Original Article

A Novel Stability Indicating RP-HPLC Method for Determination of Sofosbuvir in Bulk and Tablet Dosage Forms

G. Kumaraswamy[1], D. Sudheer Kumar[2], V. Ramya[3]

[1]Associate Professor [2]Professor [3]Student, Dept. of Pharmaceutical Analysis, Care College of Pharmacy, Warangal, Telangana 506006, India.

Abstract

The proposed work was accurate and precise stability indicating RP-HPLC method has been developed and validation of Sofosbuvir, in tablet dosage form. The separation was achieved on a Kromasil C18 (4.6×250 mm, 5µ) column using a mixture of Methanol: water (60: 40% v/v) as the mobile phase at a flow rate of 1.0 mL/min and detected 247 nm. The retention time of sofosbuvir 3.475 minutes. The linear responses in the concentration range of 10-60 µg/mL of Sofosbuvir. The method precision for the determination of assay was less than 2.0% RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Sofosbuvir; RP-HPLC; Validation; Tablet dosage forms.

Introduction

The Sofosbuvir (Fig.1) is chemically Isopropyl (2S)-2- [[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3 hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxyphosphoryl]amino] propanoate. It is white to off-white crystalline solid, slightly soluble in water with a pKa value of 9.38. It is an antiviral drug used in the treatment of Hepatitis C.
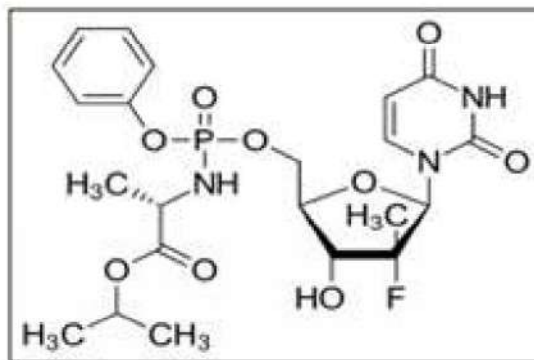

Fig. 1: Structure of Sofosbuvir

It is soluble slightly in water (pH 1.2-7.7), freely soluble in ethanol and acetone, soluble in 2-propanol, and insoluble in heptanes [3].

Literature survey review reveals a few HPLC methods, LC method have been used. The objective of the proposed work was to develop simple, rapid, accurate and specific RP-HPLC stability indicating method.

The proposed work aim was to develop and validate a simple, rapid and reliable isocratic RP method with PDA detection for the determination of Sofosbuvir tablet dosage form. The novelty of the proposed method included simple sample treatment with sonicator of small amount of powder sample at ambient temperature, short retention time (less than 5 min) good precision (R.S.D. below 2%).

Corresponding Author: G. Kumaraswamy, Associate Professor, Dept. of Pharmaceutical Analysis, Care College of Pharmacy, Warangal, Telangana 506006, India.

Email: kumaraswamy.gandla@gmail.com

The aim of the current work was to develop and validate a simple, fast and reliable isocratic RP.

Method with UV detection for the determination of Sofosbuvir in bulk form. The important features and novelty of the proposed method included simple sample treatment with sonicator of small amount of powder sample at ambient temperature, shot elution time (less than 5 min) SFS, good precision (R.S.D. less than 2%).

Conformation of the applicability of developed method validated according to the international conference on Harmonization (ICH) [6].

Materials and Methods

Chemicals

Sofosbuvir and other chemicals were procured from Sura Pharma Lab Pvt. Ltd. Hyderabad [7].

Reagents

Methanol (HPLC grade), Water (HPLC grade), Potassium dihydrogen phosphate (GR grade), Orthophosphoric acid (GR grade).

Instruments and Equipments

All the chemicals High Performance Liquid Chromatography (Waters 2695 HPLC, Class) with 2487 pumps, auto injector with loop volume of 10 µl (Rheodyne), Programmable variable wavelength PDA detector [8].

Preparation of mobile phase

Mix a mixture of Methanol 60 ml and HPLC grade Water 40 mL (60:40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of standard and sample solutions

Acurately weighed and transferred in to the clean and dry 100 ml volumetric flask and made up to the mark with the diluent (1000 µg/mL). Standard solutions of SFS were prepared in the range of 20 µg/mL to 100 µg/mL by diluting the stock solution with mobile phase. The eluate was monitored at 260 nm. Each solution was then injected into the column and chromatogram was recorded.

Degradation study

The proposed research work to determine whether the analytical methods were stable for Sofosbuvir dosage forms are stressed on the different conditions to applied degradation studies. The ICH guidelines are expressed in ICH Q2A, Q3B, Q2B & FDA 21 CFR section of 211 all the required for development & for the validation of stability study.

The Forced degradation studies was conducted on sofosbuvir and the equivalent to the weight of each tablet was transfer into 100 ml flask & it was treated under the acidic, alkaline, thermal, oxidizing and photolytic conditions. When degradation was complete the solution were left to equilibrate to the room temperature and diluted with mobile phase to furnish the solutions of a concentration equivalent to a 30 µg/mL of Sofosbuvir. The specific degradative conditions are described below mentioned Table 5.

Acid degradation study: The Acid degradation was done by sample was treated with 3 ml of 1N hydrochloric acid and kept for 10 hrs at 60°C. After 10 hrs the solution was neutralized with 3 ml of 1N sodium hydroxide, made the volume up to the mark with mobile phase and analyzed using HPLC. The degrading drug content was found up to 10.5% in the acidic condition.

Alkaline degradation: The Alkaline degradation was done by sample was treated with 3 ml of 1N sodium hydroxide and kept the sample for 10 hr. After 10 hr solution was neutralized to add 3 ml of 1N hydrochloric acid, made the volume up to the mark with irrelevant media and analyzed using HPLC. In alkali degradation study, it was found to be 8.05% of the degraded drug.

Oxidative degradation: The oxidative degradation was done by sample was mixed with 3 mL of 30% v/v aqueous hydrogen peroxide solution and kept for 10 hrs. After 10 hrs made the volume upto the mark with mobile phase and analyzed using HPLC. In oxidative degradation, it was found to be 14.15% of the degraded drug.

Photolytic degradation: The photolytic degradation was done by exposing of drug content under the UV light for 15 mins to 7 days. There is 8.45% of the drug degradation observed in the above specific photolytic degradation condition.

Thermal degradation: The Thermal degradation is to be performing by the exposing the solid drug at the 80°C for 15 mins to 60 mins and at 220°C for 2-5 mins. Resultant chromatogram of thermal degradation study was indicates that the drug was found to be slightly stable under thermal condition. It was only 2.64% of the drug content were degraded.

G. Kumaraswamy, D. Sudheer Kumar, V. Ramya / A Novel Stability Indicating
RP-HPLC Method for Determination of Sofosbuvir in Bulk and Tablet Dosage Forms

15

Results and Discussion

The present study was developed and validated RP-HPLC using RP-C_{18} column for using entire the procedure. Atypical Chromatogram obtained by using the mobile phase (Fig. 2). The precision and Accuracy of the method was determined. The precission study was performed in two consecutive days. The method was validated for linearity, precision and accuracy parameters [9]. The calibration curve of the method was studied by injecting six concentrations of drug prepared in the mobile phase in the range 10-60 µg/mL and solutions are analyzed through the high pressure liquid chromatographic technique (Fig. 3). The peak area were plotted against concentration was subjected to linear plot and the results present in table (Table 3). Precision of this method was studied in inter day and intraday variation [12]. The precision of intraday studies was repeated on two consecutive days. The developed method was found to be precise as the percentage of RSD values for inter-day and intra–day precision studies were found to be less than 2%.

Table 2: Optimized method of parameters

Column C_{18}	Hypersil C18 (4.6×150mm) 5µ
Mobile Phase	Methanol:Water (60:40%)
Flow Rate	1.0 mL /min.
Run Time	6 min.
Column Temp.	Ambient
Volume Of Injection Loop	10µL
Detection Wave Length	247 nm
Linearity Range	10-60 µg/Ml

Table 3: Calibration data of Sofosbuvir

Concentration µg/ml	Average Peak Area
10	983048
20	1873321
30	3655166
40	7263921
50	1406038
60	28932421
Slope (m)	43935
Intercept (C)	15821
r2	0.9995

Table 1: Results of system suitability for Sofosbuvir

S.No	Peak Name	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	Sofosbuvir	3.47	2857505	7462	1.1
2	Sofosbuvir	3.47	2868475	7462	1.1
3	Sofosbuvir	3.49	2855847	6472	1.1
4	Sofosbuvir	3.48	2862642	7183	1.1
5	Sofosbuvir	3.49	2841645	7428	1.1
Mean			2835223		
Std. Dev.			7114.704		
% RSD			0.24075		

Table 4: Precision studies of Sofosbuvir

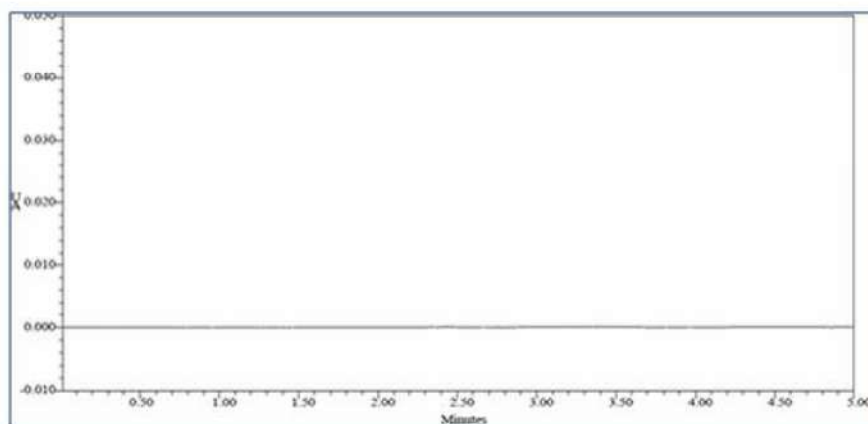| S. No	Peak name	Retention time	Area (µV*sec)	USP Plate Count	USP Tailing
1	Sofosbuvir	3.478	2958333	7583	1.1
2	Sofosbuvir	3.486	2951049	7593	1.1
3	Sofosbuvir	3.474	2959294	8674	1.1
4	Sofosbuvir	3.499	2953391	7958	1.1
5	Sofosbuvir	3.492	2950744	9745	1.1
Mean			2954562		
Std.dev			4028.083		
%RSD			0.136334		

Fig 2: Chromatogram showing blank (mobile phase preparation)

Table 5: Percentage of degradation of Sofosbuvir.

Drug Name		Acid	Alkali	Oxidative	Photolytic	Thermal
Sofosbuvir	Std Area			799605		
	Sample Area	290837	2828744	297136	289461	265381
	% of Degradation	2.1%	5.05%	3.14%	8.43%	2.68%
% Average of Degradation		3.5%	4.05%	5.15%	8.45%	2.64%
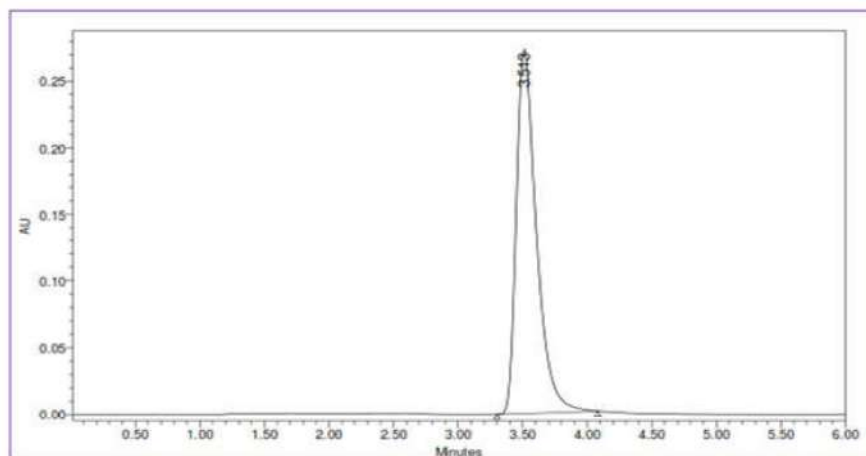
Fig 3: Chromatogram of standard sofosbuvir
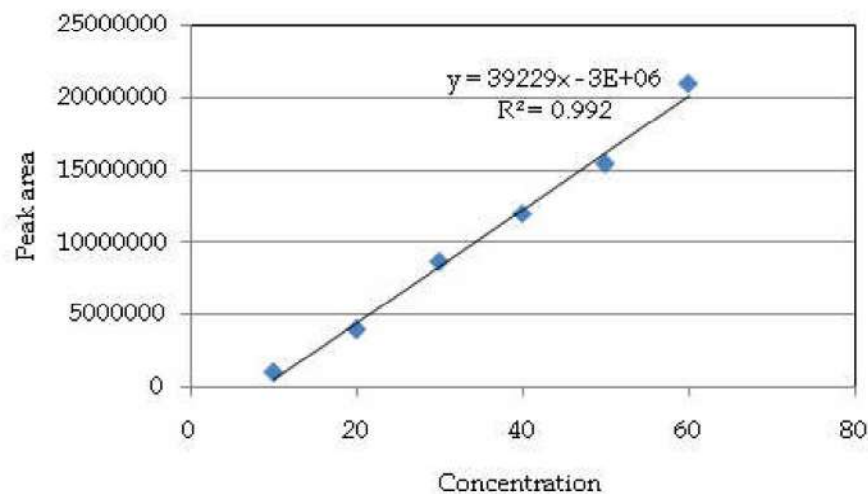
Fig. 4: Calibration graph of Sofosbuvir

Conclusion

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of Sofosbuvir from pure and its dosage forms. The mobile phase is simple to prepare and economical. The developed method is accurate, precise and reliable for the analysisof Sofosbuvir in Pharmaceutical formulations. This method was validated for linearity, accuracy and precision of sofosbuvir drug. The RSD values for all parameters were found to be <2, which indicates the validity of method and results obtained by this method is with fair agreement. Hence, this method can be easily and conveniently adopted for routine analysis of Sofosbuvir in pure form and also can be used for dissolution or similar studies.

G. Kumaraswamy, D. Sudheer Kumar, V. Ramya / A Novel Stability Indicating
RP-HPLC Method for Determination of Sofosbuvir in Bulk and Tablet Dosage Forms

17

References

1. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition Academic press, 1997.pp.24-37.

2. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A John Wiley & Sons, Inc., Publication, 2007.pp.15-23.

3. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.

4. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Edn. England, John Wiley & Sons Ltd, 2004.pp.7-8.

5. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, 2004.pp.421–426.

6. Introduction to Column. (Online), URL:http://amitpatel745.topcities.com/index_files/study/column care.

7. Detectors used in HPLC (online)URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC.

8. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, 1995.p.1126.

9. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 1996.pp.1- 8.