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NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ANTIVIRAL DRUG (SOFOSBUVIR) IN BULK DRUG AND DOSAGE FORMS

Rikshaya Tamang*, Dr. C. Sreedhar, Akkamma H. G., Katikala Dharmendra, Kripa Shrestha and Mohamed Adam Ahmed Mohamed

Department of Pharmaceutical Analysis, Karnataka College of Pharmacy, Thirumenahalli, Hegdenagar Main Road, Bangalore-560064.

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***Corresponding Author**
Rikshaya Tamang
Department of Pharmaceutical Analysis, Karnataka College of Pharmacy, Thirumenahalli, Hegdenagar Main Road, Bangalore-560064.

ABSTRACT

A simple, sensitive, precise, and accurate isocratic reverse phase high pressure liquid chromatographic method has been developed and validated for the estimation of sofosbuvir in bulk and tablet dosage form. To optimize, a column Eclipse plus-C8 (150 mm x 4.6 mm, 5 µm), mobile phase mixture acetonitrile: ammonium acetate as buffer having pH of 3.7 in the ratio of (50:50 v/v) was found to be an efficient system for elution of drug with good peak shape as well as retention time 2.220 min., flow rate 1.0 ml/min. at UV wavelength of 257nm. Quantitative linearity was obeyed in the concentration range of 1-32 µg/ml, the regression equations of concentration over their peak areas were found to be Y = 830031x + 575606 R^2 = 0.9992, where Y is the peak area and X is the concentration of drug. The number of theoretical plates obtained was 2604.352 which indicate the efficient performance of the column. The limit of detection was 0.106 µg/ml and limit of quantification was 0.355 µg/ml, which indicates the sensitivity of the method the high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

KEYWORDS: Sofosbuvir, RP-HPLC and method development.

INTRODUCTION

Sofosbuvir, whose chemical name is Isopropyl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2,4-dioxopyrimidin-1-yl)- 4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxyphosphorryl]amino] propanoate, has molecular formula and the weight of C22H29FN3O9P & 529.16 g/mol, respectively (Figure-1). Sofosbuvir is a direct acting pyrimidine nucleotide analog representing the first NS5B HCV polymerase inhibitor.[1] It has been marketed since 2013. Compared to previous treatments, Sofosbuvir-based regimens provide a higher cure rate, fewer side effects, and a 2- to 4-fold reduced duration of therapy. Sofosbuvir allows most patients to be treated successfully without the use of pegylated interferon (pegIFN), an injectable drug with severe side effects that is a key component of older drug combinations for the treatment of HCV. Sofosbuvir inhibits the RNA polymerase that the HCV uses to replicate its RNA. It was discovered at Pharmasset and developed by Gilead Sciences.[2]

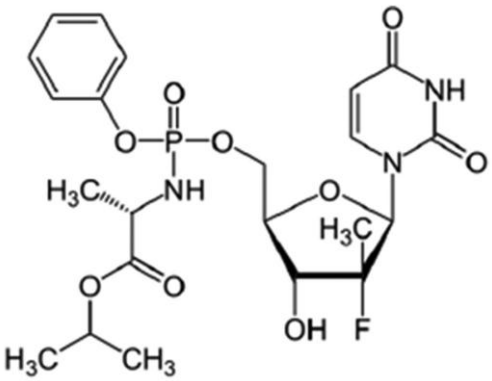

Fig. 1: Chemical Structure of Sofosbuvir.

Sofosbuvir is administered at a dosage of 400 mg once daily, taken with or without food, and has an effective activity against all HCV genotypes. Sofosbuvir is also available as a fixed-dose combination (co-formulation in one tablet) with ledipasvir, HCV NS5A inhibitor, with effective anti-HCV activity. The WHO Guidelines for the screening and treatment of persons with hepatitis C infection recommend Sofosbuvir in combination with ribavirin in genotypes 1, 2, 3 and 4 HCV infection, either with or without pegylated interferon (depending on the HCV genotype).[3] [4]

Sofosbuvir is a white to off-white powder with a solubility of ≥ 2 mg/mL across the pH range of 2-7.7 at 37°C. The partition coefficient (log P) for Sofosbuvir is 1.62 and the pKa is 9.3.[5]

MATERIALS AND METHODS

Chemicals and Reagents: The reference sample of sofosbuvir was obtained from S.R. DRUG Laboratories Pvt. Ltd. Acetonitrile (HPLC grade), Water (HPLC grade), and ammonium acetate (GR grade).

Instrument and Equipment

Table 1: Instruments used.

S.No.	Instruments	Model
1	HPLC	Agilent 1120 Compact LC, EZ Chrome Elite software gradient pump (LC-10AT vp pump) (4MPa or 40barr), rheodyne injector, UV variable wavelength detector
2	uv-visible spectrophotometer	SHIMADZU-UV 1700
3	Analytical weighing balance	Shimadzu AUX 220
4	Sonicator	Equitron 230VAC, 50Hz
5	vacuum pump	SUPER FIT

Mobile phase optimization

Initially the mobile phase tried was acetonitrile: tris buffer and acetonitrile: ammonium acetate buffer with various combination of pH as well as varying proportions. Finally, the mobile phase was optimized to ammonium acetate buffer pH 3.7, acetonitrile in proportion of 50/50% v/v respectively.

Selection of suitable wavelength detection

Spectrum for Sofosbuvir of 10mg/ml in diluent (mobile phase) was measured from 200 to 400 nm for wavelength maxima by recording UV spectrum of standard solution. Based on the spectrum maxima, 257 nm was selected for method development and validation of Sofosbuvir drug.

Preparation of ammonium acetate buffer

Weighed accurately 0.38 g of ammonium acetate and dissolved it in 500 ml of HPLC Grade water. And adjusted the pH to 3.7 with dilute glacial acetic acid, filtered through 0.45µm nylon membrane filter and degassed.

Preparation of mobile phase

Accurately measured 250ml (50%) of above mentioned buffer and 250ml (50%) of acetonitrile HPLC grade were mixed and degassed in sonicator for 10 minutes then filtered through 0.45µm nylon membrane filter under vacuum filtration.

Selection of diluent: The mobile phase was used as diluent.

Preparation of standard and sample solutions for Sofosbuvir

- **Standard stock solution of Sofosbuvir**

Accurately weighed and transferred 50 mg of Sofosbuvir working standard into a 50-ml volumetric flask. Added about 30 ml of methanol and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with methanol and mixed well.

- **Preparation of standard solution**

Transferred 0.1 ml of Sofosbuvir standard stock solution into a 10-ml volumetric flask and diluted up to the mark with the diluent and mixed well.

Preparation of sample solution

Ten tablets of sofosbuvir containing 400mg of Sofosbuvir were weighed and powdered for further study. The powder equivalent to 50mg of Sofosbuvir was accurately weighed and transferred to 50 ml volumetric flask. After that drug mixture is dissolved in methanol. The volume is maintained with methanol and is sonicated for 10 min. The above solution was carefully filtered through Whatmann filter paper (No. 41). From this solution, required dilutions for HPLC method was prepared by using mobile phase as a solvent.

Table 2: Optimized chromatographic conditions for estimation of Sofosbuvir.

S.NO.	***PARAMETERS***	***ESTIMATION OF SOFOSBUVIR***
1	Mobile phase optimized	ammonium acetate (buffer): acetonitrile 50:50% v/v pH-3.7
2	Stationary phase	C8 5µm 150 X 4.6 mm (Agilent Eclipse Plus C8 Column)
3	Flow rate (ml/min)	1
4	Run time (min)	5
5	Column Temperature (℃)	25±1
6	Volume of injection (µl)	20
7	Detection wavelength (nm)	257
8	Retention time (R_t)	2.220

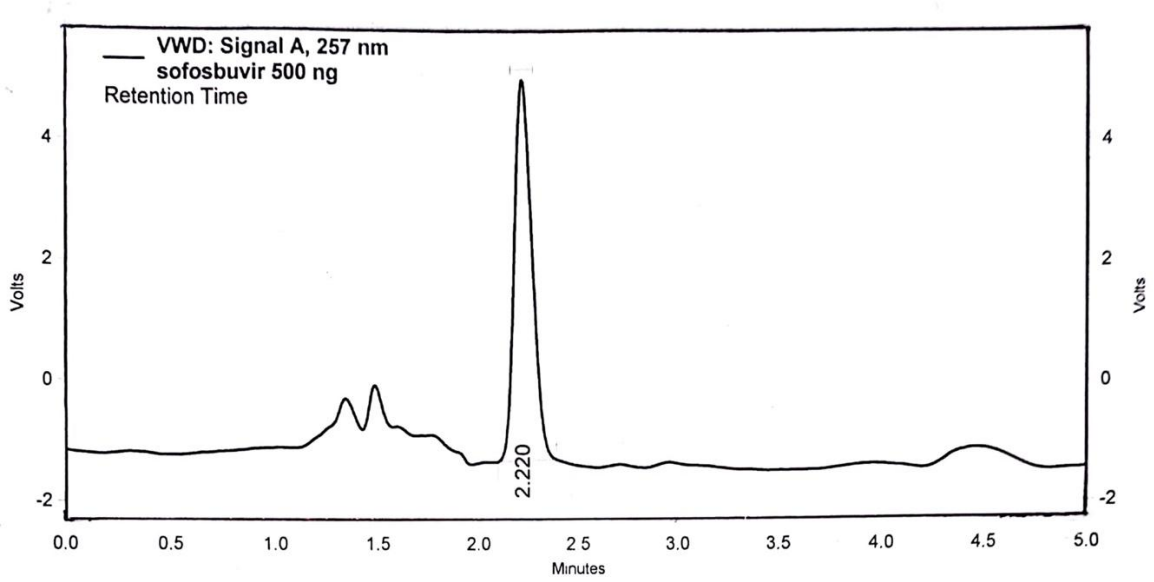

Fig. 2: System Suitability.

Table 3: Results of System Suitability Parameters for Sofosbuvir.

Name	**Sofosbuvir**
Retention time	2.220 mins
Number of Theoretical Plates per meter	2838
Tailing Factor	1.19623
Capacity Factor	0.00909

RESULT AND DISCUSSION

1. Linearity

The linearity of estimation of Sofosbuvir was determined by the analysis of analyte concentration across 1µg/ml to 32µg/ml of Sofosbuvir. They were prepared and then tested at 257 nm. Absorbance is plotted graphically as a function of analyte concentration.

Table 4: Linearity data of Sofosbuvir.

S. No.	CONCENTRATION (µg/ml)	PEAK AREA AT 257nm OF SOFOSBUVIR
1	1	1446598
2	2	2547947
3	4	3545539
4	8	6979850
5	16	14158076
6	32	27067607

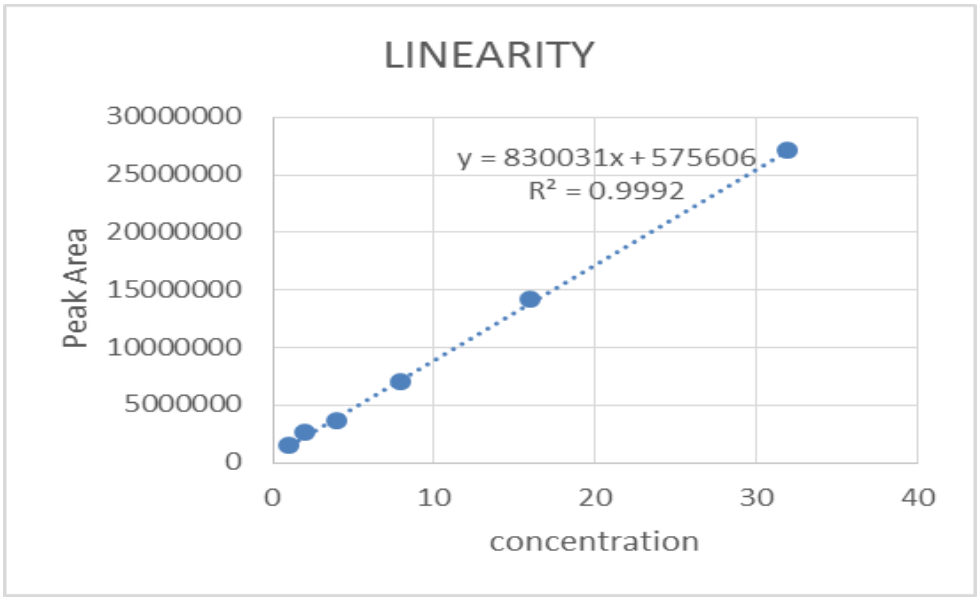

Fig. 3: Linearity curve for estimation of Sofosbuvir.

Correlation coefficient (R_2): 0.9992

2. Precision

Precision of the analytical method was studied by analysis of multiple sampling of homogenous sample.

The inter day (between 2 days) and intraday (at the same days: morning and evening) precision were carried out. The variation of results was calculated and %RSD was determined.

Calculations

Table 5: Data for Intraday Precision (morning).

S.NO. (Injection (4µg/ml)	AREA	AVERAGE	SD	%RSD
1	3581653	3575662.667	25677.6781	0.71
2	3598080			
3	3592364			
4	3552540			
5	3593850			
6	3535489			

Table 6: Data for Intraday Precision (afternoon).

S.NO. (Injection (4µg/ml)	AREA	AVERAGE	SD	%RSD
1	**3562728**	**3543733**	**21189.82**	**0.59**
2	**3548432**			
3	**3549899**			
4	**3547051**			
5	**3502017**			
6	**3552271**			

Table 7: Data for Interday Precision (Day-1).

S.NO. (Injection (4µg/ml)	AREA	AVERAGE	SD	%RSD
1	3576203	3559665	11028.76093	0.30
2	3547054			
3	3556061			
4	3556475			
5	3569711			
6	3552486			

Table 8: Data for interday precision (Day-2).

S.NO. (Injection (4µg/ml)	AREA	AVERAGE	SD	%RSD
1	3528190	3549865.167	27808.85405	0.78
2	3553495			
3	3583485			
4	3581727			
5	3533667			
6	3518627			

3. Accuracy

The accuracy for estimation of Sofosbuvir using HPLC grade methanol was determined by adding known amount of the analyte. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

Table 9: Accuracy for Sofosbuvir.

S.N.	Level of Percentage Recovery	Amount Present (mg/table)	Amount Standard drug added	Area Response	Mean	Standard Deviation	Relative Standard Deviation	Total Amount Recovered (mg)	% Recovery
1.	80%	400	320	3551372 3572302 3543349	3555674.33	14948.2978	0.42	401	100.25
2.	100%	400	400	6911198 6977835 6960031	6949688	34501.53155	0.49	398.24	99.56
3.	120%	400	480	14114471 14171281 14181270	14155674	36030.69	0.25	399.72	99.93

4. Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples. A signal to noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

The values of LOD and LOQ were given in Table no 9.

Table 10: LOD and LOQ for estimation of Sofosbuvir.

S.N.	Name of validation parameter	Signal/noise Ratio (S/N)	LOD (µg/ml)	LOQ (µg/ml
1	**Sofosbuvir**	14.065	0.106 µg/ml	0.355 µg/ml

CONCLUSION

The proposed RP-HPLC method was suitable technique for the determination of Sofosbuvir. All the parameters analyzing Sofosbuvir met the criteria of ICH guidelines for Method Validation. In the present investigation, we have developed a simple, sensitive, precise and accurate RP-HPLC method for the quantitative estimation of Sofosbuvir in bulk and pharmaceutical formulations. The recoveries achieved were found to be within the limits by this method. The HPLC method is more sensitive, precise and accurate compared to the spectrophotometric methods. The HPLC method developed may be recommended for the routine determination of Sofosbuvir in bulk drug and pharmaceutical formulations. In addition to requirements for analytical methods, the striking advantage of all the developed method is that they are economical, cheap and precise.

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