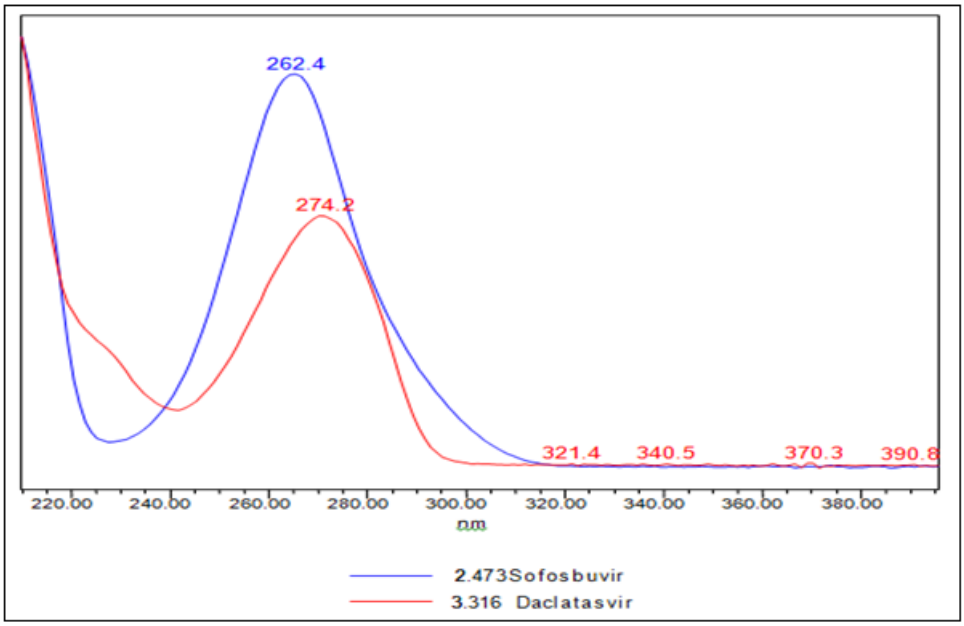

Fig-2: Overlay UV spectum of Sofosbuvir and Daclatasvir

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-3A, 3B and 3C respectively.

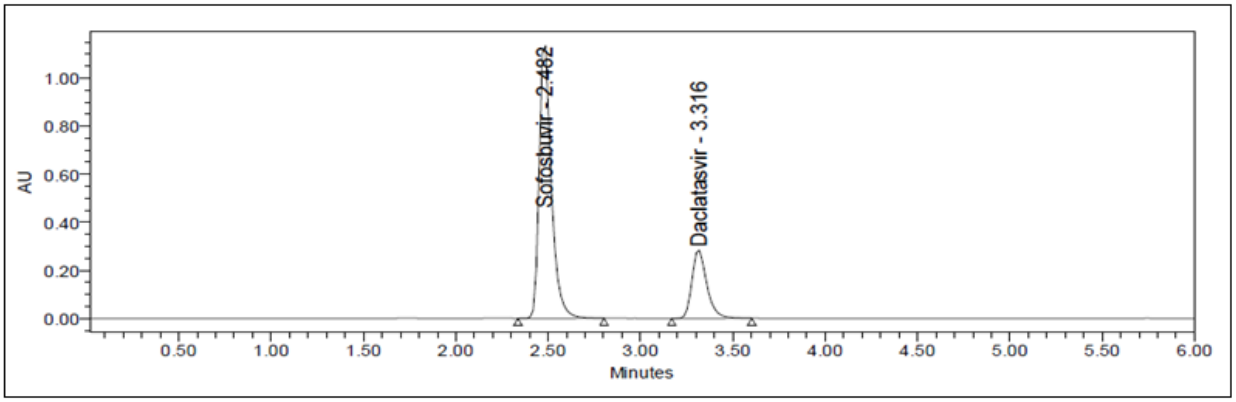

Fig-3A: Standard Chromatogram

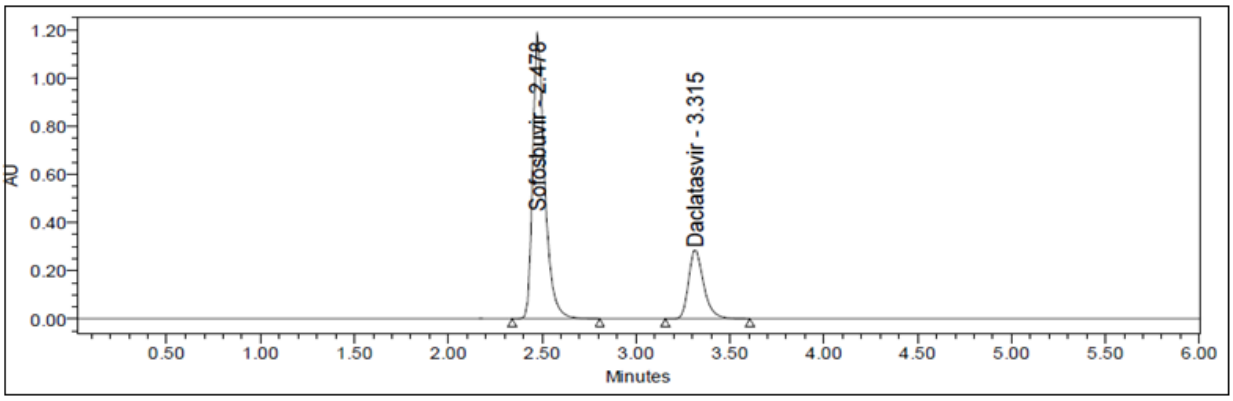

Fig-3B: Sample Chromatogram

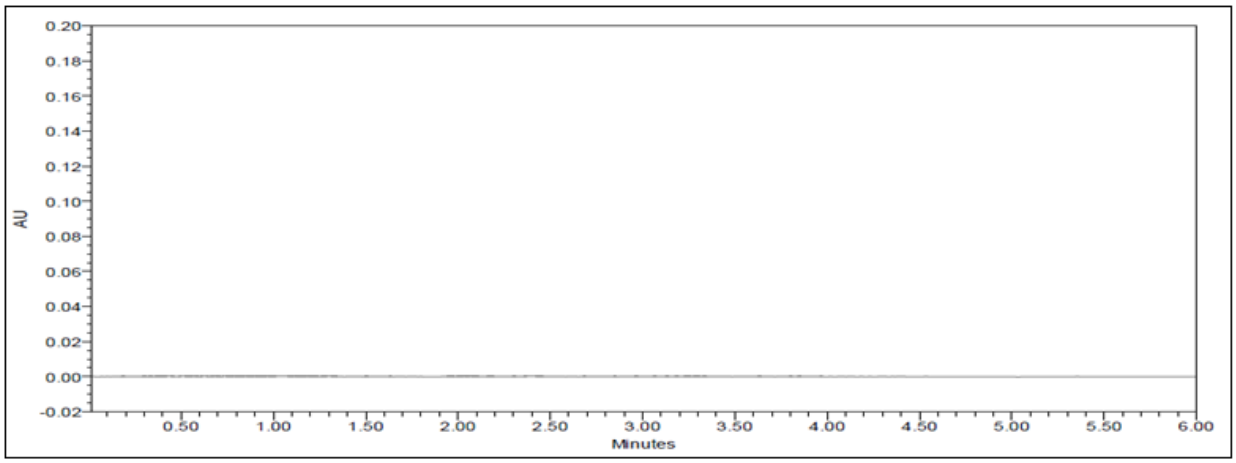

Fig-3C: Blank Chromatogram

The linearity of the method was determined by preparing serial dilutions of Sofosbuvir and Daclatasvir in the concentration range of 100µg/ml – 600µg/ml and 15µg/ml – 90µg/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-4A and 4B.

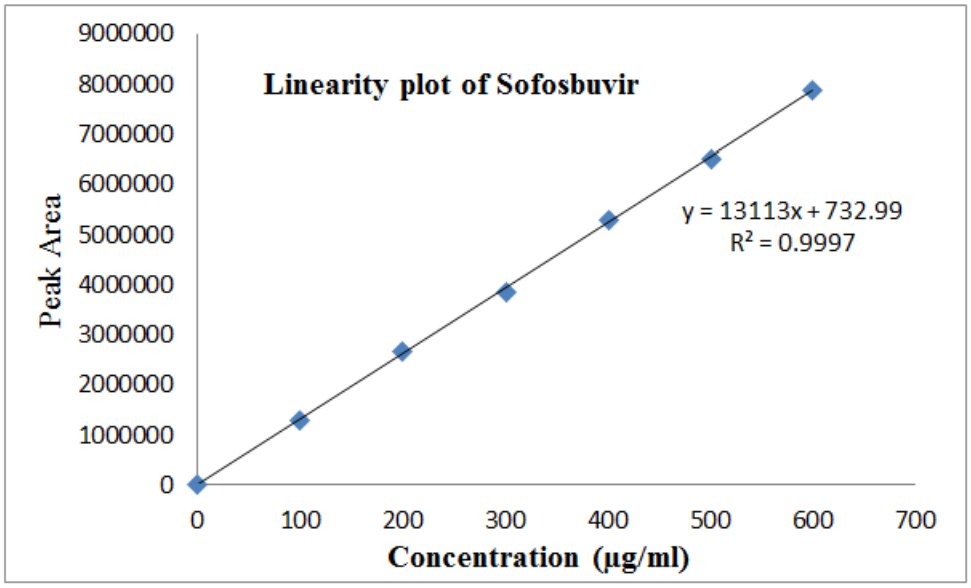

Fig-4A: Linearity plot of Sofosbuvir

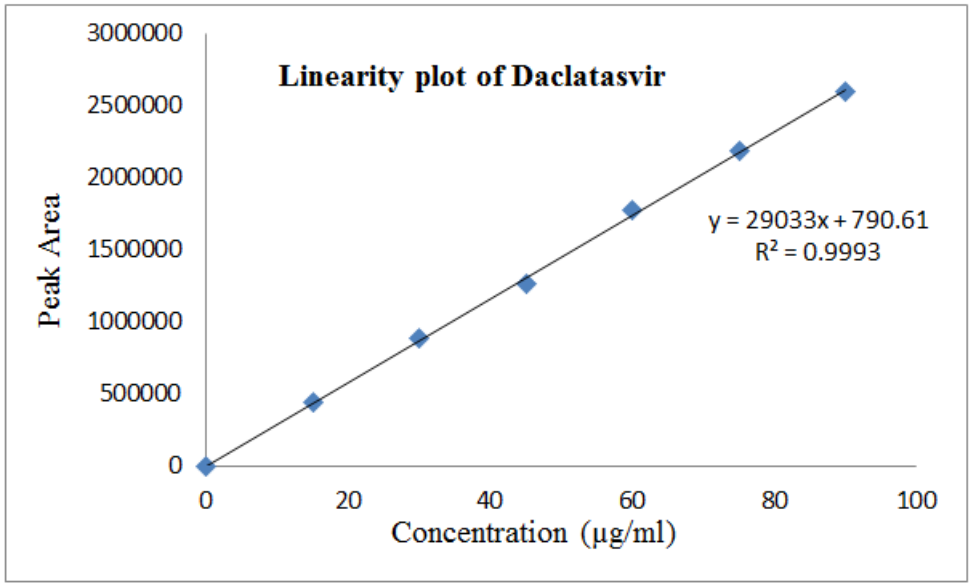

Fig-4B: Linearity plot of Daclatasvir

The % RSD was found to be 0.8 for Sofosbuvir and 0.5 for Daclatasvir and % recovery was found to be 99.90% - 100.03% for Sofosbuvir and 99.90% - 99.93% for Daclatasvir, indicating the method to be accurate and precise. The method was found to be rugged, robust and stable up to 24 hours. The developed method was found to be specific for the drugs, as there was no interference of placebo peaks with the retention time of drugs. The placebo chromatogram was shown in figure-5. The system suitability parameters and validation parameters results are summarized in Table-1.

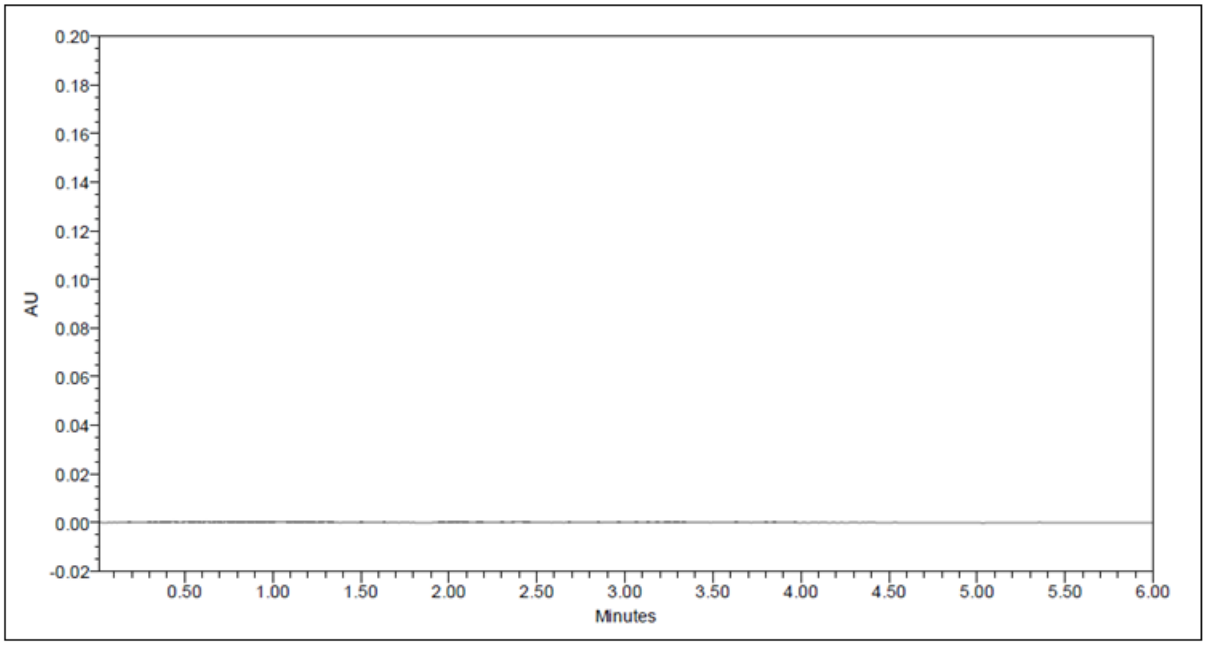

Fig-5: Placebo chromatogram

Table-1: System suitability and validation parameter results

Parameter	Sofosbuvir		Daclatasvir	
Precision (%RSD)	0.8		0.5	
Accuracy (% Recovery)	99.90% - 100.03%		99.90% - 99.93%	
Specificity	Specific, No interference		Specific, No interference	
Linearity range (µg/mL)	100 – 600		15 – 90	
Correlation coefficient, r	0.9998		0.9996	
Limit of Detection(µg/mL)	0.02		0.01	
Limit of Quantification (µg/mL)	0.07		0.03	
Ruggedness (%RSD)	Day 1	Day 2	Day 1	Day 2
	0.4	0.5	0.6	0.5
Robustness (%RSD)	Less flow rate	More flow rate	Less flow rate	More flow rate
	0.3	0.2	0.2	0.1
	Less mobile phase	More mobile phase	Less mobile phase	More mobile phase
	0.3	0.4	0.1	0.6
	Less column temperature	More column temperature	Less column temperature	More column temperature
	0.4	0.2	0.4	0.2
Solution stability	Day 1	Day 2	Day 1	Day 2
	0.4	0.5	0.6	0.5
USP Plate count	7657		8510	
USP Tailing factor	1.34		1.28	
USP Resolution			6.3	

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 6.

Table-2: Forced degradation studies results

Stress condition	Sofosbuvir				Daclatasvir				% area of degradation peak
	% Assay	Peak purity Angle	Peak Purity Threshold	% Degradation	% Assay	Peak purity	Peak purity angle	% Degradation threshold	
Acidic	95.58	1.058	1.904	4.42	95.74	0.184	0.308	4.26	-
Basic	94.75	1.193	1.548	5.25	95.59	0.116	0.317	4.41	-
Oxidative	92.08	0.621	0.745	7.92	94.91	0.139	0.341	5.09	12.72
Neutral	99.23	1.462	1.539	0.77	99.85	0.118	0.324	0.15	-
Photolytic	98.29	1.293	1.550	1.71	98.93	0.121	0.321	1.07	-
Thermal	97.77	1.146	1.812	2.23	97.98	0.122	0.317	2.02	-

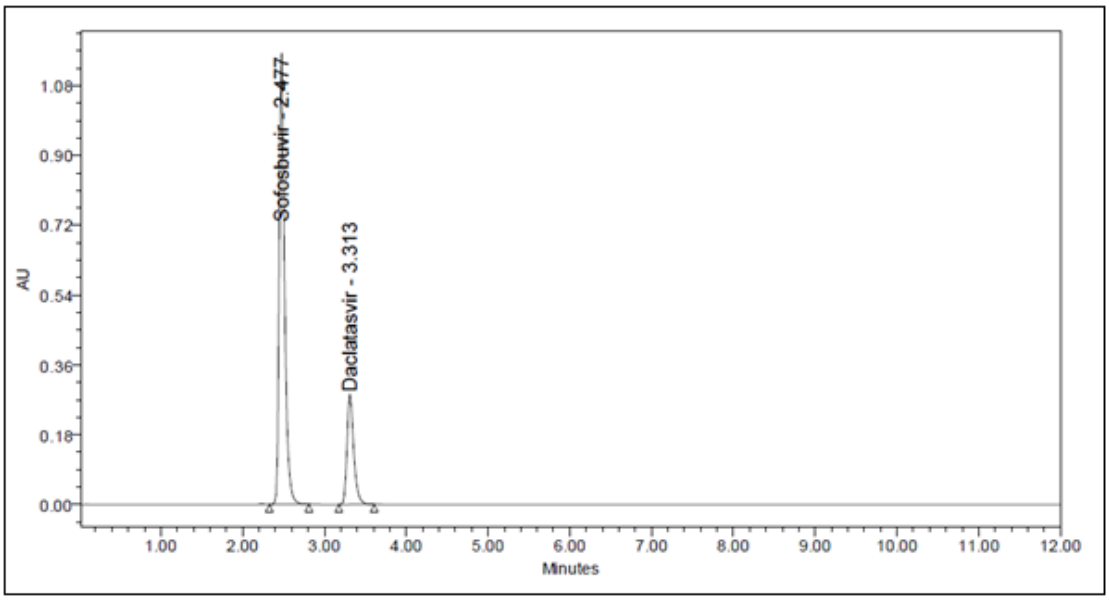

Fig-6A: Acid degradation chromatogram

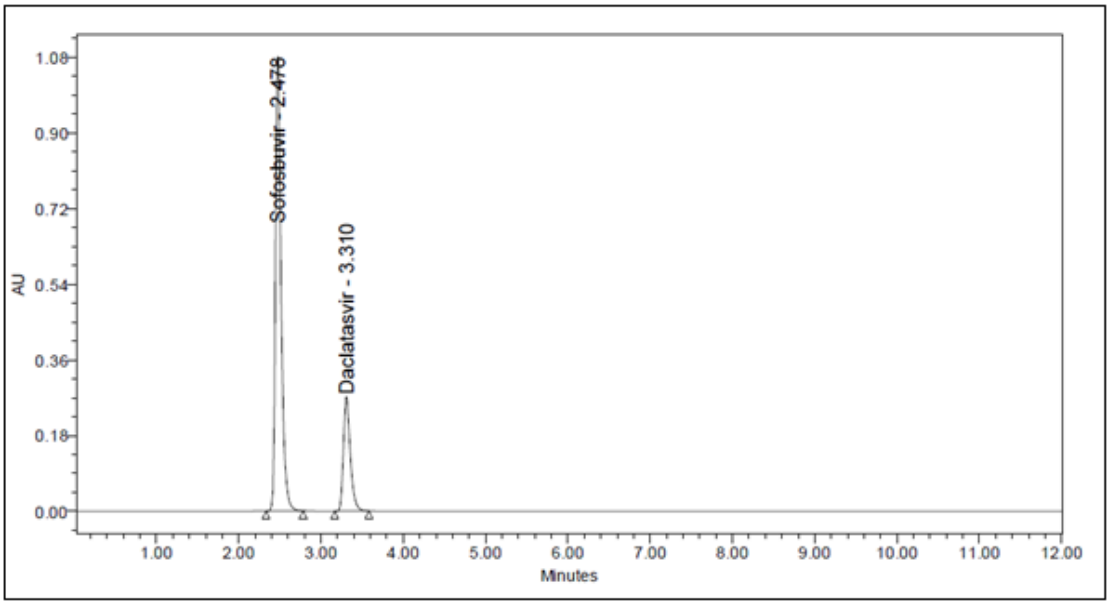

Fig-6B: Base degradation chromatogram

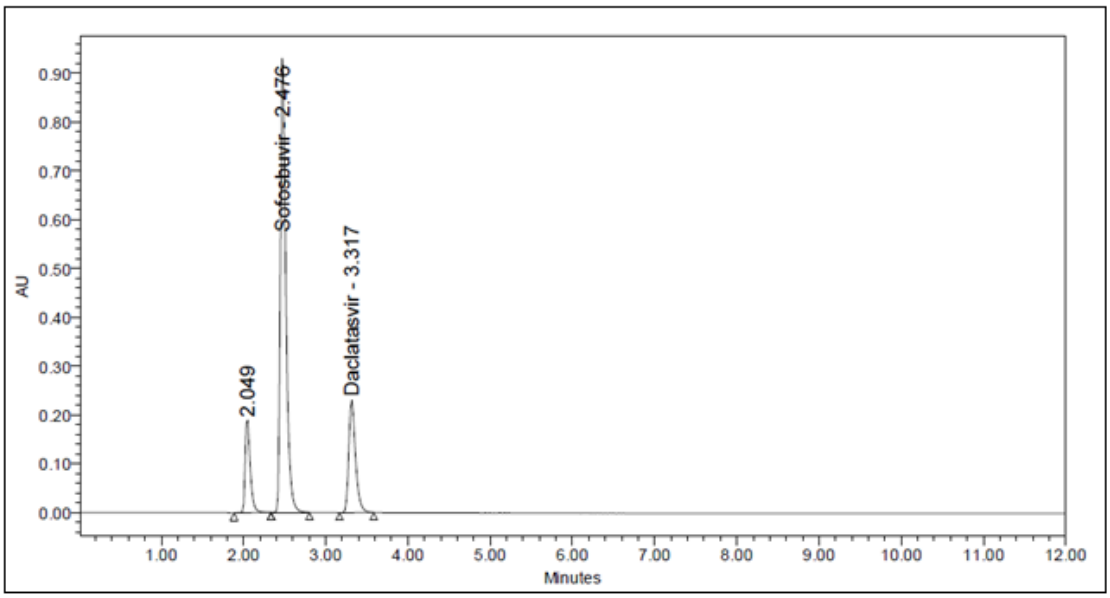

Fig-6C: Peroxide degradation chromatogram

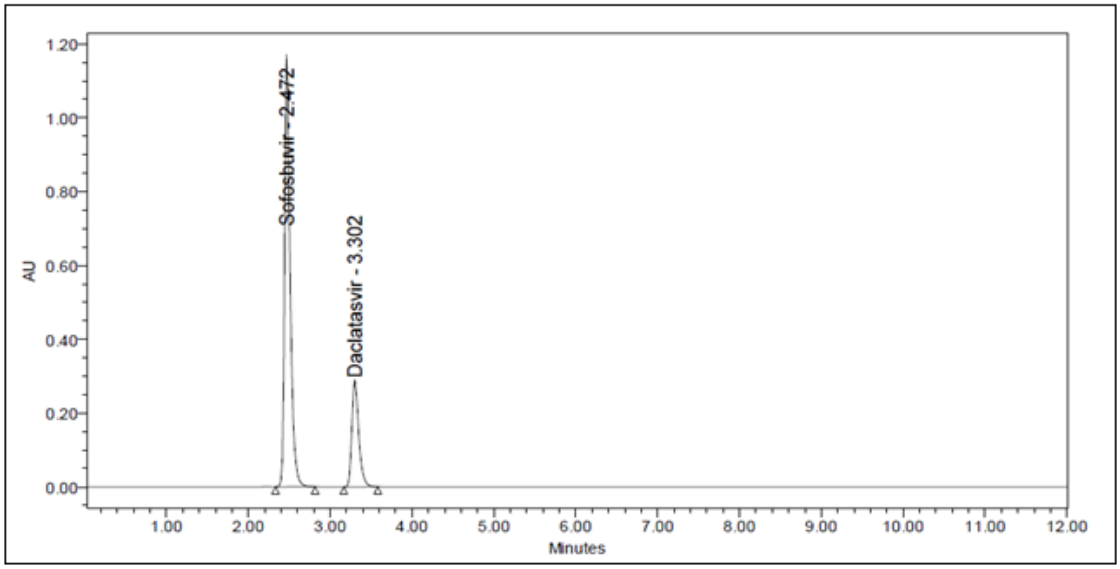

Fig-6D: Water stress study chromatogram

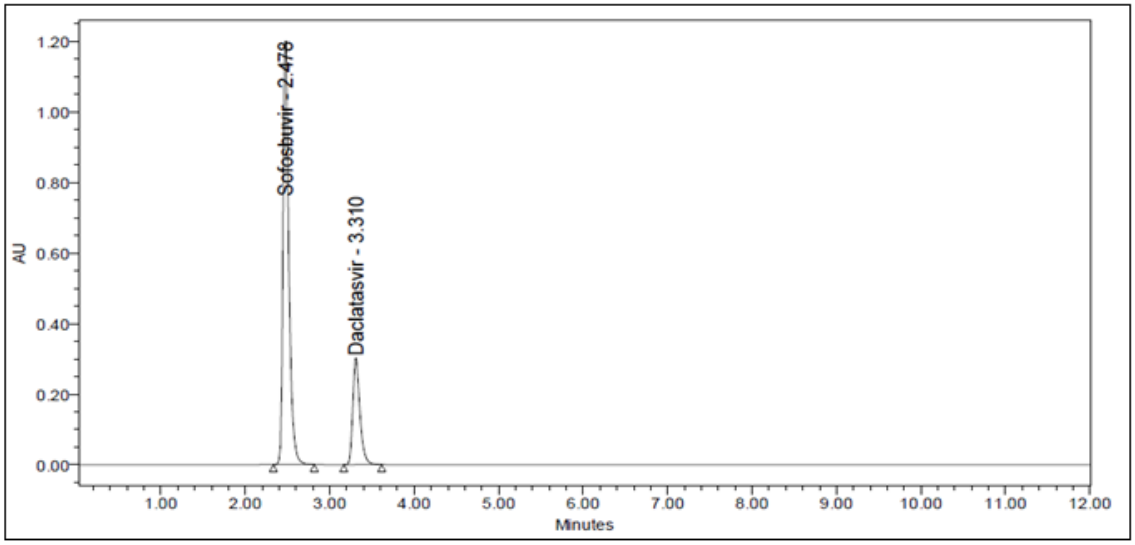

Fig-6E: Photo stability degradation chromatogram

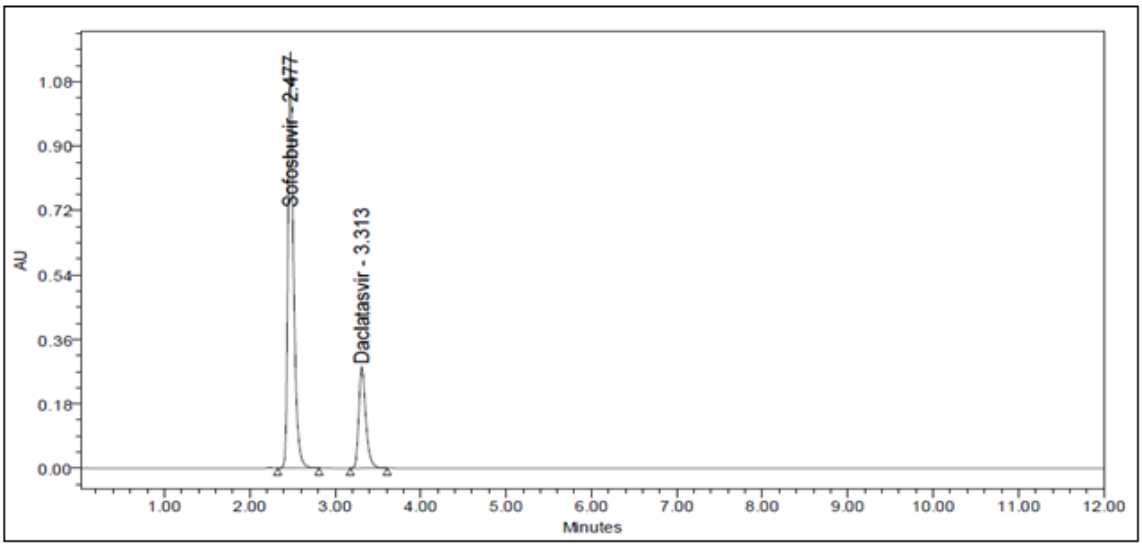

Fig-6F: Dry heat study chromatogram

CONCLUSION

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

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