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NOVEL STABILTY INDICATING RP-HPLC METHOD SIMULTANEOUS DETERMINATION OF SOFOSBUVIR AND VELPATASVIR IN BULK AND COMBINED TABLET DOSAGE FORMS

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ABSTRACT

A accurate and precise RP-HPLC method has been developed for the validated of Sofosbuvir and Velpatasvir in bulk and combined Tablet dosage form. Separation was carried out on a Primesil C_{18} (4.6 x 250mm, 5µm) column using a mixture of Acetonitrile: 0.1%perchloricacid (50:50 v/v) as the mobile phase at a flow rate of 1.2 mL/min, The detection was carried out at 262 nm. The retention time of the Sofosbuvir and Velpatasvir 4.25, 6.05 min respectively. The method produce linear responses in the concentration range of 25-150 µg/mL for Velpatasvir, and 100-600µg/ml of Sofosbuvir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS

Sofosbuvir and Velpatasvir, RP-HPLC, PDA Detection and Validation.

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INTRODUCTON

Sofosbuvir

Sofosbuvir is a medication used for the treatment of hepatitis C. It is only recommended with some combination of ribavirin, peginterferon-alfa, simeprevir, ledipasvir, or daclatasvir. Cure rates are 30 to 97% depending on the type of hepatitis C virus involved. Safety during pregnancy is unclear; while, some of the medications used in combination may result in harm to the baby. It is taken by mouth and chemically it 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 Molecular

formula $C_{22}H_{29}FN_3O_9P$ Molecular Weight 529.453 g/mol and Soluble in Methanol, Acetonitrile and water.

Velpatasvir is an NS5A inhibitor which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.

MATERIALS AND METHODS[13-14]

Materials and Reagents

The reference standards of Sofosbuvir and Velpatasvir were procured from Sura Pharma Labs, Dilshuknagar, Hyderabad, India. The branded tablet formulation Epclusa (sofosbuvir 400 mg and velpatasvir 100 mg) was purchased from the local market. All the HPLC solvents and analytical reagent grade chemicals were purchased from S.D. Fine Chemicals, Hyderabad, India.

Instrumentation

A Waters HPLC system equipped with a 2695 binary pump, an auto sampler and a 2996 photo diode array detector was employed for the study. The output signal was monitored and processed with Empower software.

Chromatographic conditions

The separation of the drugs was achieved on a Discovery® C18 HPLC Column (250 x 4.6 mm; 5µ particle size) by running a mobile phase containing a 50:50 v/v mixture of 0.1% perchloric acid in water and acetonitrile at a flow rate of 1.2 mL/min. The injection volume was 10 µL. The column temperature was maintained at 30°C and the analytes in the eluates were monitored at 262 nm. The run time was 9.0 min. A 50:50 v/v mixture of 0.1% perchloric acid and acetonitrile was used as the diluent to prepare drug solutions.

Preparation of standard solution

Accurately weighed and transferred 25 mg of Velpatasvir and Sofosbuvir working standard into 25 mL of clean dry volumetric flask add about 15mL of Diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Tablet sample solution

Twenty tablets of "Epclusa" (velpatasvir 100 mg and sofosbuvir 400 mg) were accurately weighed and the average weight of the tablet was calculated. The tablets were finely powdered and a quantity of the powder equivalent to one tablet was transferred into a 100 mL volumetric flask. 70 mL of the diluent was added to it and sonicated for 5 minutes. Then the volume was made up with the diluent and mixed well to prepare the sample stock solution. This solution was filtered through a 0.45 µm nylon filter. 2.0 mL of the filtrate was transferred to a 20 mL volumetric flask and the volume made up to give final theoretical concentrations of 100µg/mL and 400µg/mL of Sofosbuvir and Velpatasvir respectively.

Method development and Validation

Different mobile phases were considered for simultaneous separation of the two drugs on a Primesil C_{18} HPLC Column. Selection of the mobile phase was done on the basis of ideal resolution among Sofosbuvir and Velpatasvir and also their impurities formed during forced degradation studies. The required chromatographic conditions were optimized. The developed method was validated for precision, specificity, accuracy (recovery), linearity and robustness as per the ICH guidelines[7].

System Suitability

System suitability was established for initial evaluation of the method before running the sample for the validation parameters. The test was performed according to the USP[10]. The standard solutions prepared as per the proposed method were analyzed. The results of the system suitability study are presented in Figure No.3. The acceptance criterion is % RSD $\leq$ 2.0. A percent % RSD Sofosbuvir and Velpatasvir of 0.26 and 0.17 indicates good system precision of the method. The tailing factor obtained from the standard injection is 2.18 and 1.68 and Theoretical plates obtained from the standard injection are 4881 and 6036 respectively.

Linearity

The linearity were observed for in the concentration rages from 25-150µg/mL for Velpatasvir and 100-600 µg/mL. The Linearity of the method was demonstrated by preparing different concentrations of drug substance and analyzing as per the proposed method. A plot of the area of the peak as a function of analyte concentration was prepared and its regression equation computed. The linearity data of the two drugs are given in Table No.6 and Figure No.4.

From the above the LOD values of Sofosbuvir and Velpatasvir were found to be 0.19 and 0.63µg/m l respectively. The LOQ values of Sofosbuvir and Velpatasvir were found to be 0.59and 1.91µg/ml respectively. Thus the method developed was found to be sensitive.

Precision

In the precision study,% RSD was found to be less than 2 % for Velpatasvir 0.6% and Sofosbuvir 0.2 which indicates the system has a good reproducibility for precision studies 5 replicate studies of Sofosbuvir and Velpatasvir formulation (method precision) was performed.% RSD was determined for peak areas of Sofosbuvir and Velpatasvir and the acceptance limits should be NMT 2% and the results were found to be within the acceptance limits The chromatograms of precision were showed in Figures 7.22-7.26. The results were reported in Table No.7.25.

Accuracy

The accuracy studies were shown as % recovery for Sofosbuvir and Velpatasvir at 50%, 100%, 150%, the limits of recovery should be in range of 98-102% the limits obtained for Sofosbuvir and Velpatasvir were found to be within the limits. Hence the method was found to be accurate. The accuracy studies shows % recovery of the Velpatasvir 100% and Sofosbuvir and the limits of % recovery of drugs were 98-102% and from the above results its indicates that the method was accurate and also revealed that the commonly used excipients present in the pharmaceutical information do not interfere in the proposed method. The chromatograms of shown in results were shown Tables No.7 and 8.

FORCED DEGRADATION STUDIES

Acid degradation

Degradation was observed by the additon of 0.5 N HCl.

Alkaline degradation

Degradation was observed by the additon of 0.5N NaoH.

Thermal degradation

Degradation was observed when the sample solution was kept under heat at 60-80^0 C for 3 hours.

Peroxide degradation

Degradation was observed by the additon of 3% H_2O_2.

Hydrolysis degradation

Degradation was observed by sunlight exposre.

Table No.1: Optimized Chromatographic condition

S.No	Parameters	Chromatographic conditions
1	Mobile phase ratio	Acetonitrile: Water(50:50% v/v)
2	Column	Primesil C_{18} (4.6×250mm) 5µ
3	Detector	UV-VIS Detector
4	Column temperature	Ambient
5	Wavelength	262 nm
6	Flow rate	1.2 ml/min
7	Injection volume	10 µl
8	Run time	9 minutes

Table No.2: Result of system suitability parameters

S.No	Parameter	Sofosbuvir	Velapatasvir
1	Retention time	4.23	6.05
2	Theoretical plates	6036	4881
3	Tailing factor	1.68	2.18
4	Area	2275297	1815105

Table No.3: Linearity Results of Velpatasvir

S.No	Linearity Level	Concentration (µg/mL)	Peak Area
1	1	25	80022
2	2	50	1443902
3	3	75	2139373
4	4	100	2856272
5	5	125	3589707
6	6	150	4220592
	Correlation coefficient	0.999%	

Table No.4: Linearity Results of Sofosbuvir

S.No	Linearity Level	Concentration (µg/mL)	Peak Area
1	1	100	1316161
2	2	200	2730754
3	3	300	4257943
4	4	400	5904229
5	5	500	7185983
6	6	600	8790138
	Correlation coefficient	0.999%	

Limit of detection and Limit of Quantification (LOD and LOQ)

Table No.5: Data of LOD and LOQ

S.No	Drug	LOD	LOQ
1	Velpatasvir	2.74	8.31
2	Sofosbuvir	7.71	23.37

Table No.6: Data of precision

S.No	No. Injections	Velpatasvir Peak Area	Sofosbuvir Peak Area
1	Injection1	2475114	6397432
2	Injection2	2475284	6396243
3	Injection3	2528371	6468822
4	Injection4	2515584	6462955
5	Injection5	2515624	6463145
6	Injection6	2515624	6462841
7	Average	2504268	6438573
8	S.D	23052	34985
9	% RSD	0.92	0.62

Table No.7: Accuracy Results of Velpatasvir

S.No	%Concentration (at specification Level)	Peak Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Mean Recovery
1	50%	2075760	50	49.9	99.8	99.6%
2	100%	2676350	100	99.8	99.8	
3	150%	3064030	150	151.0	100.1	

Table No.8: Accuracy Results of Sofosbuvir

S.No	%Concentration (at specification Level)	Peak Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
1	50%	8971513	50	49.9	99.8%	99.4%
2	100%	12607635	100	99.8	99.4%	
3	150%	14838255	150	150.1	99.2%	

Robustness

Table No.9: System suitability data of Results for Sofosbuvir and Velpatasvir

S.No	Flow Rate (ml/min)	Name of drugs	System Suitability Results		
			USP Plate count	USP Tailing	Retention time(min)
1	1.2mL/min	Sofosbuvir	8971513	2.18	4.23
		Velpatasvir	2075760	1.68	5.78

Table No.10: Data of degradation studies

S.No	Type of degradation	Area of sample		Assay content (% w/w)	
		Velpatasvir	Sofosbuvir	Velpatasvir	Sofosbuvir
1	Acid (0.5N HCl)	859527	2831919	89.02	94.6
2	Base (0.5N NaOH)	968847	2904346	91.4	96.3
3	Peroxide (3% H_20_2)	890779	28036451	91.2	94.9
4	Thermal (at 60-80^0 c)	975570	2884978	93.2	95.3
5	Hydrolysis	1038182	3036541	92.9	95.7

Table Summary for RP-HPLC Method

S.No	PARAMETERS	ACCEPTANCE CRITERIA	RESULTS OBTAINED
1	System suitability	Theoretical Plates- NLT 2000	Velpa- 4881 Sofos-6036
		Tailing factor - NMT 2	Velpa -2.18 Sofos -1.68
		Retention time	Velpa -5.78 Sofos -4.23
2	Precision	% RSD of Velpa -NLT 2 % RSD of Sofos -NLT 2	Velpa -0.92 Sofos -0.62
3	ID Precision	% RSD of Velpa -NLT 2 % RSD of Sofos -NLT 2	DAY-1 Velpa -0.83 Sofos -0.73 DAY-2 Velpa -1.02 Sofos -0.87
4	Linearity	Correlation coefficient NLT 0.999	Velpa -0.999 Sofos -0.999
5	Accuracy	Percentage Recovery 98-102%	Velpa -99.6% Sofos -99.4%
6	Limit of Detection	1:3	Velpa -2.74µg/ml Sofos – 7.71 µg/ml
7	Limit of quantitation	1:10	Velpa -8.31µg/ml Sofos – 23.37µg/ml

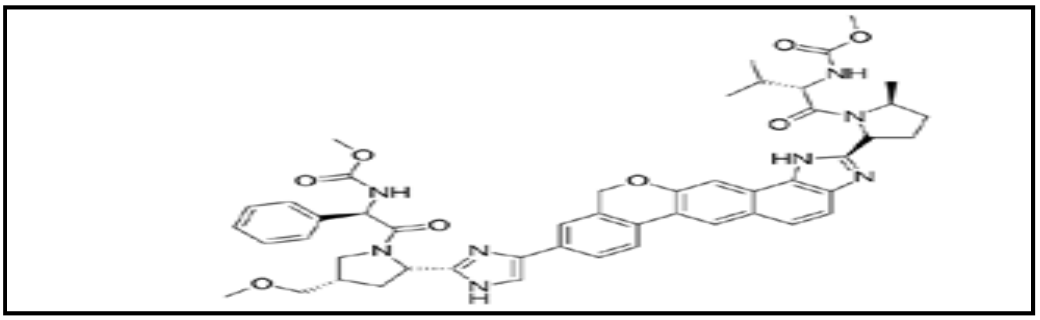

Figure No.1: Chemical Structure of Velpatasvir

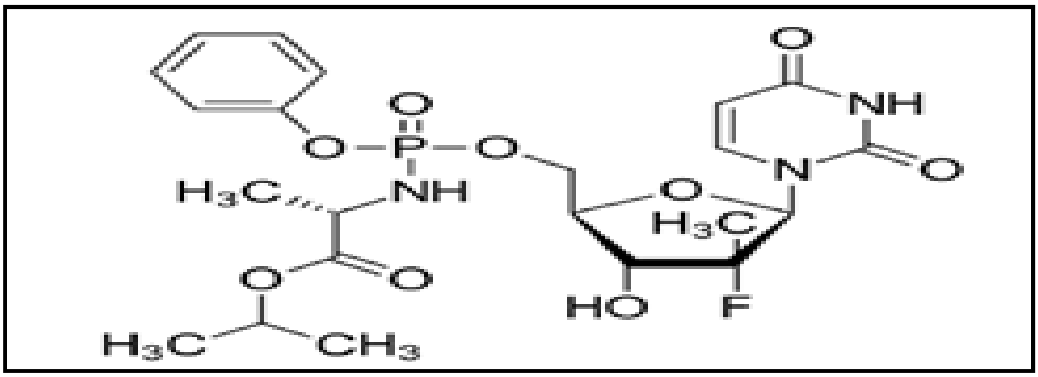

Figure No.2: Chemical structure of Sofosbuvir

Specificity

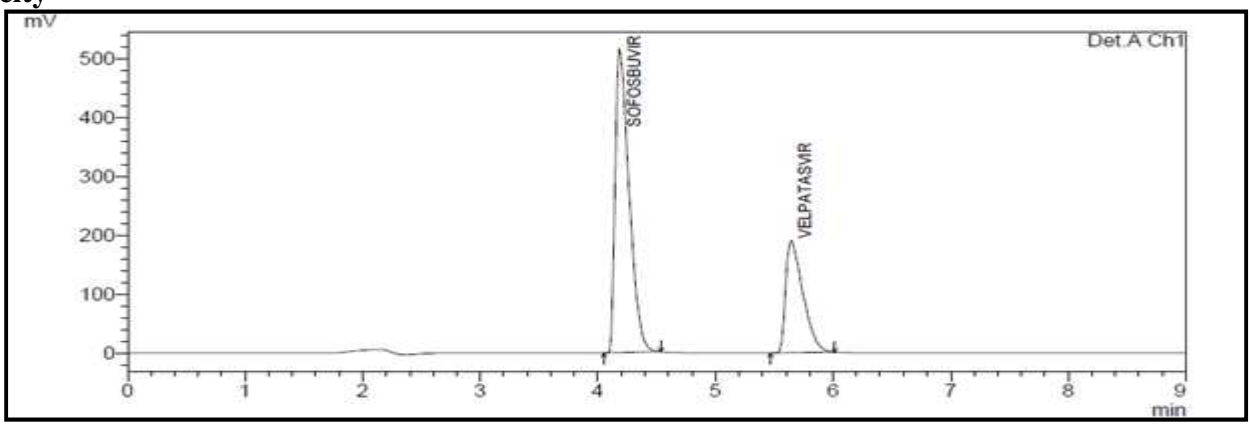

Figure No.3: Typical chromatogram of Sofosbuvir and Velpatasvir

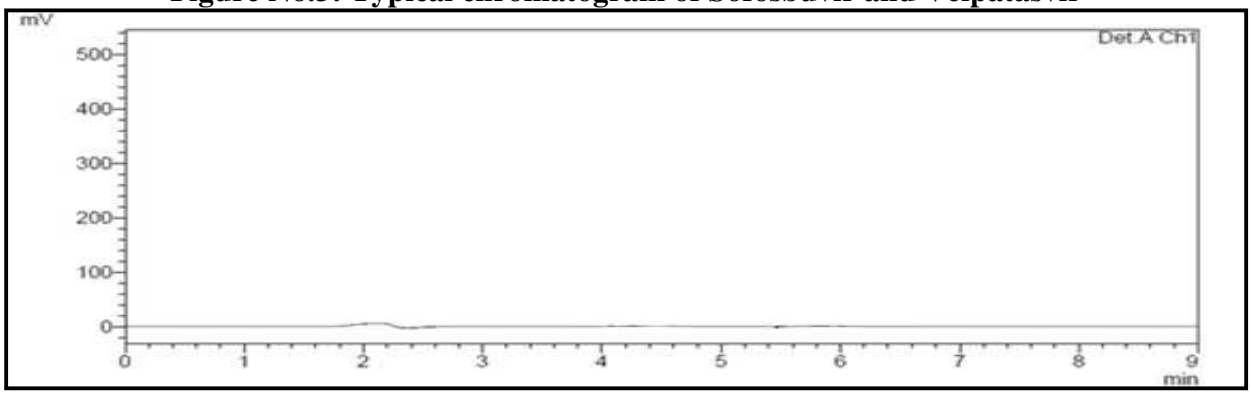

Figure No.4: Chromatogram of blank (Solvent system)

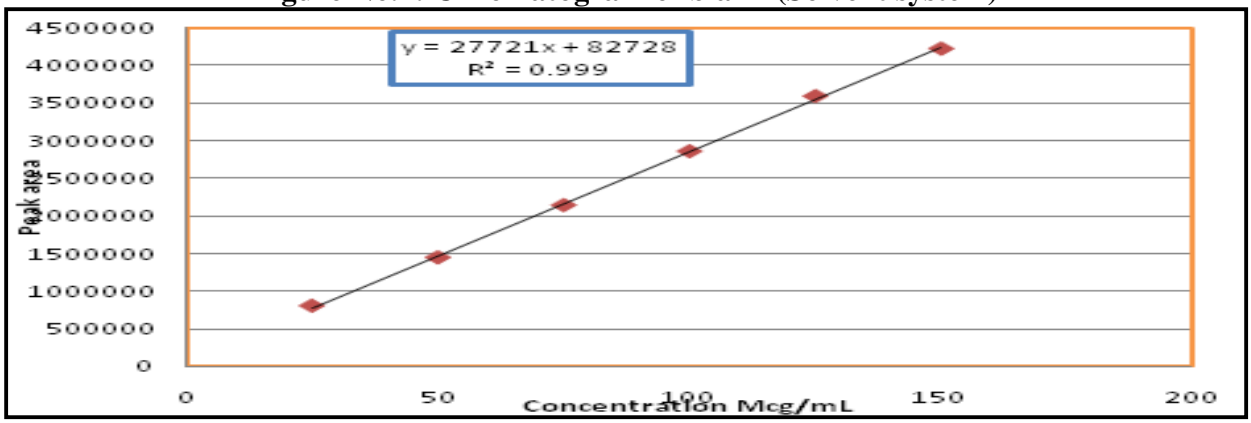

Figure No.4: Calibration Curve for Velpatasvir

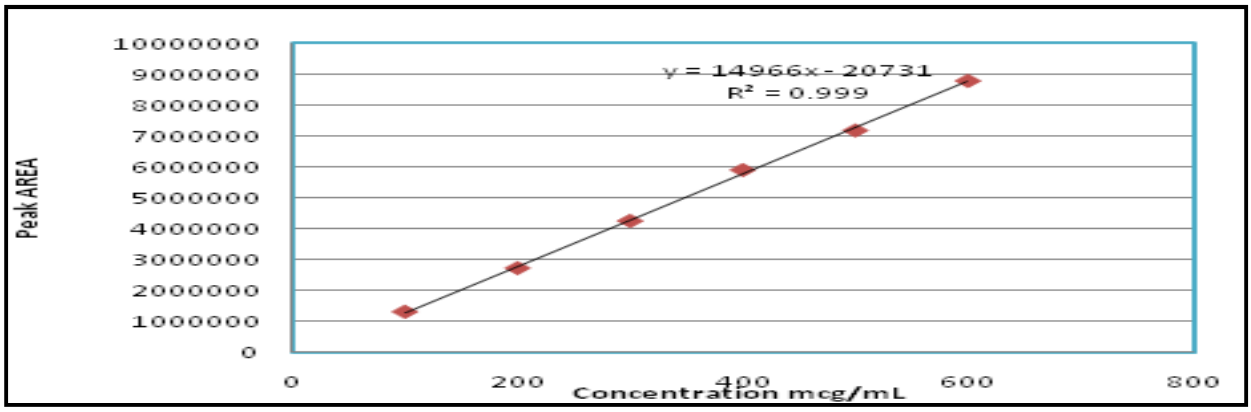

Figure No.5: Calibration Curve for Sofosbuvir

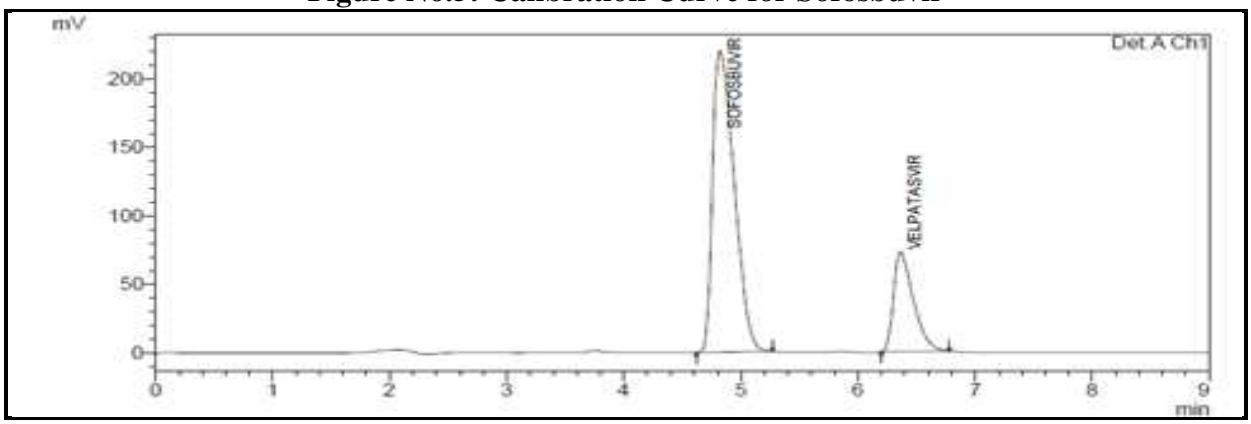

Figure No.6: The chromatogram showing effect of acid degradation

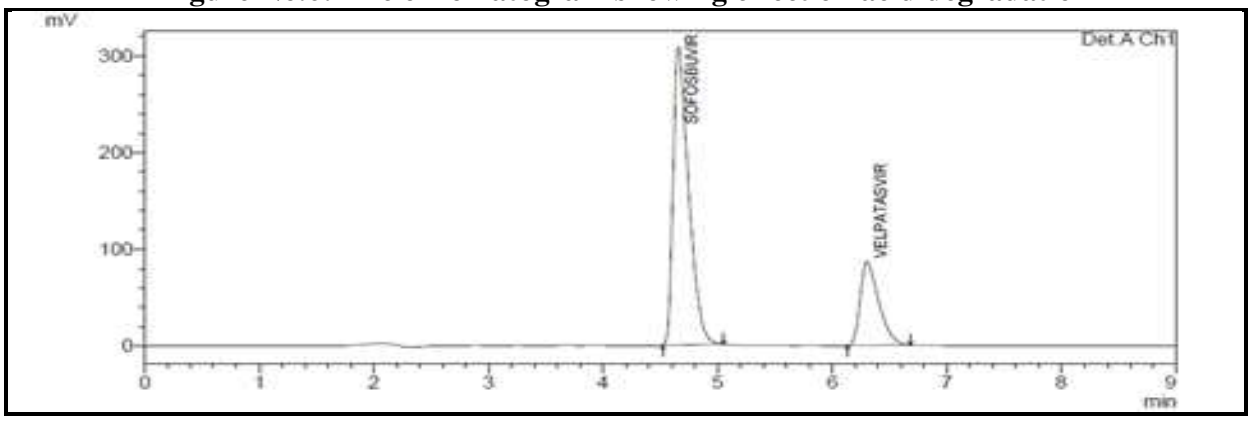

Figure No.7: The chromatogram showing effect of Alkaline degradation

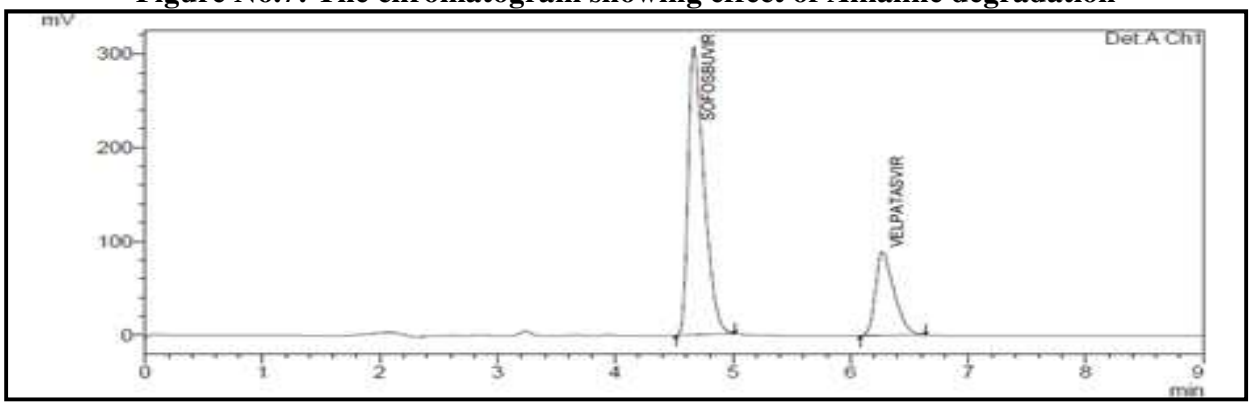

Figure No.8: The chromatogram showing effect of Thermal degradation

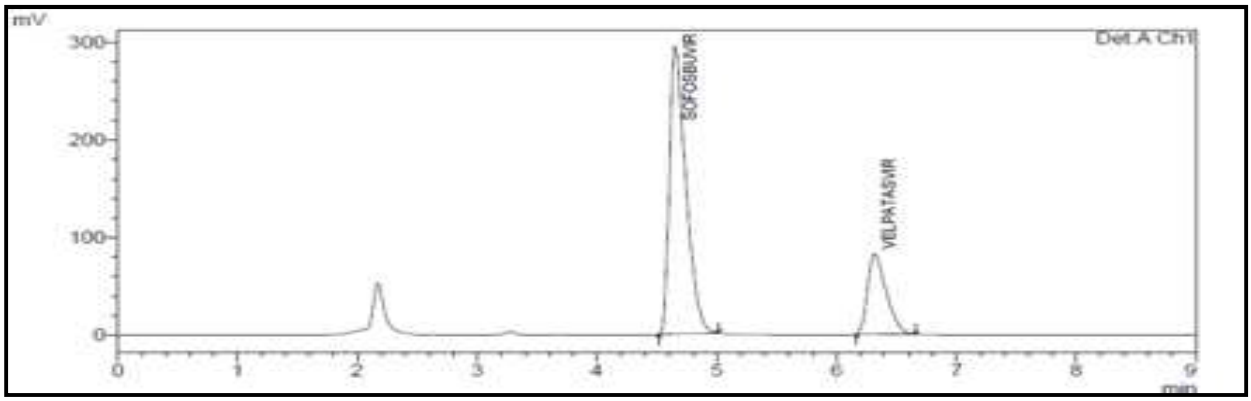

Figure No.9: The chromatogram showing effect of peroxide degradation

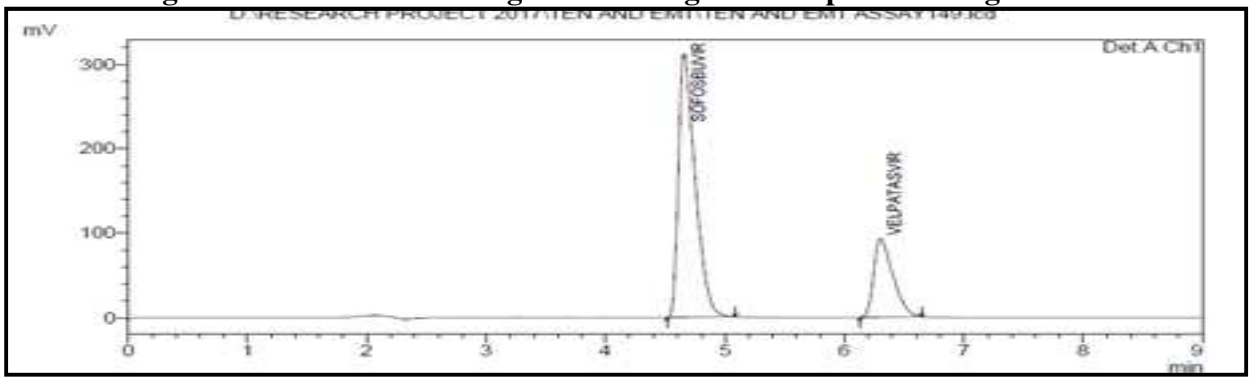

Figure No.10: The chromatogram showing effect of Photolytic degradation

SUMMARY AND CONCLUSION

SUMMARY

RP-HPLC method was developed for simultaneous estimation of Sofosbuvir and Velpatasvir in pharmaceutical dosage form. Chromatographic separation was performed on Premisil C18 (4.6×250mm) 5µ column, with mobile phase comprising of mixture of Acetonitrile: 0.1%Perchlioric acid in the ratio of 50:50% (v/v), at the flow rate 1.2ml/min. The detection was carried out at 262nm.

CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Sofosbuvir and Velpatasvir in tablet dosage form. It was also proved to be convenient and effective for the determination of Sofosbuvir and Velpatasvir in the bulk and combined dosage form. It inferred the method found to be simple, accurate, precise and linear. The method was found to be have a suitable application in routine laboratory analysis with high degree of accuracy and precision.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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