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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF SOFOSBUVIR IN ITS SYNTHETIC MIXTURE

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ABSTRACT

A stability indicating HPTLC method was developed and validated for estimation of Sofosbuvir in its synthetic mixture. Chromatographic separation was performed on pre-coated silica gel $60F_{254}$ aluminum plates using the mobile phase Toluene: Acetone: Acetic Acid: Water (6.5:3.5:1:0.2v/v). The R_f value for Sofosbuvir was found to be 0.67. The spots were scanned densitometrically at 267 nm. The solvent system was able to separate Sofosbuvir and its degradation products formed under acidic, alkaline and oxidative condition. Developed HPTLC method was validated as per ICH Q2 (R1) guideline. The linear regression analysis data for the calibration plots showed a good linear relationship with R^2 value of Sofosbuvir is 0.997 in the concentration range of 200-1000 ng/band for Sofosbuvir. Percent recovery of drug was found in the range of 98.40-101.76 % by developed method. Limit of detection and limit of quantitation was found to be 16.08 ng/band and 48.73 ng/band for Sofosbuvir respectively. The method was applied to estimate Sofosbuvir in its Synthetic mixture.

1. INTRODUCTION

Sofosbuvir is designated chemically as Isopropyl(2S)-2-[[[(2R,3R,4R,5R)-5-(2,dioxopyrimidin1yl)-4-fluoro-3-hydroxy-4tetrahydrofuranyl]methoxy-phenoxy-phosphoryl] amino]propanoate. Sofosbuvir is Antiviral agent.[1-9] Chemical structure is shown in figure 1.

The literature review described UV-visible spectrophotometric,[10,11] RP-HPLC,[12] method for determination of Sofosbuvir in bulk and tablet dosage form. The literature review described stability indicating LC-MS-MS [13] method for Sofosbuvir. The literature review also described characterization of forced degradation products and in silico toxicity prediction of Sofosbuvir by LC-ESI-QTOF-MS/MS.[14] The present paper describes development and validation of stability indicating HPTLC method for estimation of Sofosbuvir in its synthetic mixture.

2. MATERIALS AND METHODS

2.1 Instrumentation

The HPTLC system (Camag, Switzerland) consisting of Linomat V automatic sample applicator, TLC Scanner IV (Camag), Flat bottom twin-trough developing chamber (10 × 10 cm) (Camag), UV cabinet with dual wavelength (254/366 nm) UV lamps, win-CATS software (Camag), Micro syringe (100 µl- Hamilton), Pre coated silica gel aluminum plate 60 F_{254}, (10 × 10cm with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologies, Mumbai, India), Electronic analytical balance (AUX-220-Shimadzu) and Whatman Filter paper no.42, Sonicator (Janki Impex Pvt Ltd) and controlled temperature water bath (Durga Scientific Equipment).

2.2 Chemicals and Reagents

Sofosbuvir was received as gift sample from Sunrise Remedies, Pvt. Ltd, Ahmedabad, Gujarat, India. Hydrocholric acid (AR grade, 35.4%), Sodium Hydroxide flakes (LR grade), Acetone (AR grade, 99.8%), Toluene (AR grade, 99.5%) and Acetic acid (AR grade, 80%) were purchased from LOBA Chemie, Vadodara. Hydrogen Peroxide IP 6% v/v was purchased from Gujarat Pharma lab Pvt. Ltd. Double distilled water was prepared in laboratory.

3. Preparation of solutions

3.1 Preparation of standard stock solution of Sofosbuvir

Accurately weighed quantity of Sofosbuvir 10 mg was transferred into 10 ml volumetric flask, dissolved in 5 ml of methanol and diluted up to mark with same (1000 µg/ml).

3.2 Preparation of working standard solution of Sofosbuvir

1 ml aliquot of Sofosbuvir stock solution was transferred into 10 ml volumetric flask and diluted up to mark with methanol (100 µg/ml). From resulting solution, 4 ml was further diluted to 10 ml with methanol (40 µg/ml).

3.3 Preparation of Forced Degradation Solutions

Degradation of Sofosbuvir was studied in acidic, alkaline and oxidative conditions.

3.3.1 Acidic Hydrolysis

Accurately weighed 10 mg of Sofosbuvir was transferred into 10 ml volumetric flask, dissolved in 5 ml methanol and diluted up to the mark with 0.2 N HCl. The solution was kept for 30 min at room temperature. Aliquot of 1 ml was transferred to 10 ml volumetric flask and neutralized using 0.1 N NaOH and diluted up to mark with methanol.

3.3.2 Alkaline Hydrolysis

Accurately weighed 10 mg of Sofosbuvir was transferred into 10 ml volumetric flask, dissolved in 5 ml methanol and diluted up to the mark with 0.02 N NaOH. The solution was kept for 30 min at room temperature. Aliquot of 1 ml was transferred to 10 ml volumetric flask and neutralized using 0.01 N HCl and diluted up to mark with methanol.

3.3.3 Oxidative Degradation

Accurately weighed 10 mg of Sofosbuvir was transferred into 10 ml volumetric flask, dissolved in 5 ml methanol and diluted up to the mark with 6% H_2O_2. The solution was kept for 3 hours at room temperature. Aliquot of 1 ml was transferred to 10 ml volumetric flask and diluted up to mark with methanol.

3.4 Solution Stability Study

The freshly prepared working standard solution of Sofosbuvir (40 µg/ml) was stored at room temperature for 24 hour. The solution was analysed immediately after preparation and after 24 hour using optimized chromatographic conditions. Areas of peak of Sofosbuvir obtained at 0 hour and 24 hour were compared to check the stability of solution.

4. Chromatographic conditions

Chromatographic condition was performed on Silica gel 60 F_{254} aluminium plates (10×10 cm) as stationary phase, using mobile phase comprised of Toluene : Acetone : Acetic acid: Water (6.5:3.5:1:0.2 v/v/v/v). The working standard/sample solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen gas using a Camag Linomat V semi-automatic sample applicator. A constant application rate of 100nl/sec was employed and space between two bands was fixed at 11.6 mm. Ascending development to 80 mm was performed in Camag (10 x 10cm) twin trough glass chamber saturated with the mobile phase for 30 min at room temperature. The developed TLC plate was air dried and scanned between 200 to 400nm using Camag TLC scanner IV. Both drug and degradation product showed reasonably good absorbance at 267 nm keeping the slit dimension of 4.00 x 0.30 mm and scanning speed of 20 mm/s with absorption/reflectance mode.

4.1 Selection of wavelength

From working standard solution and forced degraded samples, 25 µl was spotted on the same TLC plate. The plate was developed using mobile phase comprised of Toluene: Acetone: Acetic acid: Water (6.5:3.5:1:0.2 v/v/v/v) in previously saturated twin-trough chamber and dried in oven. The plate was scanned in the range of 200-400 nm for selection of wavelength.

4.2 Preparation of calibration curve

Aliquots of 5, 10, 15, 20 and 25 µl of working standard solution (40 µg/ml) were spotted on pre-coated TLC plate under nitrogen stream using Linomat V semiautomatic sample applicator. The plate was developed, dried and analyzed as per optimized chromatographic conditions. The calibration curve was constructed by plotting area versus respective concentration (ng/band).

4.3 Method Validation

4.3.1 Linearity and Range

The linearity was expressed in terms of correlation coefficient of linear regression analysis. The linearity range was determined by analysing 5 independent levels of calibration curve in the range of 200-1000 ng/band for Sofosbuvir. The calibration curve was prepared by plotting peak area vs. concentration and correlation co-efficient and regression line equation for Sofosbuvir was calculated.

4.3.2 Specificity

The spots of Sofosbuvir from synthetic mixture were confirmed by comparing the Rf value and absorbance/reflectance spectrum with that of the standard. The peak purity of Sofosbuvir was determined by comparing the UV spectra of sample scanned at peak start (S), peak apex (M) and peak end (E) positions of the spot.

4.3.2 Precision

4.3.2.1 Repeatability of sample application

Repeatability of sample application was determined by application of 15 µl of working standard solution (40µg/ml) seven times on a same TLC plate. The plate was developed, dried and analysed as per the optimized chromatographic condition. The peak areas of seven spots was measured and % RSD was calculated.

4.3.2.2 Repeatability of peak area measurement

From working standard solution 15µl was spotted on a TLC plate. Plate was developed, dried and analysed as per the optimized chromatographic condition. The separated spot of Sofosbuvir was scanned seven times without changing plate position and % RSD for measurement of peak area was computed.

4.3.2.3 Intermediate precision

Intraday and Interday precision was performed using the optimized chromatographic condition. These method was evaluated by analyzing the entire calibration range of

Sofosbuvir (200-1000 ng/band), three times on same day. The % RSD of peak area at each level was calculated. Interday precision of the proposed method was evaluated by analyzing the entire calibration range of Sofosbuvir (200-1000 ng/band) on three consecutive days. The % RSD of peak area at each level was calculated.

4.3.3 Limit of Detection (LOD)

The limits of detection of the developed method was calculated from standard deviation of intercepts and mean slope of calibration curves of Sofosbuvir using the equation given below

$$LOD = 3.3 \times \sigma/S$$

Where, σ = the standard deviation of the Y-intercepts of the five calibration curves

S = mean slope of the five calibration curves

4.3.4 Limit of Quantification (LOQ)

The limits of Quantification of the developed method was calculated from standard deviation of intercepts and mean slope of calibration curves of Sofosbuvir using the equation given below

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the Y-intercepts of the five calibration curves

S = mean slope of the five calibration curves

4.3.5 Accuracy

The accuracy of the method was determined by calculating recovery of Sofosbuvir using the standard addition method. The quantity of synthetic mixture of placebo equivalent to 10 mg of Sofosbuvir was transferred to four individual 10 ml volumetric flasks. Standard Sofosbuvir 8 mg, 10 mg and 12 mg was spiked in second, third and fourth volumetric flasks, respectively. All four flasks were filled to about 80% with methanol, sonicated for 30 minutes and diluted up to the mark with methanol. These solutions were filtered using whatman filter paper no. 42 individually. From each filtrate, 1 ml was diluted up to 10 ml with methanol individually. Aliquot of 3 ml of each resulting solution was diluted to ml with methanol. Each resulting solution (10 µl) was individually spotted on TLC plate. Plate was developed and analyzed as per the optimized chromatographic condition. From calibration curve, the amount of Sofosbuvir recovered was calculated and % recovery was determined.

4.4 Preparation of Synthetic mixture

Synthetic mixture conitaining Sofosbuvir 400 mg prepared in laboratory by using suitable excipients (prepared by mixing 400 mg SOF, 68 mg Lactose Monohydrate, 90 mg Microcrystalline Cellulose, 24 mg Croscarmellose Sodium, 50 mg Colloidal Silicon Dioxide, 12 mg Magnesium Stearate to 644 mg of synthetic mixture).

4.5 Assay of synthetic mixture

The powder equivalent to 10 mg of Sofosbuvir was accurately weighed and transferred into a 10 ml volumetric flask and 5 ml methanol was added. The flask was sonicated for 20 min and volume was made up to 10 ml with methanol. The solution was mixed well and filtered through Whatman filter paper No.42. and 1 ml solution was further diluted to 10 ml with methanol. From the resulting solution aliquot of 4 ml was further diluted to 10 ml with methanol. A volume 15 µl of resulting solution was applied in triplicate on TLC plate followed by as per the optimized chromatographic condition. The amount of Sofosbuvir present in sample solution was determined by fitting area values of corresponding peak into the equation representing calibration curve of Sofosbuvir.

4.6 Analysis of forced degraded sample

For the analysis of forced degradation samples, 15µl of each forced degraded solution was spotted on TLC plate and analyzed and percentage of drug degraded was calculated from calibration curve.

5. RESULTS AND DISCUSSION

5.1.1 Optimization of mobile phase

Different solvent systems were tried for stability study but better result was found to be in Toluene: Acetone: Acetic acid: Water (6.5: 3.5: 1: 0.2 v/v/v/v). The R_fs value for Sofosbuvir was 0.67±0.02.

5.1.2 Selection of wavelength of detection

The wavelength of maximum absorbance for Sofosbuvir was found to be 267 nm. It was selected as detection wavelength.

5.2 Solution Stability Study

Solutions were kept at room temperature (25±2℃) for 24 hours and analysed by the developed HPTLC method as per the optimized chromatographic condition. At initial, peak area was 4079.51±61.04 and after 24 hours, peak area was 4051.53±62.09. No significant change was observed in peak area of chromatogram, implies that solutions are stable at room temperature. The working standard solution Sofosbuvir was found to be stable for 24 hours at room temperature (25±2℃).

5.3 Calibration Curve of Sofosbuvir

Calibration data for 200-1000 ng/band of Sofosbuvir is presented in the table 1.Calibration curve of peak area versus concentration is shown in figure 2. Linearity was determined from regression line equation.

5.3.1 Method validation

5.3.1.1 Linearity and Range

The calibration curve was prepared by plotting peak area versus respective concentration. The peak area of Sofosbuvir was linear with respect to concentrations over the range of 200-1000 ng/band. Data is shown in table 2 and calibration graph is shown in figure 2. The results show excellent correlation between peak area and concentrations (R^2 = 0.9978).

5.3.1.2 Specificity

The standard and sample showed same Rf value of 0.67 (figure 3). Peak purity of Sofosbuvir was assessed by comparing spectra acquired at start, middle and end of band obtained from scanning the spot. The values of r(s,m) and r(m,e) for standard are 0.9995 and 0.9956 respectively. The values of r(s,m) and r(m,e) for sample are 0.9995 and 0.9951 respectively. Hence, the method was found to be specific as excipients did not interfere with analysis.

5.3.1.3 Precision

Percent RSD for repeatability of sample application and measurement were found to be 0.92% and 0.41% respectively. % RSD for intraday and interday precision were found to be 0.46 – 0.84 % and 0.99 – 1.79 % respectively.

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

The LOD and LOQ were found to be 16.08 ng/band and 48.73 ng/band respectively.

5.3.1.5 Accuracy

Percent recovery ranges for Sofosbuvir was found to be between 98.4 - 101.76 %. Summary of validation parameter in depicted in table 3.

6. Assay of Synthetic mixture

The developed HPTLC method was used to estimate Sofosbuvir in synthetic mixture. Amount of Sofosbuvir was calculated using equation y=5.205x+891.5 (where, y=peak area and x=concentration in ng/band). Data for estimation of synthetic mixture is presented in table 4.

7. Analysis of forced degraded samples

The Sofosbuvir was degraded in acidic and alkaline condition used for study. It was stable in oxidative degradation condition. In comparison with standard chromatogram of drug, one additional peak (Rf = 0.15) and three additional peak (Rf= 0.17, 0.50 and 0.56) were observed in chromatogram of acid and alkaline degraded conditions respectively as depicted in figure 4 and 5. No additional peak was observed in oxidative condition used in the experiment as depicted in figure 6. Percentage degradation of Sofosbuvir is found to be 15.03% and 35.38% in acid and alkaline hydrolysis respectively.

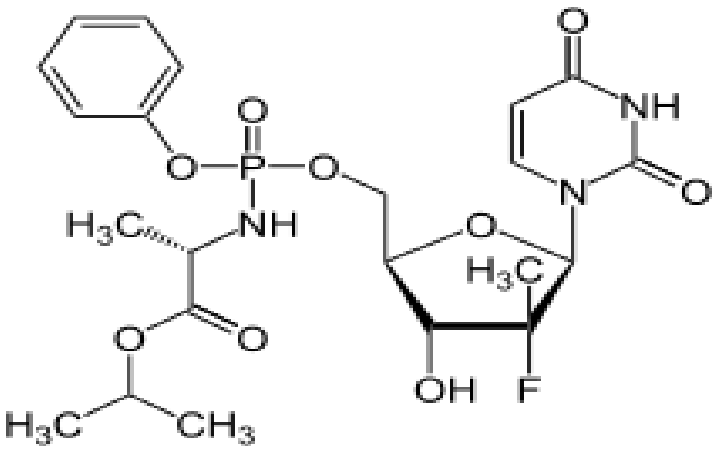

Fig. 1: Structure of Sofosbuvir.

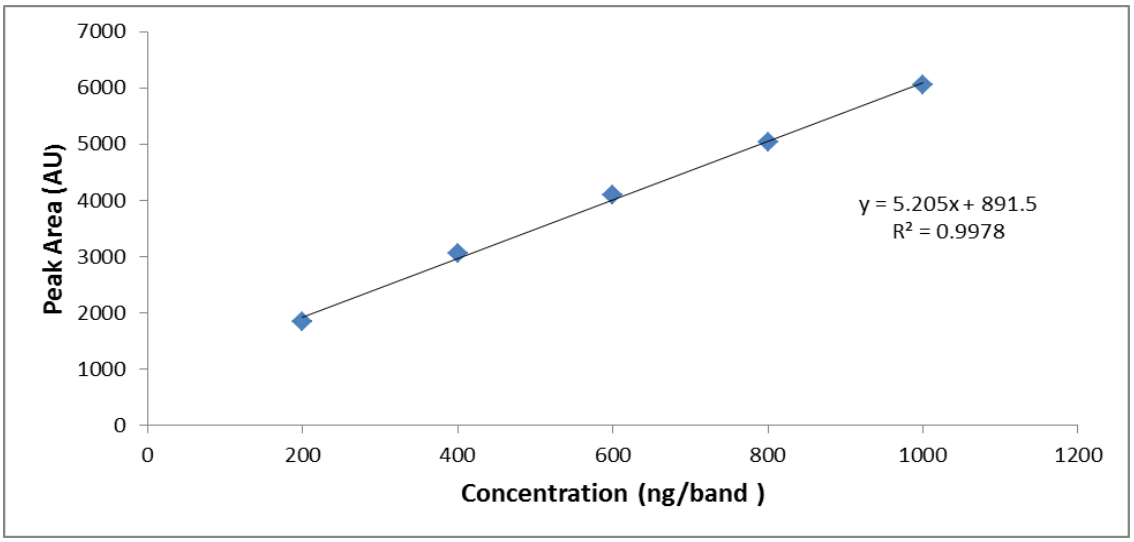

Fig. 2: Calibration curve for Sofosbuvir (200 - 1000 ng/band).

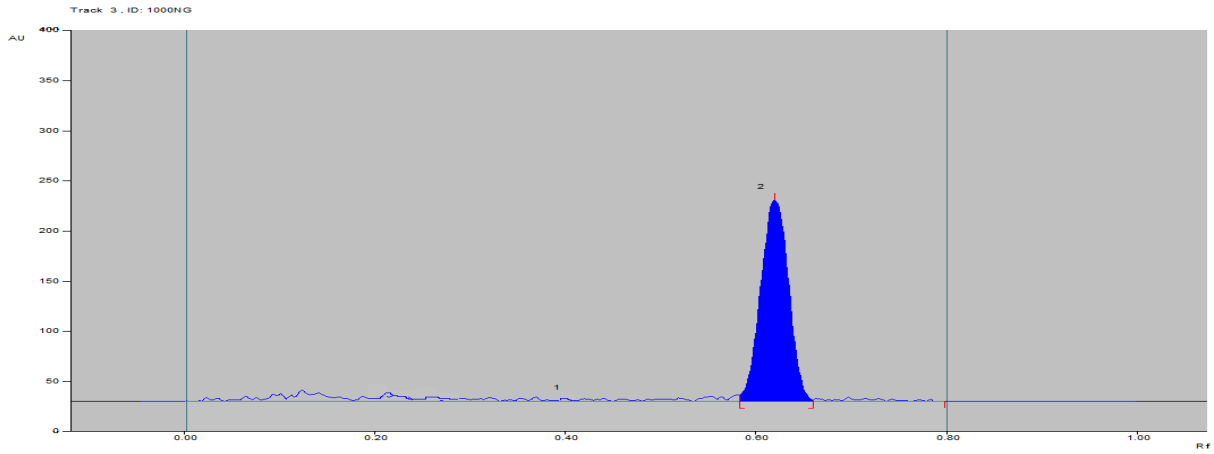

Fig. 3: Chromatogram showing standard Sofosbuvir (600 ng/band) at R_f = 0.67.

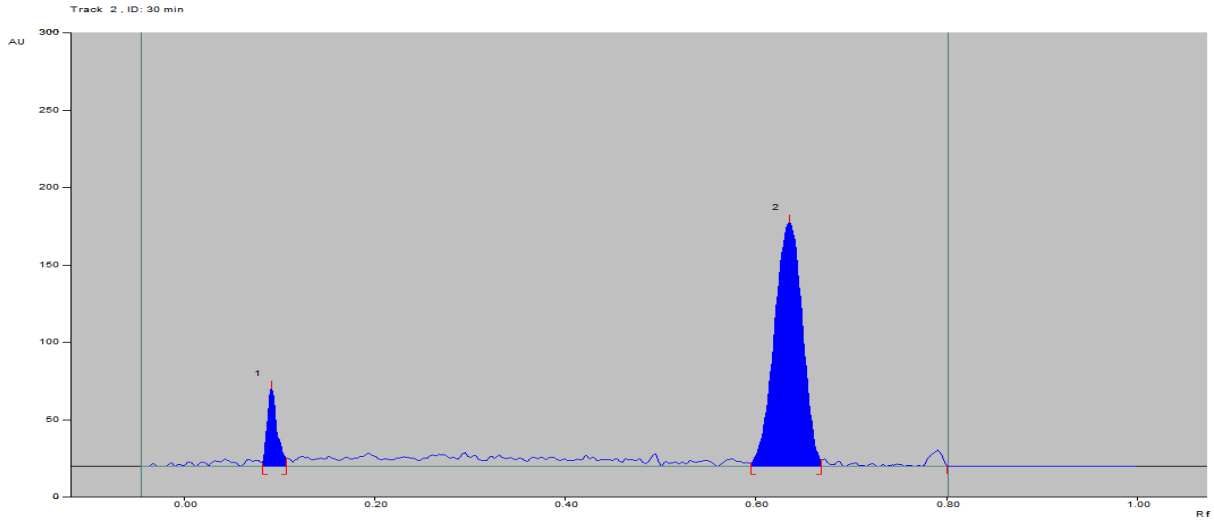

Fig. 4: Chromatogram of Sofosbuvir in acidic condition: (0.1 N HCl/ / room temperature for 30 min); Sofosbuvir (R_f = 0.67); degradation product 1 (R_f = 0.15).

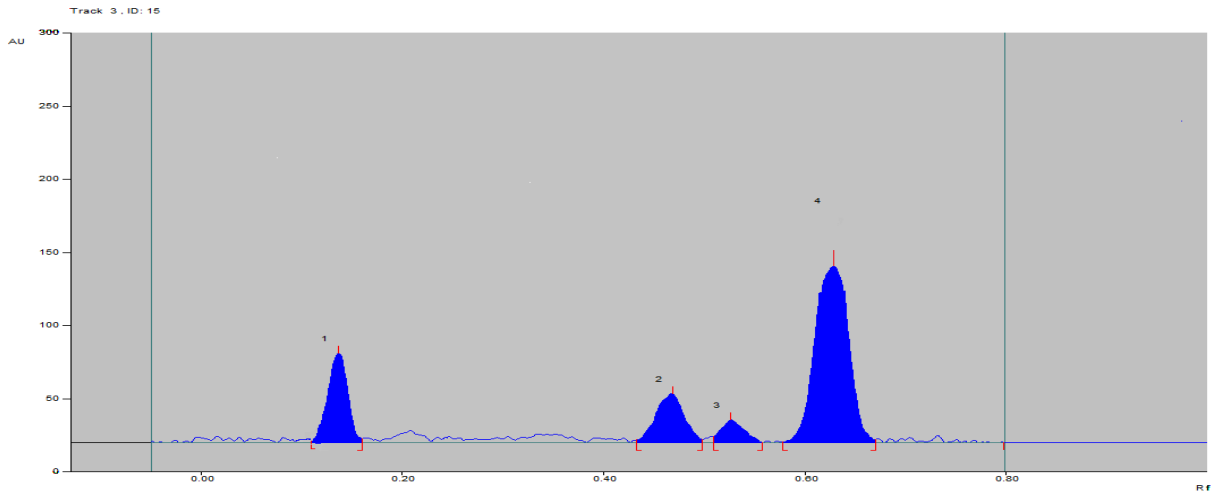

Fig. 5: Chromatogram of Sofosbuvir in alkaline condition (0.01 N NaOH/ room temperature/ 30 min); Sofosbuvir (R_f = 0.67); degradation product 1 (R_f = 0.17),2(R_f =0.50),3(R_f =0.56).

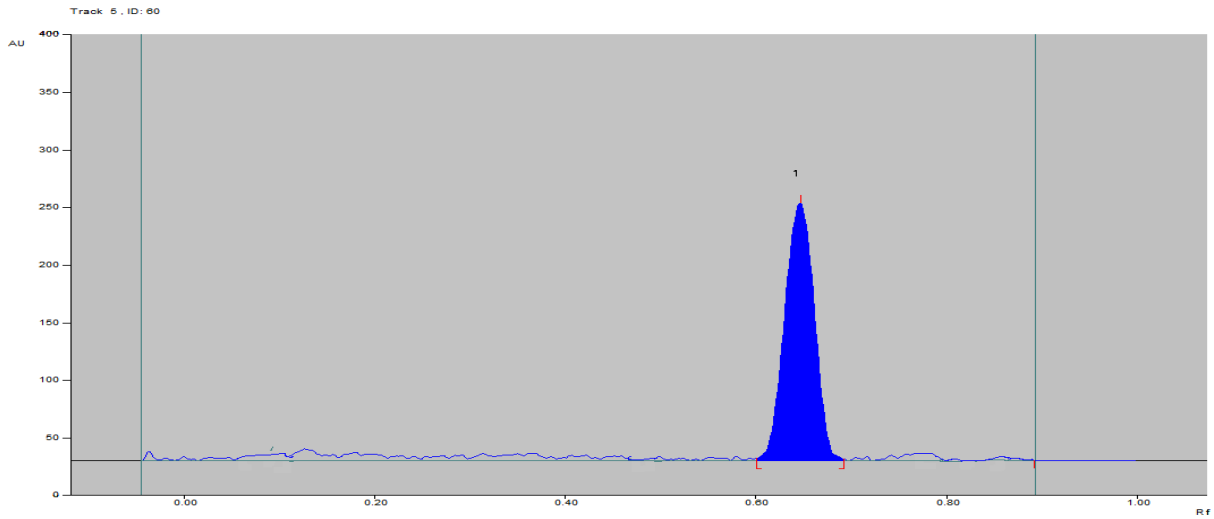

Fig. 6: Chromatogram of Sofosbuvir in oxidative condition: (3 % H_2O_2 / Room temperature/ 3 hours); Sofosbuvir (R_f = 0.67).

Table 1: Calibration data of Sofosbuvir.

Concentration	Peak Area (Mean ± S.D.)	%RSD
200	1839.98 ± 18.31	0.99
400	3053.41 ± 31.66	1.03
600	4094.83 ± 48.12	1.17
800	5025.83 ± 22.78	0.45
1000	6058.85 ± 30.48	0.51

Table 2: Results of linearity.

Parameters	Results
Linearity range (ng/band)	200-1000
Regression line equation	Y=5.205x+891.5
Slope ± S.D.(n=5)	5.2046 ± 0.37
Y -intercept ± S.D.(n=5)	891.48 ± 25.365
Correlation coefficient (R^2)	0.9978

Table 3: Summary of Validation parameters.

Parameters	Results
Linearity Range (ng/band)	200-1000
Regression line Equation	y = 5.205x + 891.5
Correlation co-efficient (R^2)	0.9978
Precision (%RSD)	
Repeatability of Peak area measurement (n=7)	0.41
Repeatability of Sample Application (n=7)	0.92
Intra-day precision (n=3)	0.46 – 0.84
Inter-day precision (n=3)	0.99 – 1.79
% Recovery	98.40 – 101.76
Limit of Detection (LOD) (ng/band)	16.08
Limit of Quantitation (LOQ) (ng/band)	48.73

Table 4: Recovery data of Sofosbuvir in Synthetic mixture (n=3).

Synthetic mixture	Amount taken of Sofosbuvir obtained (mg)	Average amount obtained (mg)	Average amount (%)
Quantity equivalent to	10 mg	9.95 ± 0.35 mg	99.47%

8. CONCLUSION

The stability indicating HPTLC method was developed and validated for estimation of Sofosbuvir in synthetic mixture. Forced degradation study involved formation of degradation product under acidic and alkaline hydrolysis. No degradation product was observed in oxidative conditions used. The developed method successfully separated drug substance from degradation products

formed. The method was successfully applied for assay of Sofosbuvir in its synthetic mixture. The methods was validated as per ICH Q2(R1) guidelines. The linearity range was found to be 200-1000 ng/band with Regression Coefficient 0.9978. The % RSD of all precision studies were found to be less than 2% indicating developed method is precise. The % recovery of Sofosbuvir was found to be in the range of 98-102 %. Hence the method is accurate. The developed method was applied for assay of synthetic mixture and the results are in agreement with spiked amount of Sofosbuvir.

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10. REFERENCES

1. Waugh A, Grant A. Anatomy and Physiology in Health and Illness.12th ed. International Edition, 2014; 324-326.
2. The World Health Organization's (WHO) 2016 Guidelines for the screening, care and treatment, May2016. (http://apps.who.int/medicinedocs/documents/s21419en/s21419en.pdf)
3. Asselah T. Sofosbuvir for the Treatment of Hepatitis C Virus, Expert opinion pharmacotherapy, 2016; 15(1): 121-130.
4. Q1A (R2): Stability Testing Of New Drugs And Products, International Conference On Harmonization Of Technical Requirements For Registration Of Pharmaceuticals For Human Use, ICH harmonised tripartite guideline, 2003; 1-18.
5. FDA Organisation, Mechanism of Action Oct 25, 2013. www.fda.gov/downloads/advisorycommittees/.../drugs/.../ucm375286 pdf.
6. Shethi PD. High Performance Thin Layer Chromatography-Quantitative analysis of pharmaceutical formulations. 1st ed. New Delhi: CBS Publishers, 1996; 1-68.
7. Drug profile of Sofosbuvir https://www.drugbank.ca/drugs/DB08934 (Accession Date Jan 1, 2014).
8. Klein R, Struble k. Approval of Sovaldi Tablets for the treatment of chronic hepatitis C Federal Drug Administration, 2013; 1-4.
9. Conteduca v, Sansonno D, Russi S, Pavone F, Dammacco F.Therapy of chronic hepatitis C virus infection in the era of direct-acting and host-targeting antiviral agents. J infect, 2014; 68: 1-20.
10. Chakravarthy V, Sailaja B, Kumar A. Method Development and Validation of Ultraviolet-Visible Spectroscopic method for the Estimation of Hepatitis-C Drug- Daclatasvir and Sofosbuvir in Active Pharmaceutical Ingredient Form. Asian Journal Pharmaceutical and Clinical Research, 2016; 9(3): 61-66.
11. Abdel S, Abdel N. Simple Chromatographic Spectrophotometric Determination of Sofosbuvir in pure and tablet forms. European Journal of Chemistry, 2016; 7(3): 375-379.
12. Vejendla R, Subramanyam C, Veerabhadram G. Estimation and validation of Sofosbuvir in Bulk and Tablet Dosage form By RP-HPLC .International Journal of Pharmacy, 2016; 6(6): 121-127.
13. Nebsen M, Eman S. Stability-Indicating Method and LC-MS –MS Characterization of Forced Degredation Product of Sofosbuvir. Journal of Chromatographic Science, 2016; 2(3): 1-10.
14. Bahrami M, Mohammadi B, Miraghaei S, Babaei A, Ghaheri M, Bahrami G. Quantification of Sofosbuvir in human serum by liquid chromatography with negative ionization mass spectrometry using the parent peak and its source-induced fragment: Application to a bioequivalence study. Journal of Separation Science, 2016; 1-22.