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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SOFOSBUVIR TABLETS BY RP-HPLC

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ABSTRACT

This paper describes the analytical method suitable for validation of Sofosbuvir by reversed Phase High Performance Liquid Chromatography (RP-HPLC) method. The method utilized RP-HPLC (Water 2695 with PDA detector) model and a column Agilent C18 4.5×100 mm 3.0 µm. The mobile phases were comprised with 60:40 of Methanol: Water at a flow rate of 1.0 ml/min. UV detection at 235 nm MTS were eluted with retention times of 2.351min. The method was continued and validated accordance with ICH guidelines. Validation revealed the method is rapid, specific, accurate, precise, reliable, and reproducible. Calibration curve plots were linear over the concentration ranges 320-480µg/mL (R^2 = 0.9993). Limit of detection (LOD) was 1.5µg/ml and limit of quantification (LOQ) was 4.7µg/mL. The method showed good recoveries (99.1 - 99.9%). Statistical analysis was proves the method is suitable for the analysis of Sofosbuvir as a bulk, in tablet dosage form without any interference from the excipients. It was also proved study for degradation kinetics. It may be extended for its estimation in plasma and other biological fluids.

Keywords: *RP-HPLC, Sofosbuvir, Analytical method, Quality control, Validation.*

INTRODUCTION

Sofosbuvir is chemically Isopropyl (2*S*)-2-[[[(2*R*,3*R*,4*R*, 5*R*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetra hydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate. Sofosbuvir is a nucleotide analog used in combination with other drugs for the treatment of hepatitis virus (HCV) infection. It has been marketed since 2013. Compared to previous treatments, sofosbuvir-based regimens provide a higher cure rate, fewer side effects, and a two- to four-fold reduced duration of therapy [1-3]. Sofosbuvir allows most patients to be treated successfully without the use of peginterferon [4], an injectable drug with severe side effects [5] that is a key component of older drug combinations for the treatment of HCV.

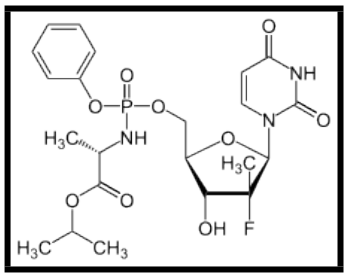

Fig. 1: Chemical structures of Sofosbuvir

In the scientific literature, analysis of Sofosbuvir has been reported as individual ingredients and in combination with other compounds. Analytical methods have included estimation of Sofosbuvir [6] individually.

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No other chromatographic methods are found for analysis of Sofosbuvir in a tablet dosage form. The method described is rapid, economical, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH guidelines [7-10].

MATERIALS AND METHOD

Apparatus:

The analysis was performed by using the analytical balance G285 (Mettler Toledo), the HPLC used is of Water 2695 with PDA detector. Column used in HPLC Agilent C18 4.5×100 mm 3.0 µm with a flow rate of 1.0 ml/min (Gradient). The mobile phase consists of 60:40 of Methanol**:** Water which is degassed in a sonicator for about 10 minutes the injection volume is 10µl and the ultra violet detection was at 235 nm.

Reagents and solutions:

Pure sample of Sofosbuvir and other ingredients such as methanol and water used were of HPLC and milli-q grade. Optimized chromatographic conditions are listed in table no.1.

Stock and Standard Preparation:

Accurately weigh and transfer 10mg of Sofosbuvir into a 10 mL of volumetric flask, add about 10 mL of diluent, sonicate to dissolve, make up to volume with diluent (1000 µg/ml). Transfer 4.0 mL of the above solution into 10 mL volumetric flask, dilute to the volume with mobile phase and mix well. And the final concentration of standard sample is 400µg/ml Filter the solution through the 0.45 mm filter.

Sample preparation:

Weigh and powder the 20 tablets. An accurately weigh and transfer the powder equivalent to 10 mg of Sofosbuvir into 10 ml volumetric flask. Add about 10 ml of mobile phase and sonicate for 20 minutes with occasional swirling to dissolve. Cool it and makeup to the volume with mobile phase. Centrifuge the solution at 3000 rpm for 15 minutes.

Transfer the 4 ml of the above supernatant solution into 10 ml volumetric flask, dilute to the volume with mobile phase and mix well. Filter the solution through the 0.45 mm filter.

Linearity & Range:

The Linearity of detector response is established by plotting a graph to concentration versus area of Sofosbuvir standard and determining the correlation coefficient. A series of solution of Sofosbuvir standard solution in the concentration ranging from about 320-480 µg/ml level of the target concentration (400µg/ml of Sofosbuvir) was prepared and injected into the HPLC system.

Accuracy:

Accuracy for the assay of Sofosbuvir tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of Sofosbuvir standard is added at different levels (50%, 100%, and 150%). The sample were filtered through 0.45mm membrane filter and injected into the chromatographic system.

Precision:

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as %RSD. The %RSD was found to be 0.75% in the results of precision.

RESULTS AND DISCUSSION

Sofosbuvir standard having concentration 400 µg/ml was scanned in UV- region between 200-400 nm. λ max of Sofosbuvir was found to be at 235 nm.

The Sofosbuvir peak in the sample was identified by comparing with the Sofosbuvir standard and the Retention time was found to be around 2.351 minutes.

The estimation of Sofosbuvir tablets was carried out by RP-HPLC using Mobile phase having a composition of 600 volumes of methanol and 400 volumes of water. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes. The column used was Agilent C18 4.5×100 mm 3.0 µm. Flow rate of Mobile phase was 1.0 ml/min, System suitability parameters such as RSD for six replicate injections was found to be less than 2%, theoretical plates - 12582.8, and tailing factor – 1.03.

The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability 0.31% which shows that the method is repeatable.

The acceptance criteria of Method Repeatability is RSD should be not more than 2.0% and the method show Method Repeatability 0.75% which shows that the method is precise.

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 320-480 µg/ml of target concentration for Sofosbuvir standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of (R^2) = 0.9993, which shows that the method is capable of producing good response in PDA detector.

The Accuracy limit is the % recovery should be in the range of 99.1 - 99.9%. The validation of developed Method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy.

Table No. 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Agilent C18 4.5×100 mm 3.0 µm
Mobile Phase	60:40 v/v (Methanol**:** Water)
Flow rate (ml/min)	1.0
Run time (minutes)	10.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	10
Detection wavelength (nm)	235
Drugs RT (min)	2.351

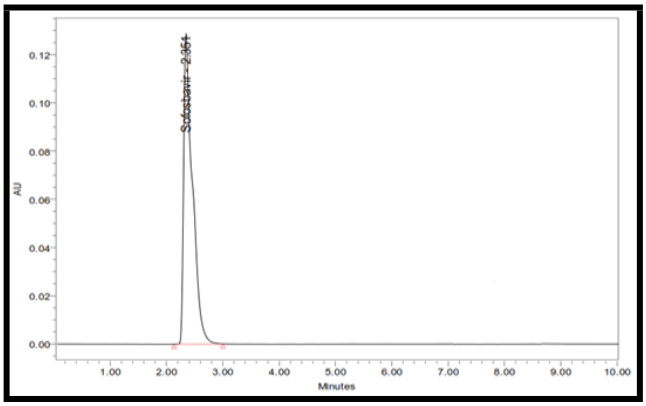

Fig. 2: Chromatogram of Sofosbuvir at 235nm

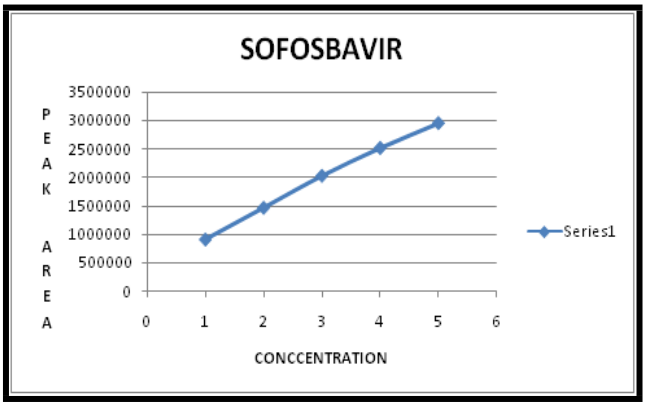

Fig. 3: Linearity Curve of Standard Sofosbuvir

Table No. 2: System suitability parameters

Parameter	Sofosbuvir
Calibration range (mg/ml)	320-480
Theoretical plates	12582.8
Tailing factor	1.03
Correlation Coefficient(r2)	0.9993
% Recovery	99.1 - 99.9%
System Suitability %RSD	0.31%
Method Repeatability %RSD	0.75%

CONCLUSION

A new RP-HPLC method described in this manuscript provides a simple, convenient and reproducible approach for the estimation and quantification of Sofosbuvir in routine quality control analysis.

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