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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UV SPECTROSCOPIC METHOD FOR ESTIMATION OF SOFOSBUVIR

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ABSTRACT

A stability-indicating UV Spectrophotometric method has been developed for analysis of the drug in the presence of the degradation products and is validated as per ICH Q2 R1 guidelines. Sofosbuvir in water shows maximum absorbance at 260.5 nm. The data of linear regression analysis indicated a good linear relationship over the range of 10-100 µg/ml concentrations with a correlation coefficient (R^2) of 0.9984. The LOD and LOQ were found to be 0.269 µg/ml and 0.814 µg/ml respectively. A recovery of Sofosbuvir in tablet formulation was observed in the range of 99.524-101.208 %. Percentage assay of Sofosbuvir tablets was found to be in the range of 99.812-101.740 %. Sofosbuvir was subjected to different stress testing conditions. Degradation of Sofosbuvir was mainly found in alkaline condition. The developed method was found to be simple, accurate and precise for analysis of Sofosbuvir and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

KEY WORDS

Method development, Sofosbuvir, Stability indicating method, Ultraviolet spectroscopy, Validation.

INTRODUCTION:

Sofosbuvir is a direct acting pyrimidine nucleotide analog representing the first NS5B HCV polymerase inhibitor. The drug is approved by the US FDA and the European Medicines Agency and has become commercially available for the treatment of Hepatitis C in the US in late 2013 and in several European countries in early 2014. Sofosbuvir is used in the treatment of chronic HCV genotype 1, 2, 3, or 4 infections in adults, including those with hepato cellular carcinoma awaiting liver transplantation and those with HIV co-infection. Limited data is available for treatment of chronic HCV infection caused by genotype 5 or 6. Chemically it is (S)-Isopropyl 2- ((S) - (((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetra hydrofuran-2-yl) methoxy) -(phenoxy) phosphoryl amino) propanoate. [1] (www.drugbank.ca/drugs/DB08934).

Literature survey reveals that several analytical methods have been reported for the estimation of Sofosbuvir in pharmaceutical dosage form including spectroscopic methods[2-4] and high performance liquid chromatography (HPLC) in single as well as in combination with other drugs,[5-7] high performance thin layer chromatography,[8] ultra-high performance liquid chromatography[9-10] and in biological fluids by RP-HPLC.[11] Although few reports are available on stability indicating HPLC methods in single as well as in combination with other drugs,[12-16] stability indicating HPTLC methods[17] but no method is available on stability indicating UV spectroscopy hence we have tried to develop stability indicating UV spectroscopic method for estimation of Sofosbuvir in bulk and pharmaceutical dosage form. The present work describes a simple stability indicating UV spectroscopic method for the determination of Sofosbuvir in bulk and pharmaceutical dosage form (MyHep™-400mg) according to the

International conference on harmonization (ICH) guidelines.

MATERIALS AND METHODS:

Reagents and chemicals:

The formulation MyHep™ tablets labeled to contain Sofosbuvir 400 mg was procured from local market. AR grade water was collected at college using ELGA water purification system. Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH); all AR grade were purchased from LobaChemie Pvt. Ltd., Mumbai.

Preparation of standard stock solution:

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 10 ml of water to get concentration of 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with water to get concentration of solution 100 µg/ml. Further 2 ml of this solution was diluted to 10 ml with water to get concentration of solution 20 µg/ml.

Selection of detection wavelength:

From the standard stock solution (1000 µg/ml) further dilutions were made using water and scanned over the range of 200-400 nm and the spectra were obtained. It was observed that the drug showed linear, stable and considerable absorbance at 260.5 nm. Representative UV spectrum of Sofosbuvir is shown in Fig. 1.

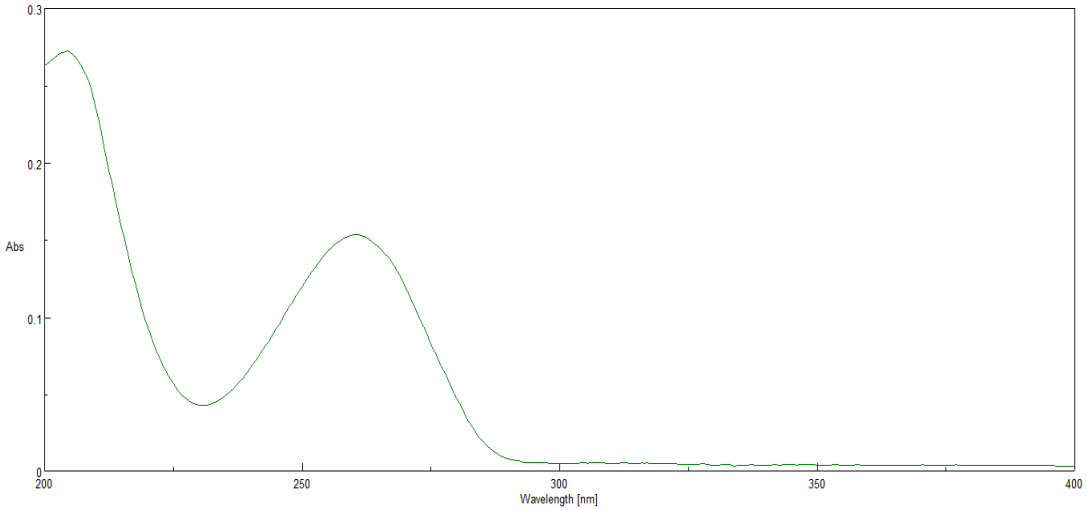

Fig. 1: The UV spectrum of Sofosbuvir (10 µg/ml).

Table 1: Intra-day variation studies data for Sofosbuvir.

Replicates	Conc. (µg/ml)		
	20	30	40
1	0.300	0.454	0.600
2	0.301	0.451	0.600
3	0.302	0.457	0.610
Mean	0.301	0.454	0.603
SD	0.001	0.003	0.006
%RSD	0.419	0.692	0.935

Preparation of sample solution:

20 tablets each containing 400 mg of Sofosbuvir (MyHep™- 400 mg) was weighed and powdered. A quantity of powder equivalent to 10 mg of Sofosbuvir was transferred to a 10 ml volumetric flask containing 5 ml of water. The mixture was ultra-sonicated for 10 min and the resulting sample stock solution was filtered with Whatman filter paper- 41 and the volume was made up with the water to get concentration of 1000 µg/ml. From this solution 1 ml was diluted to 10 ml with water to get concentration of solution 100 µg/ml. Further dilution in water was done to get concentration 20 µg/ml.

STRESS DEGRADATION STUDIES OF BULK DRUG: [18]

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like acidic, alkaline, neutral hydrolysis, oxidation, dry heat and photolytic degradation. Dry heat and photolytic degradation were carried out in the solid state. All studies were carried out at concentration level of 100 µg/ml.

Alkaline hydrolysis:

To 1 ml stock solution of Sofosbuvir (1000 µg/ml), 1 ml of 0.1 N NaOH was added. The above solution was kept for 26 hours at room temperature. After exposure the volume was made up to 10 ml with water to get the concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under alkaline hydrolysis, percent recovery obtained for Sofosbuvir was 70.17%. The representative UV spectrum is shown in Fig. 2.

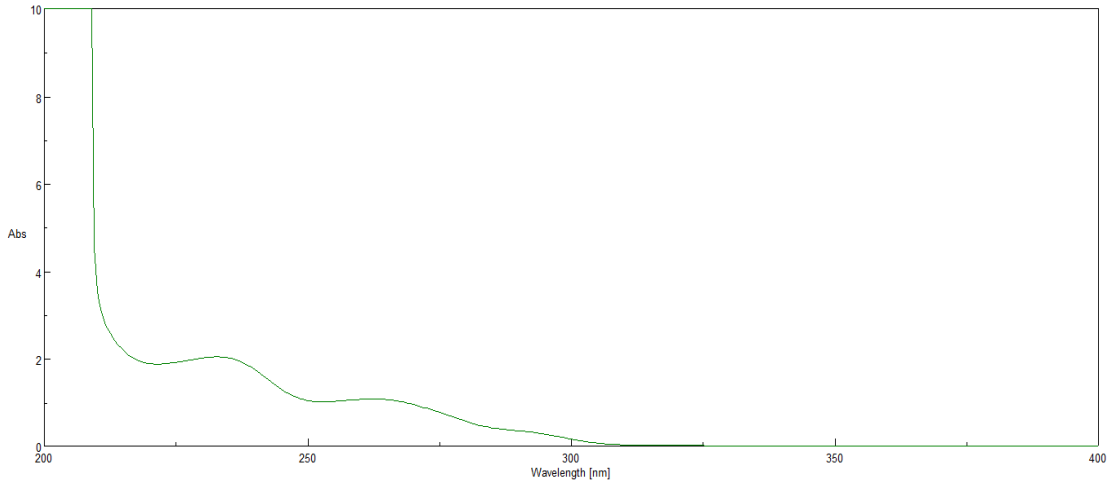

Fig. 2: UV spectrum of Sofosbuvir after alkaline degradation.

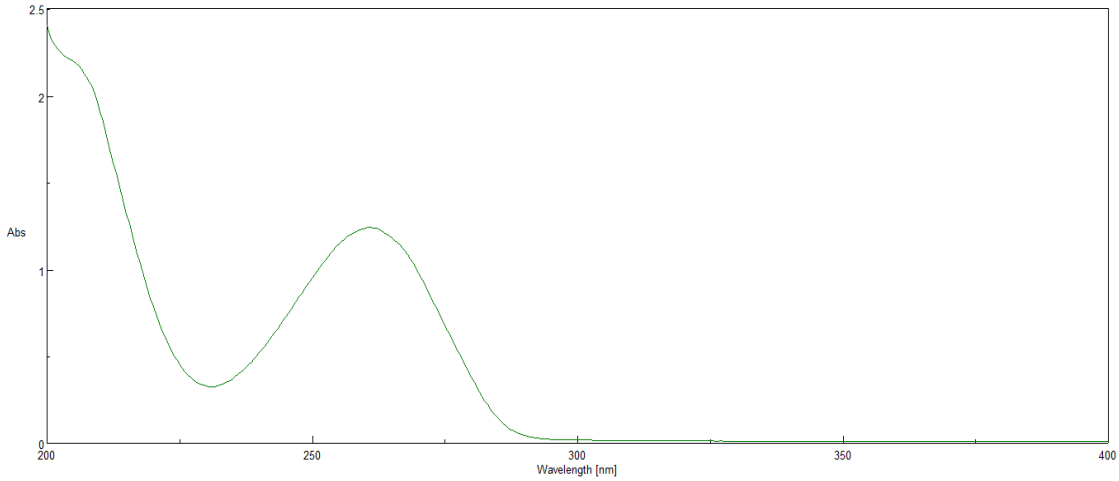

Fig. 3: UV spectrum of Sofosbuvir after acidic degradation.

Acid hydrolysis:

To 1 ml stock solution of Sofosbuvir (1000 µg/ml), 1 ml of 0.1 N HCl was added. The above solution was kept for 26 hours at room temperature. After exposure the volume was made up to 10 ml with water to get the concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under acid hydrolysis, percent recovery obtained for Sofosbuvir was 81.13%. The representative UV spectrum is shown in Fig. 3.

Table 2: Inter-day variation studies data for Sofosbuvir.

Replicates	Conc. (µg/ml)		
	20	30	40
1	0.300	0.455	0.603
2	0.300	0.456	0.600
3	0.303	0.458	0.608
Mean	0.301	0.456	0.603
SD	0.002	0.001	0.004
%RSD	0.617	0.249	0.644

Table 3: Assay of marketed formulation.

Sr. No.	Sofosbuvir		
	Absorbance	Amount Recovered (µg/ml)	% Recovery
1	0.303	20.267	101.334
2	0.301	20.161	100.807
3	0.304	20.348	101.740
4	0.301	20.145	100.727
5	0.301	20.163	100.816
6	0.298	19.962	99.812
Mean	0.301	20.175	100.873
SD	0.002	0.130	0.651
%RSD	0.665	0.645	0.645

Table 4: Accuracy of Sofosbuvir.

Level	Conc. of Sample solution (µg/ml)	Conc. of Standard solution spiked (µg/ml)	Absorbance	Amount recovered (µg/ml)	% recovery
50%	20	10	0.451	29.857	100.212
			0.457	30.242	
			0.454	30.092	
100%	20	20	0.606	39.923	100.352
			0.610	40.198	
			0.611	40.302	
150%	20	30	0.758	49.844	100.465
			0.770	50.604	
			0.765	50.249	

Neutral hydrolysis:

To 1 ml stock solution of Sofosbuvir (1000 µg/ml), 1 ml of water was added. The above solution was kept for 72 hours at room temperature. After exposure the volume was made up to 10 ml with ELGA water to get the concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under neutral hydrolysis, percent recovery obtained for Sofosbuvir was 76.97%. The representative UV spectrum is shown in Fig. 4.

Degradation under oxidative condition:

To 1 ml stock solution of Sofosbuvir (1000 µg/ml), 1 ml of 30% H_2O_2 was added. The above solution was kept for 26 hours at room temperature. After exposure the volume was made up to 10 ml with water to get the concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under oxidative degradation, percent recovery obtained for Sofosbuvir was 82.79%. The representative UV spectrum is shown in Fig. 5.

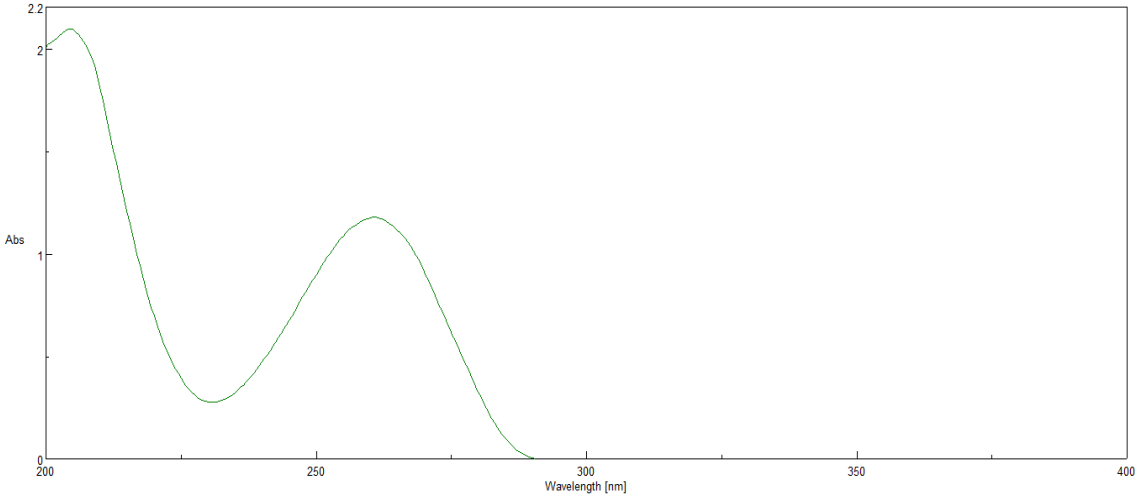

Fig. 4: UV spectrum of Sofosbuvir after neutral degradation.

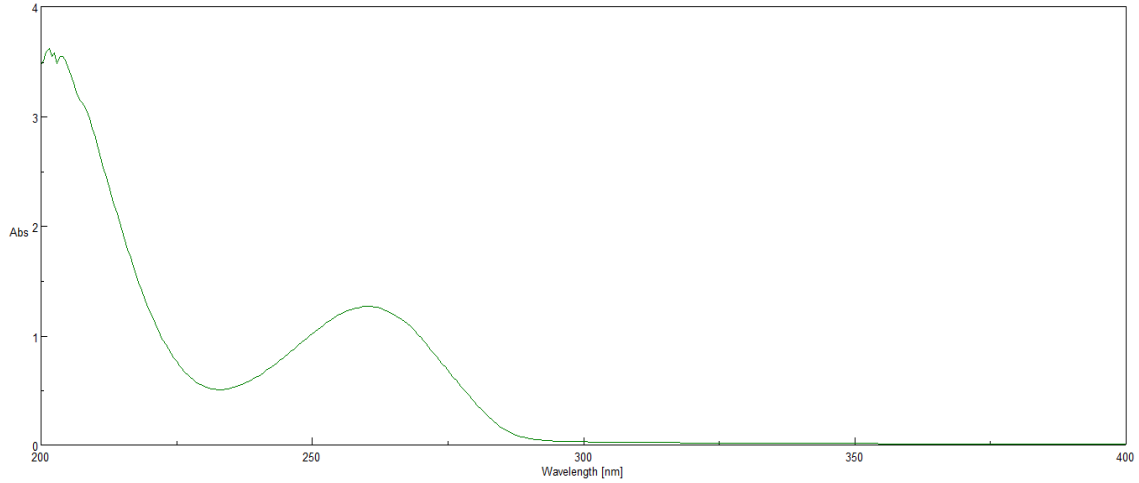

Fig. 5: UV spectrum of Sofosbuvir after oxidative degradation.

Table 5: Robustness study.

% RSD Found For Robustness Study (Absorbance)		
DETECTION WAVELENGTH (± 1 nm)		
259.5	**260.5**	**261.5**
1.394	0.501	1.363

Degradation under dry heat:

Dry heat studies were performed by keeping drug sample in oven (80^0C) for a period of 72 hours. A sample was withdrawn and transferred to 10 ml volumetric flask then dissolved in water to get concentration of solution 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with water to get concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under dry heat degradation condition, percent recovery obtained for Sofosbuvir was 88.65%. The representative UV spectrum is shown in Fig. 6.

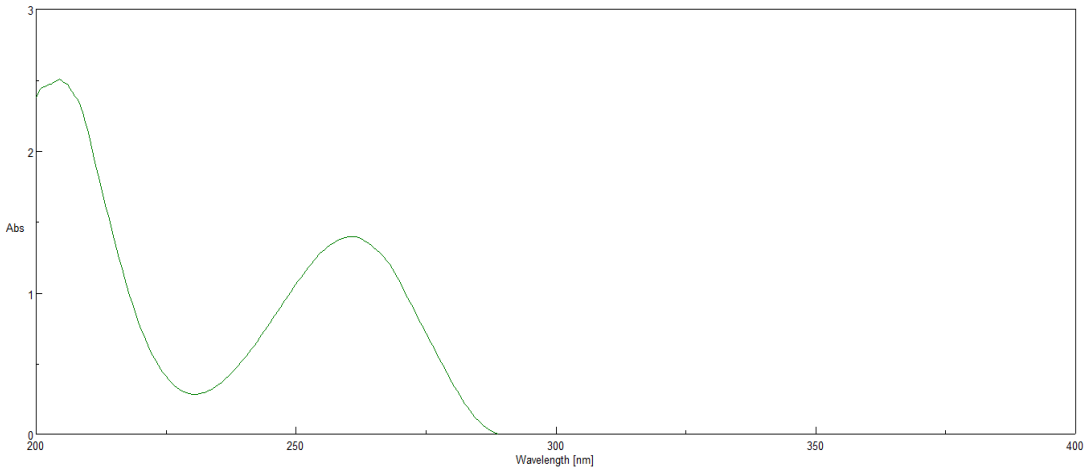

Fig. 6: UV spectrum of Sofosbuvir after dry heat degradation.

Table 6: Summary of Validation Parameters.

Sr. No.	Validation parameters	Sofosbuvir
1.	Detection wavelength (nm)	260.5
	Linearity equation	y = 0.0154x - 0.0092
2.	R^2	R^2 = 0.9984
	Range	10-100 µg/ml
	Precision	(%RSD)
3.	Intra-day	0.669
	Inter-day	0.492
4.	Assay	100.873% ± 0.645
5.	Accuracy	Mean ± %RSD
	50	100.212% ± 0.645
	100	100.352% ± 0.487
	150	100.465% ± 0.757
6.	Limit of detection	0.269 µg/ml
7.	Limit of quantitation	0.814 µg/ml
8.	Robustness	Robust

Photo-degradation studies:

1. UV illumination for solid drug sample:

The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200-watt hr/m^2. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with water to obtain concentration of solution 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with water to get concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under photo degradation study by UV light, percent recovery obtained for Sofosbuvir drug solid sample was 82.46%. The representative UV spectrum is shown in Fig. 7.

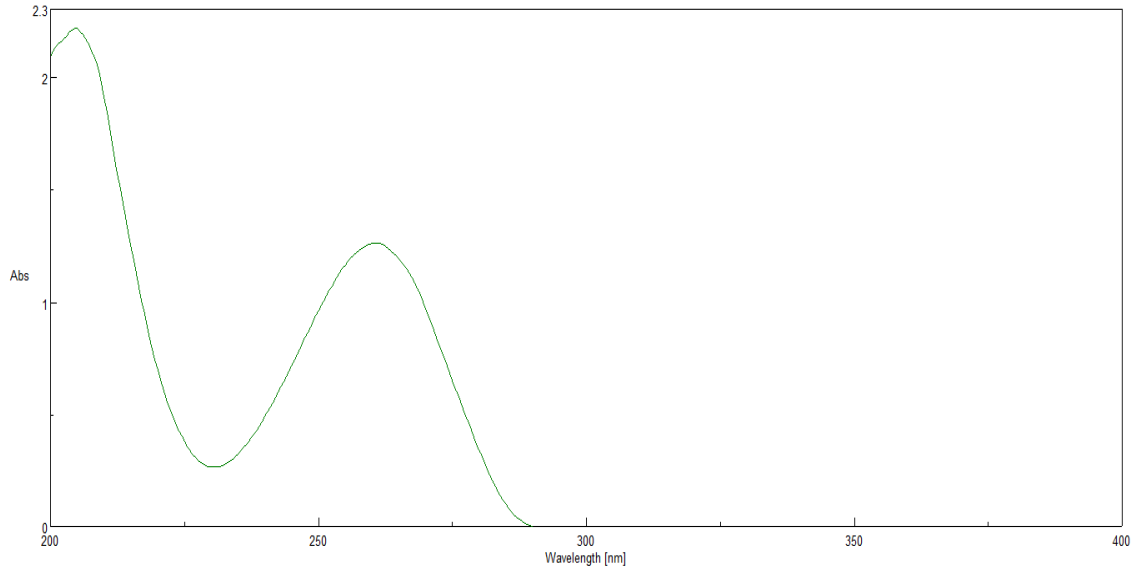

Fig. 7: UV spectrum of Sofosbuvir after photolytic UV degradation of solid drug sample.

2. UV illumination for drug solution:

From stock solution (1000 µg/ml) of Sofosbuvir 1 ml was taken in 10 ml volumetric flask and volume was made up with water to obtain concentration of solution 100 µg/ml. And expose this drug solution to UV light providing illumination of NLT 200-watt hr/m^2. After exposure 2 ml of resulting solution was taken in cuvette and absorbance was recorded. Under photo degradation study by UV light, percent recovery obtained for Sofosbuvir drug solution was 76.06%. The representative UV spectrum is shown in Fig. 8.

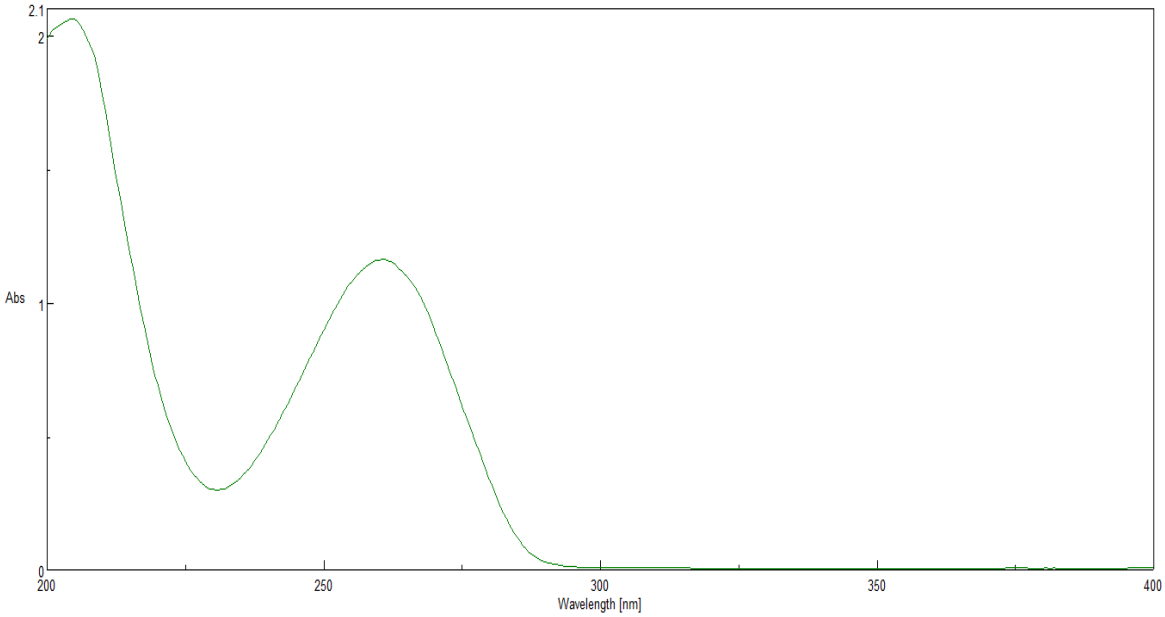

Fig. 8: UV spectrum of Sofosbuvir after photolytic UV degradation of drug solution.

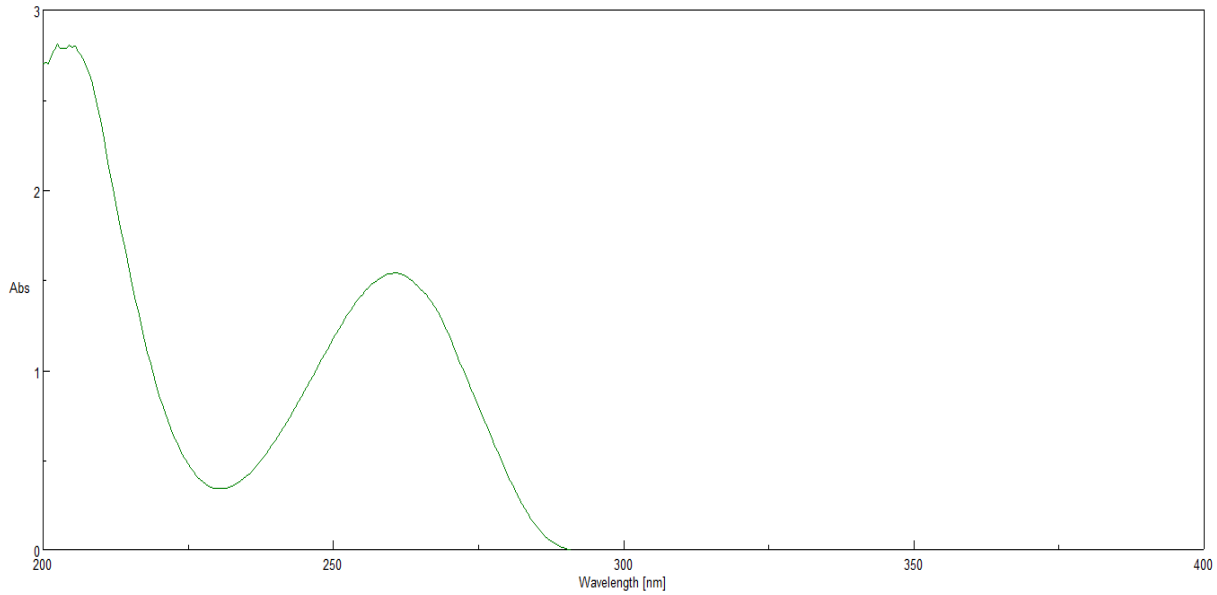

Fig. 9: UV spectrum of Sofosbuvir after photolytic fluroscent light degradation.

3. Fluroscent light:

The photo degradation study of the drug was studied by exposing the drug to fluroscent light providing illumination of NLT 1.2×10^6 Lux hr of fluroscent light. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with water to obtain concentration of solution 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with water to get concentration of solution 100 µg/ml. From the resulting solution, 2 ml was taken in cuvette and absorbance was recorded. Under photo degradation study by fluroscent light, percent recovery obtained for Sofosbuvir was 87.47%. The representative UV spectrum is shown in Fig. 9.

VALIDATION OF ANALYTICAL METHOD: [19]

Linearity:

From the standard stock solution (1000 µg/ml) of Sofosbuvir, solution was prepared containing 100 µg/ml of Sofosbuvir in water. This solution was further diluted with water to get range of solution containing different concentrations 10-100 µg/ml. Absorbance was taken at λ_{max} 260.5 nm. The linearity (relationship between absorbance and concentration) was determined by analyzing ten solutions over the concentration range of 10-100 µg/ml. The equation of calibration curve was found to be y = 0.0154x - 0.0092. The absorbance of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 10.

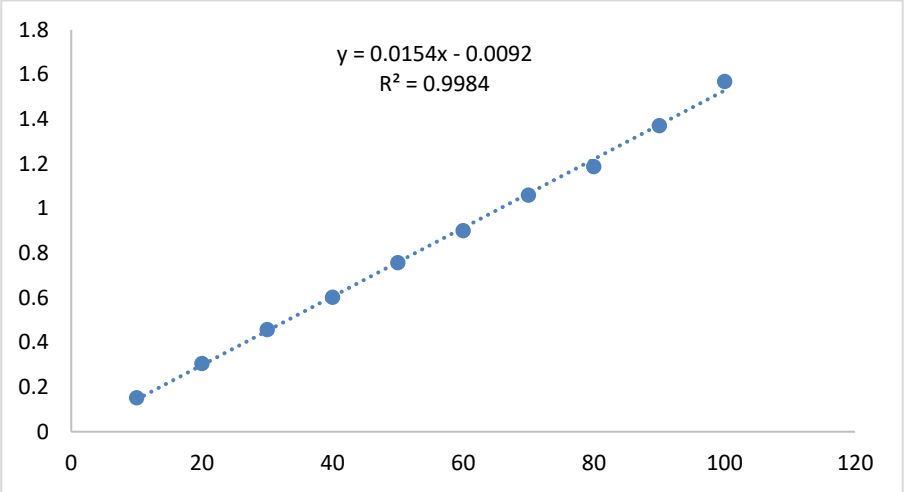

Fig. 10: Linearity curve of Sofosbuvir (10-100 µg/ml).

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter-day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intra-day and inter-day variations are shown in Table 1 and Table 2.

Limit of detection (LOD) and limit of quantitation (LOQ):

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. The LOD and LOQ was found to be 0.269 µg/ml and 0.814 µg/ml, respectively.

Assay:

MyHep™- 400 mg tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Sample solution was taken in cuvette and absorbance was recorded. Basic concentration of sample chosen was 20 µg/ml from tablet solution. Procedure was repeated for six times. Concentration and % recovery was determined from linear equation. The results obtained are shown in Table 3.

Accuracy:

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the MyHep™- 400 mg tablet sample solution, at three different levels around 50, 100 and 150%. Basic concentration of sample solution chosen was 20 µg/ml of Sofosbuvir. % recovery was determined from linearity equation. The results obtained are shown in Table 4.

Robustness:

Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength (± 1 nm) was altered and the effects on the absorbance was noted. The method was found to be robust. The results obtained are shown in Table 5.

RESULTS AND DISCUSSION:

The developed method was found to be simple, sensitive, specific, accurate and repeatable for analysis of Sofosbuvir in bulk and pharmaceutical dosage form without any interference from the excipients. The results indicated the suitability of the method to study stability of Sofosbuvir under various forced degradation conditions.

CONCLUSION:

A simple, precise, accurate, reproducible and stability indicating UV spectroscopic method without interference from the excipients or from degradation products has been developed and validated for the determination of Sofosbuvir as bulk drug and in tablet dosage form. Drug found to be degraded under alkaline condition evident from change in UV spectrum pattern of drug. The developed method can be used for quantitative analysis of Sofosbuvir in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

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