International Journal of Psychosocial Rehabilitation, Vol.24, Issue 09, 2020
ISSN: 1475-7192

Degradation Study Of Sofosbuvir In Bulk Drug And Tablet Dosage Form By RP-High Performance Liquid Chromatography

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Abstract
Sofosbuvir were subjected to forced degradation conditions and the formed degradation products were well separated and resolved from the drug and excipients on SunFire C18 column (250 × 4.6 mm, 5 µ) using blend of Acetonitrile: Water (70: 30 v/v) pH 7.0 in isocratic mode at a flow rate of 0.8mL/min at an ambient temperature of 28 °C with the detection wavelength at 260nm. The retention time of Sofosbuvir was found 3.22 min. The linearity was performed in the concentration range of 20-100 ppm with correlation factor of 0.999 for Sofosbuvir. The percentage purity of Sofosbuvir tablet was found 99.98%. The drug was found to degrade under acid, alkali and oxidative condition but found stable under dry heat and photolytic conditions.

Keywords: *Sofosbuvir, RP-HPLC, Method Development, Method Validation, Stability.*

Introduction

Antiviral treatment to continual hepatitis C virus contaminated patients Sofosbuvir is used in amalgamation therapy among other with HCV genotypes 1-6, as well as to treat HCV with HIV tainted patients. [1].
Present work aim and objectives study degradation, developed as well as validation for the evaluation of Sofosbuvir in Bulk and Tablet dosage form.
Rani, J. S. and n. Devanna[2] ,Nebsen, M. and E. S. Elzanfaly [3],Bandla, J. and S. Ganapaty [4] ,Annapurna, M. M. [5] and Mastanamma, S., et al. [6] above research papers where reported on single drug or in combination but these methods not economic so developed economic and simple method for estimation of sofosbuvir in tablet dosage form as well as bulk drug.
The paper mainly focuses on how degradation products were well separated and resolved from the drug and excipients on SunFire C18 column (250 × 4.6 mm, 5 µ) using blend of Acetonitrile: Water (70: 30 v/v) pH 7.0 in isocratic mode at a flow rate of 0.8mL/min at an ambient temperature of 28 °C with the detection wavelength at 260nm.For estimation of sofosbuvir we refer some articles for research purpose [7-13].

Material And Methods

Pharmaceutical grade Sofosbuvir was kindly supplied as gift sample from Mylan Laboratories Ltd was used without any further purification. All the chemicals were AR Grade and purchased from S.D. Fine Chemicals, Mumbai (India), Membrane Filter was used of 0.45µ purchased from Pall India Ltd. Mumbai (India). Fourier-transform infrared spectroscopy Spectra were recorded on SHIMADZU IR-Affinity-1 FT-IR

Preparation of Standard Stock Solution

Quantity equivalent to 10mg of Sofosbuvir was weighed and dissolved in methanol to make 10ml. This gave 1000 µg/ml of standard stock solution of Sofosbuvir.

Preparation of calibration curve standards

The above standard stock solution (1000 µg/ml) of Sofosbuvir was diluted with mobile phase to yield five calibration curve (CC) standards with concentrations of 20, 40, 60, 80 and 100 µg/ml.

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Method Validation

Analytical method validation was carried out as per International Council for Harmonisation method validation guidelines Q2 (R1).

Analysis of marketed formulation

To determine the content of Sofosbuvir Tablet (SOFOCAST) Label claim 400mg A quantity of Powder equivalent to 500 mg of Sofosbuvir was transferred to 100 mL volumetric flask add 25 mL methanol and sonicate for 10min. Filtered with Whatman filter paper and the volume was made up to the mark with the same solvent. Pipette out the 0.05 ml solution in 10 ml volumetric flask was diluted up to 10 mL with mobile phase and subjected to chromatographic analysis stated chromatographic condition. The amount of Sofosbuvir was obtained from the regression equation of the calibration curve[8-11].

Results And Discussion

The UV Absorption spectrum of Sofosbuvir showed a λmax at 260nm in methanol shown in figure1.

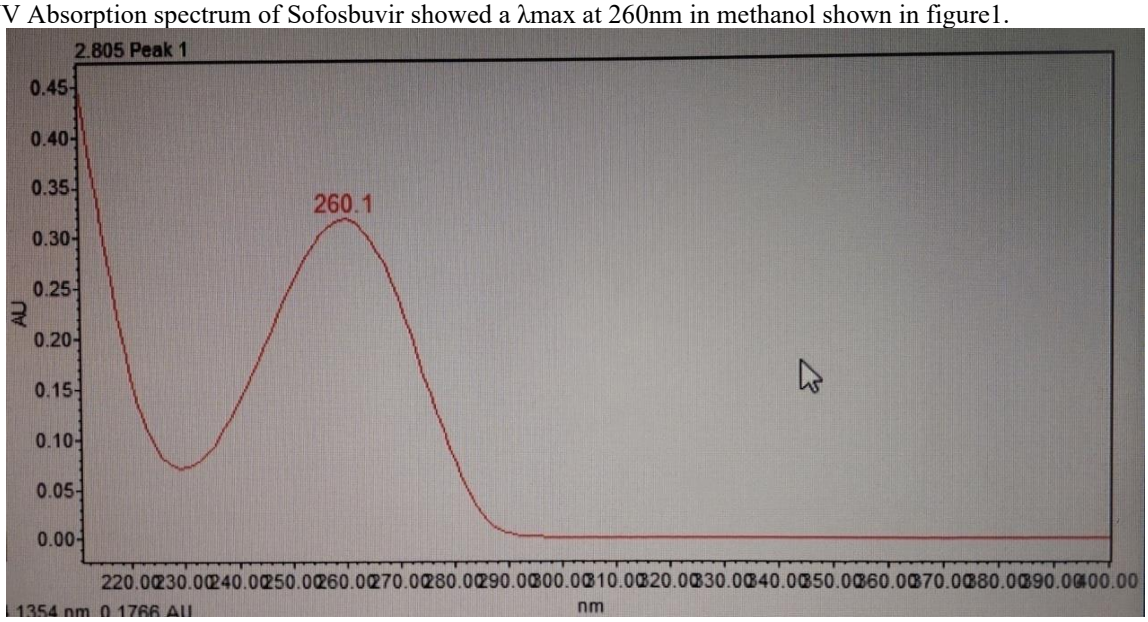

Figure 1: UV Spectrum of Sofosbuvir

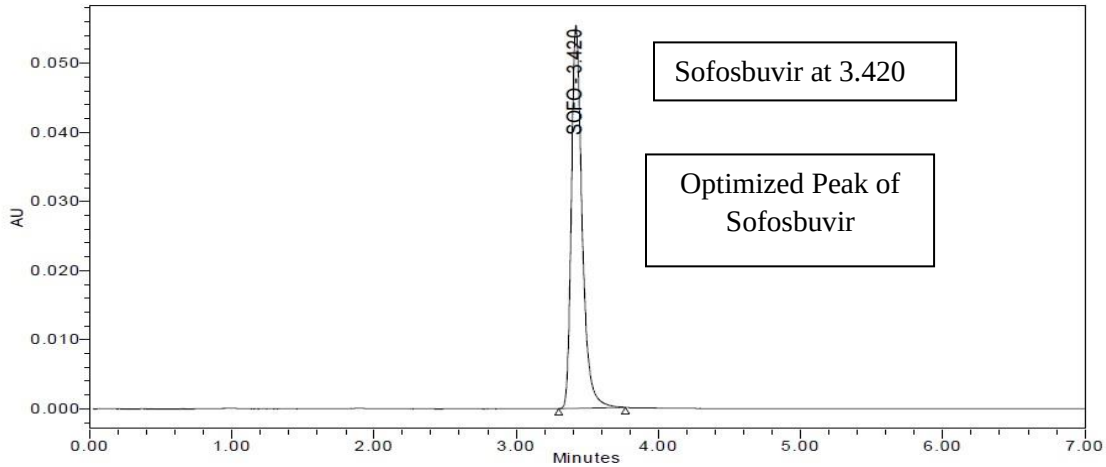

Figure 2: Representative Chromatogram of Sofosbuvir in Acetonitrile: Water (70: 30 v/v) pH 7.0, 0.8ml/min

System suitability test were evaluated for five replicate injection of drug at a concentration of 10 µg/ml. the result given table were within acceptable limits.

Forced Degradation of Sofosbuvir
Acid Degradation

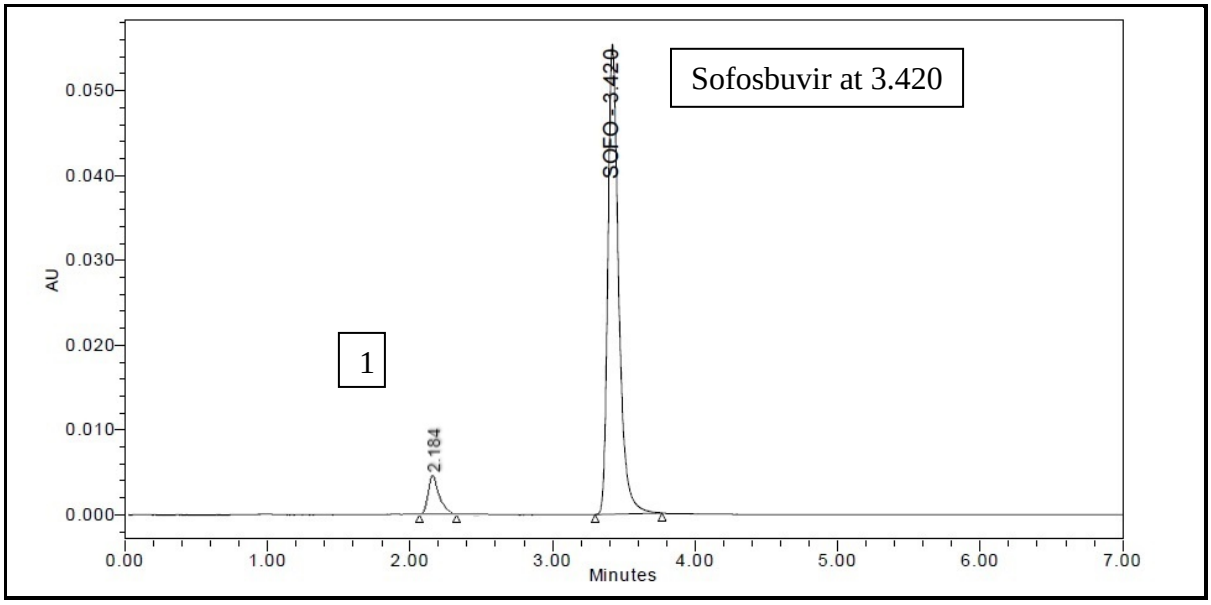

Figure 3: Acid Treated chromatogram of Sofosbuvir (1 N HCl for 1 h reflux) Peak 1: Degradant at RT: 2.184, Sofosbuvir RT: 3.420

Alkali Degradation

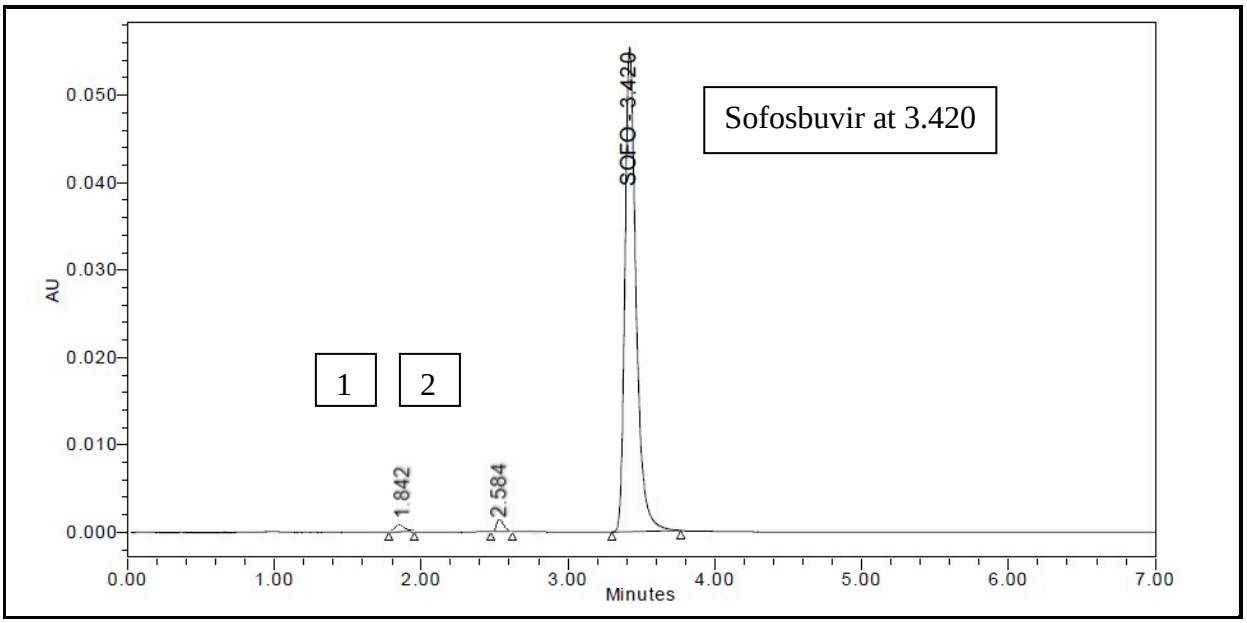

Figure 4: Representative chromatogram of Alkali treated Sofosbuvir (1 N NaOH for 24 Hrs) Peak 1: Degradant one RT: 1.842min, Peak 2: Degradant two RT: 2.584min, Sofosbuvir at RT 3.421

Oxidative Degradation

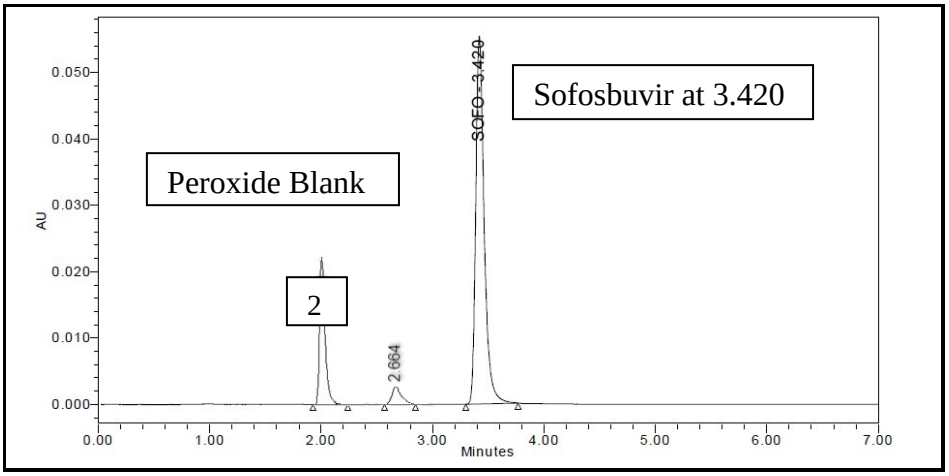

Figure 5: Representative chromatogram of oxidative treated Sofosbuvir (3 % H_2O_2 for 24 Hrs) Peak 1: Peroxide Blank at RT: 2.061, Peak 2: Degradant 2 at RT: 2.664, Peak 3: Sofosbuvir at RT: 3.420.

Photolytic Degradation

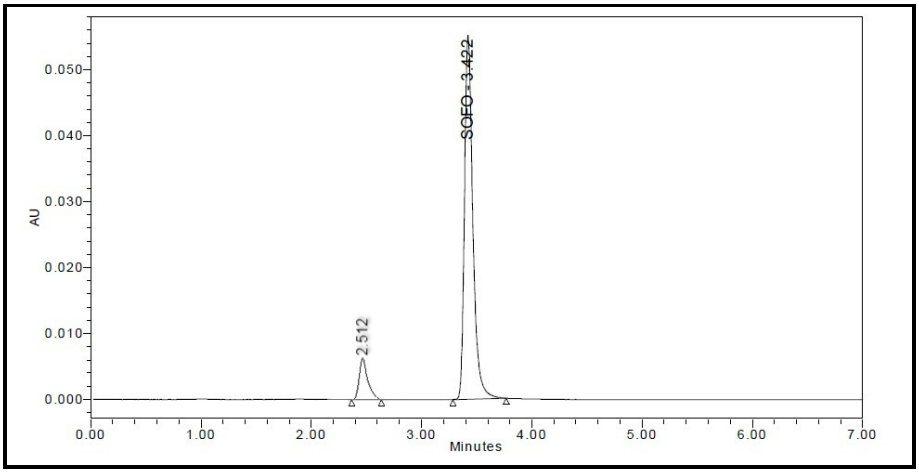

Figure 6: Representative chromatogram of photolytic degradation of Sofosbuvir (Kept in sunlight for 1 month) Peak 1: Sofosbuvir at RT: 3.420 Peak 2:512

Thermal Degradation

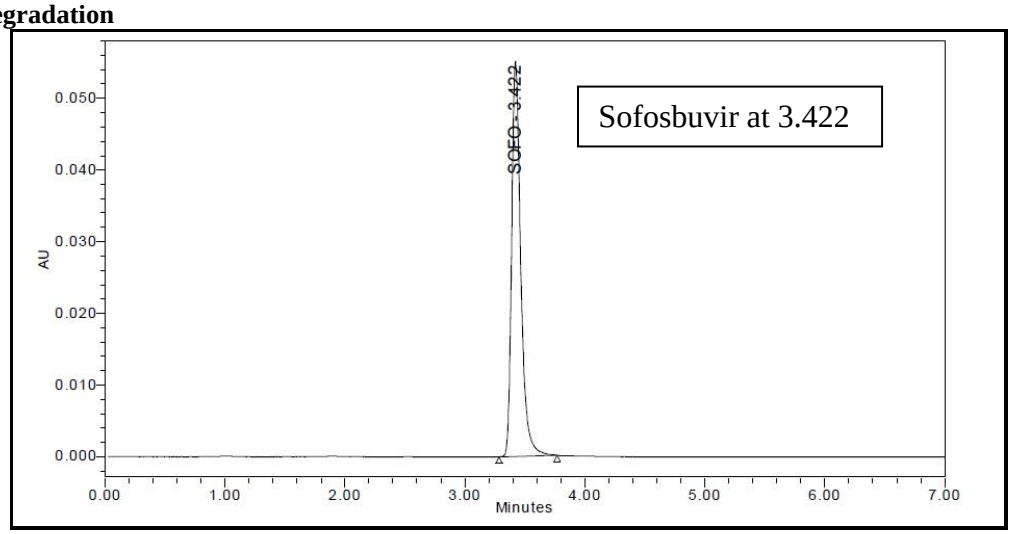

Figure 7: Representative chromatogram of dry heat degradation of Sofosbuvir (Kept in oven for 8 Hrs at 80 °C Temperature) Sofosbuvir RT: 3.422

Table 1: Forced Degradation Results

Sr. No	Stress Condition	Drug peak area at zero time sample	Drug peak area of stressed sample	Retention time of degradation product (min)	% Degradation
1	Acid Hydrolysis (1 N HCl of 1Hr)	290035	261408	1.842	9.87
2	Alkali Hydrolysis (1 N NaOH of 24 Hrs)	290035	269355	1.842, 2.584	7.13
3	Oxidative (3% v/v H_2O_2) in direct room temperature	290021	256304	2.664	11.63
4	Photolytic (exposed to direct sunlight for 1 Month)	290021	264181	2.512	8.91
5	Dry Heat 80 °C (Kept in oven for 8 Hrs)	290021	282683	No Degradation Peak	Stable

Table 2: Calibration Curve of Sofosbuvir

Sr. No	Concentration	Area
1	20	290022
2	40	581144
3	60	881070
4	80	1161087
5	100	1460225
	Slope	14602
	Intercept	1395
	Correlation	0.9999

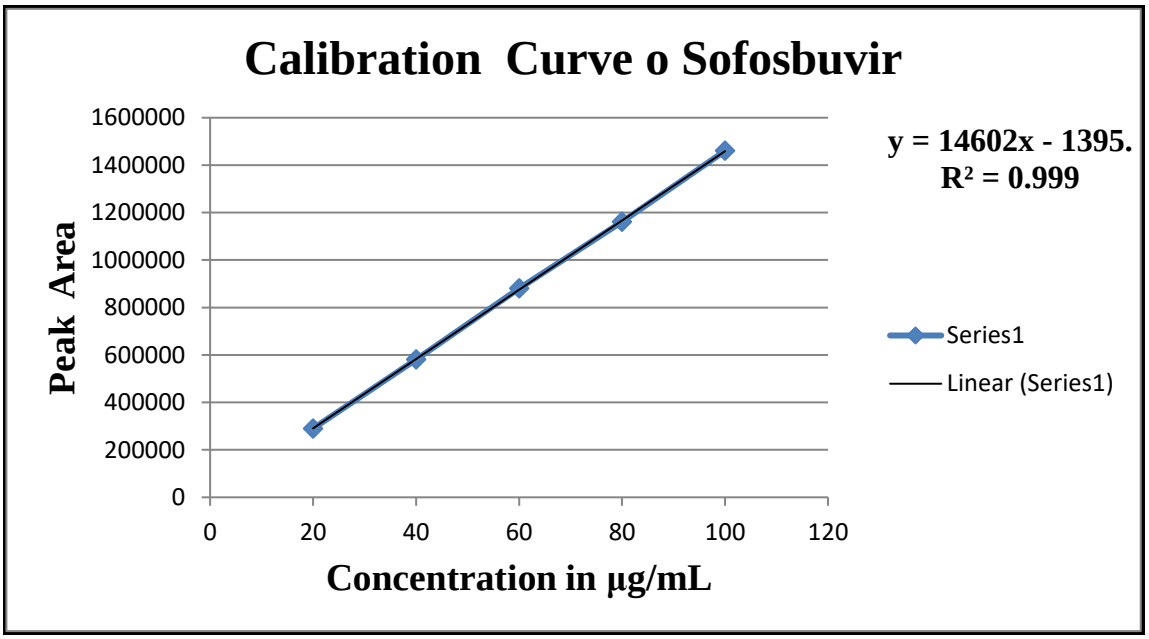

Figure 8: Calibration Curve of Sofosbuvir

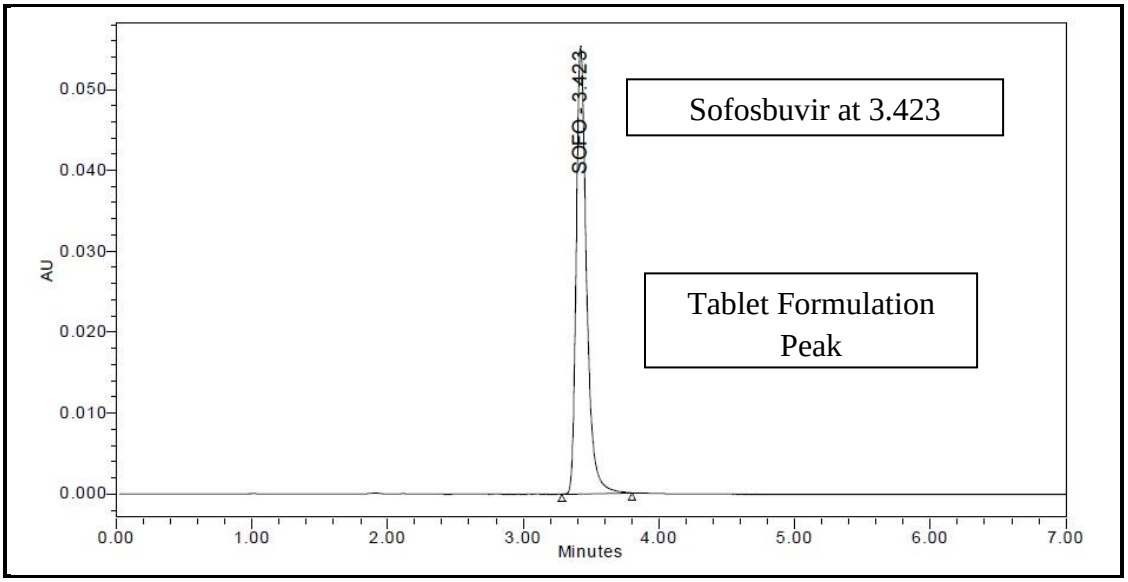

Figure 9: Chromatogram of Tablet formulation of Sofosbuvir

Table 3: Assay of Tablet of Sofosbuvir

Sr. No	Area of Std	Area of Sample	% Assay	Average % Assay	Std Deviation	% RSD
1	2900760	2899310	99.95	99.943	0.04041	0.04043
2		2900179	99.98			
3		2897859	99.90			

Conclusion

Analysis of Sofosbuvir was carried out using SunFire C18 Column (250×4.6mm, 5µ) during the stress studies Sofosbuvir was found to degrade under acidic, alkaline, oxidative condition but stable at to dry heat and photolytic condition. The degradation products and tablet excipients were well resolved from the drug using mobile phase of Acetonitrile: Water (70:30 v/v). The detection wavelength was 260nm.

The developed method was validated as per ICH Guidelines. The method was found to be accurate, precise, robust and linear in the range of 20-100µg/ml.

The LOD and LOQ were found to be 1.22µg/ml and 3.69µg/ml respectively. It can be concluded that the HPLC method developed for Sofosbuvir is capable of discriminating between the drug and degradation products. The method has the necessary accuracy and precision in the range tested can be used in routine quality control and stability studies for the assay of Sofosbuvir from tablet formulations.

Furthermore work will be carried to identify the degradation product formed as a result of normal storage condition and accelerated stability study and identification and characterization of the degradation products.

Declarations

Funding: Self Funding

Conflict of interest: No

Ethical approval: Not Required

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