Spectrofluorimetric Quantification of Sofosbuvir using MBTH Reagent and Ferric Chloride in Bulk and Tablet Dosage Form

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ABSTRACT

A new, simple and affordable spectrofluorimetric method was established for the quantification of sofosbuvir in bulk and in its tablet formulation. Native fluorescence of sofosbuvir using MBTH reagent and ferric chloride was measured at 385 nm after excitation at 340 nm. Linear relationship between fluorescence intensity and the sofosbuvir concentration was noticed in 1 – 5 μg/mL range. The method was supported by checking several validation parameters as stated by ICH guidelines. The limit of detection and quantification values (0.32 and 0.97 μg/mL, respectively) and results of validation parameters demonstrated that the method procedure was sensitive, accurate, precise and reproducible (% relative standard deviation < 2.0). The % assay in commercial formulation was found to be 99.50, which is in agreement with ICH guidelines. As a consequence of the above findings, developed method can be successfully adopted in routine analysis of sofosbuvir in pharmaceutical dosage forms.

Keywords: Sofosbuvir, MBTH, Ferric chloride, Spectrofluorimetry.

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INTRODUCTION

Sofosbuvir is chemically known as (S)-Isopropyl 2-((S)-(((2R, 3R, 4R ,5R)-5-(2,4-dioxo3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl) methoxy) -(phenoxy) phosphoryl amino) propinate (Figure 1). Being a Nucleotide Polymerase inhibitor, it is used in the treatment of Hepatitis-C. Extensive literature review of Sofosbuvir disclosed several analytical methods for its quantification either alone or in combination with other drugs. UV spectrophotometric methods in various solvents[1,4,7,8,12,14,15,16], RP-HPLC methods in combinations of stationary and mobile phases[2,3,8,11,12,17,19], UPLC method[5], LC-MS/MS methods[6,9,18], capillary electrophoresis[10], comparative chromatographic, electrophoresis, spectrophotometric methods[13] were reported in literature.

Although numerous instrumental techniques are available as on date, no spectrofluorimetric method has been reported for sofosbuvir using MBTH reagent and ferric chloride, Methanol as solvent to the best of our cognition. Chromatographic methods (RP-HPLC, UPLC, RP-UPHPLC-DAD, etc.) require costly instrumentation, skilled technicians and expensive solvents. Spectrofluorimetry attained exceptional status in the drug analysis because of its appreciable specificity and sensitivity. Unlike spectrophotometry, the analysis can be achieved at both excitation and emission wavelengths in spectrofluorimetry. Keeping these facts in view, a simple, extraction free and sensitive spectrofluorimetric method was attempted for Sofosbuvir using MBTH reagent and ferric chloride, Methanol as solvent. The method was validated as stated in ICH guidelines and the same with success utilized for quantification of Sofosbuvir in marketed dosage form.
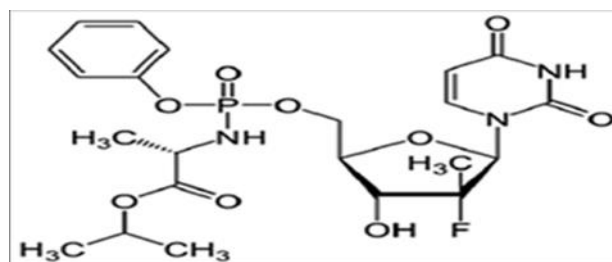

Figure 1: Sofosbuvir chemical structure

MATERIALS AND METHODS

Chemicals

Sofosbuvir (API) was procured from Mylan Laboratories Ltd as a gift sample and the marketed formulation containing Sofosbuvir (Zydus Healthcare, Ltd, East sikkim) was acquired from a drug store in Bidar, Karnataka.

Instrumentation

Various instruments like Digital balance (Shimadzu, AUX 220D, Japan), Melting point apparatus (DBK, Mumbai, India), and Spectrofluorometer (Shimadzu, RF 5301PC, Japan) were used in the present investigation. The standard statistical functions in MS-EXCEL were utilized to compute statistical parameters like arithmetic mean (AM),

standard deviation (SD) and percent relative standard deviation (%RSD).

Chemicals and reagents

Sofosbuvir stock solution:

10 mg of Sofosbuvir was weighed and dissolved in the 10 mL of methanol and used as the standard stock solution (1000µg/mL). From which 1mL was diluted with water to 10mL, produce a solution of (100µg/mL) concentration.

Preparation of 2% MBTH reagent solution:

1 gm of MBTH reagent was weighed and dissolved in 50 mL of water.

Preparation of 1.5% Ferric chloride solution:

0.75gm of ferric chloride was weighed accurately and dissolved in 50mL of 0.1 N Hydrochloric acid.

0.1 N Hydrochloric acid:

0.83mL of concentrated hydrochloric acid was taken in the volumetric flask of 100mL and made up to the volume with water.

Analytical method development

The spectrofluorimetric method development for sofosbuvir was attempted by dissolving the analyte in various solvents such as methanol, water, chloroform, and acetonitrile. Methanol was selected as solvent as it produced clear spectra. MBTH reagent and ferric chloride was found to be suitable for spectrofluorometric analysis of sofosbuvir through optimization studies.

The stock solution containing 10 µg/mL of sofsobovir in MBTH reagent and Ferric chloride, was used to identify the excitation and emission wavelengths.

The excitation wavelength was fixed and solutions were scanned to get the emission spectra. Sofosbuvir showed fluorescence at emission wavelength 385 nm following excitation at 340 nm, when used MBTH reagent and ferric chloride as blank.

Analytical method validation

The emerged method was validated as stated in International Conference on Harmonization (ICH) specifications to prove applicability of the analytical method in quality control of the sofosbuvir.

Linearity studies:

Different volumes of stock solution (100µg/mL) were accurately transferred into 10mL volumetric flasks 1-5µg/mL. To each volumetric flask 1mL of 2% MBTH and 1mL of 1.5% ferric chloride were added and a sufficient amount of water is added and the volume was adjusted to 10mL. The solutions were scanned in the range of 220-500nm, and the fluorescence intensities of the solutions were observed at λ_{ems} 385nm keeping the wavelength of excitation, as λ_{exc} 340nm.

Precision:

The intra-day and interday precision of the fluorometric method was performed by estimating the responses to the three different concentrations of Sofosbuvir (2,3,4 µg/mL) and the responses were recorded three times on the same day and different days. Both intra-day and inter-day precision results were reported as relative standard deviation (%RSD).

Accuracy:

The standard addition method was used to determine the accuracy by calculating recoveries of Sofosbuvir. Pre-qualified sample solutions of Sofosbuvir(400mg) were spiked with a standard solution of Sofosbuvir at 80%, 100%, and 120% levels. The amount of Sofosbuvir was estimated by observing the intensities of the spiked solutions at λ_{ems} of 385nm. The recovery was confirmed by the estimating of the drug in triplicate preparations at each specified concentration level.

Detection Limit (LOD) and Quantification Limit (LOQ):

The LOD and LOQ of Sofosbuvir were calculated by the signal-to-noise ratio (S/N, M i.e., 3.3 for LOD and 10 for LOQ) using the following equation as per ICH guidelines.

The Limit of Detection (LOD):

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

Where, σ = standard deviation of the response

S = slope of the calibration curve of the analyte

The Limit of Quantification (LOQ):

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

Where, σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

Determination of Sofosbuvir dosage form (Assay):

The average weight of 3 tablets was calculated (SOFOSBUVIR 400mg) powdered using a mortar and pestle. Tablet powder equivalent to 10 mg is taken in a 10 mL volumetric flask and dissolved with methanol. The contents were filtered through the Whatman filter paper, and 1 mL of the filtrate was diluted to 10 mL.

The amount of sofosbuvir was estimated by measuring the fluorescence intensity at the emission wavelengths (λ em = 340 nm). The same procedure was performed using the same concentration of drug and the corresponding readings were recorded in triplicate. The amount of sofosbuvir was estimated by substituting the readings into the linear equation representing a calibration graph.

RESULTS AND DISCUSSION

Analytical method optimization:

Fluorescence intensities of Sofosbuvir were measured in different reagents such as NQS, Salicylaldehyde, MBTH, and Ferric chloride.

Table 1: Method Optimization

S. No.	Drug solution	MBTH (2%)	Ferric chloride (1.5%)	Observation
1	1mL	1 Ml	1 mL	Observation was noticed
2	1mL	1 mL	2 mL	No observation
3	1mL	2 mL	2 mL	No observation

As the absorbance for Sofosbuvir was noticed with 1mL of 2% MBTH and 1mL of 1.5% Ferric chloride, this composition of the reagent was selected for the method development.

Selection of excitation and emission wavelengths:

The standard stock solution of Sofosbuvir was prepared by dissolving 10mg of sofosbuvir in 10 mL methanol, from the above solution 1mL was taken to that 1ml of 2% MBTH and 1mL of 1.5% $FeCl_3$ was added then the volume was made up to 10mL with water.

The solution was scanned between 220-500nm in a 1cm cell, and the excitation λ_{exc} 340nm and emission wavelength λ_{ems} 385nm were Observed.
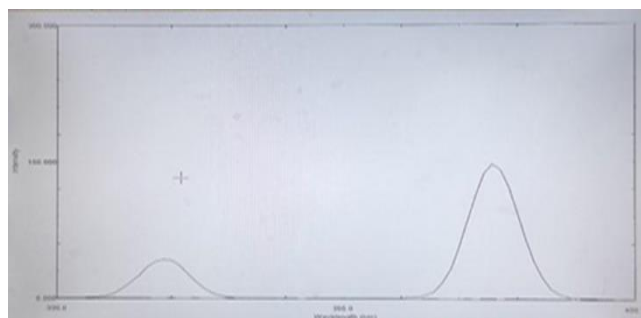

Figure 2: Excitation and Emission wavelength

Analytical method validation

Linearity:

The correlation coefficient (R^2) value for sofosbuvir was 0.9993 and the regression equation was Y=19.645x+101.86. The linearity data and calibration graph of sofosbuvir was given in Table 2 which indicated an increase in intensity with an increase in drug concentration at emission wavelength 385nm.
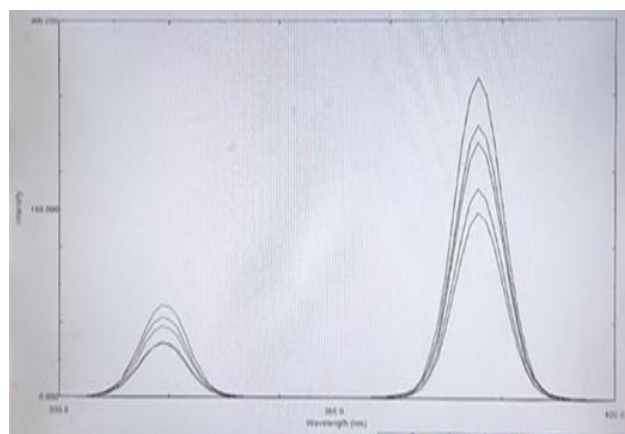

Figure 3: Overlay emission spectra of Sofosbuvir (1-5µg/mL) at λ_{exc} 340nm and λ_{ems} 385nm

Table 2: Linearity data of Sofosbuvir

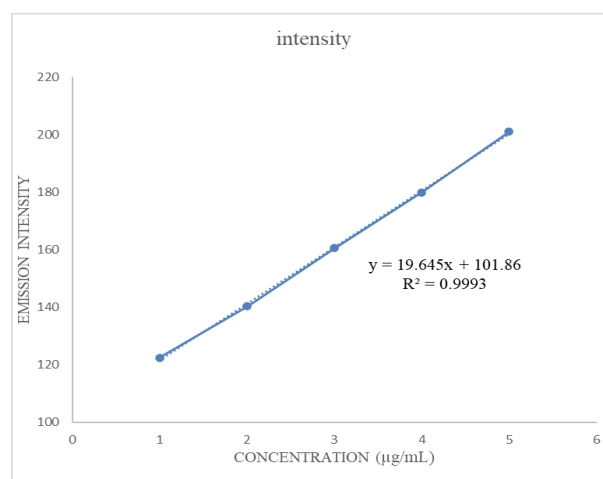| S. No	Concentration (µg/mL)	Intensity (AM ± SD) (n=3)
1	0	0
2	1	122.403 ± 0.576
3	2	140.338 ± 0.602
4	3	160.524 ± 0.774
5	4	179.819 ± 0.831
6	5	200.888 ± 0.90

Figure 4: Standard calibration plot of Sofosbuvir

Precision:

Both intra-day and inter-day precision results were reported as relative standard deviation (%RSD) in Table 3. The %RSD values were less than 2.0, confirming that developed method was precise.

Accuracy:

The recovery at each specified concentration level was found to be in range of 98.44-99.80%, which is satisfactory.

Detection Limit (LOD) and Quantification Limit (LOQ):

The LOD and LOQ values obtained for the spectrofluorimetric method are reported.

Table 3: Precision data of Sofosbuvir

Concentration (µg/mL)	Intra-day Precision		Inter-day Precision	
	Concentration Found (µg/mL) AM ± SD (n=3)	%RSD	Concentration Found (µg/mL) AM ± SD (n=3)	%RSD
2	1.7 ± 0.01	0.5	1.85 ± 0.015	0.82
3	2.9 ± 0.025	0.8	2.8 ± 0.036	1.07
4	3.5 ± 0.05	1.4	3.7 ± 0.05	1.35

Acceptance criteria: % RSD should not be more than 2

Table 4: Accuracy data of Sofosbuvir (400mg) (Recovery studies)

Level of accuracy, %	Theoretical Concentration, (µg/mL)	Concentration found (µg/mL) (AM ± SD) (n=3)	% Recovery	% RSD
80%	1.6	3.51 ± 0.01	99.8	0.2
100%	2	3.97 ± 0.0208	99.5	0.5
120%	2.4	4.47 ± 0.01	98.44	1.56

Acceptance criteria: % RSD should not be more than 2

Table 5: LOD & LOQ data of Sofosbuvir

Validation parameters	Values (µg/mL)
LOD	0.32
LOQ	0.97

Analysis of marketed formulations (Assay):

The accuracy of the proposed method was evaluated by the assay of Sofosbuvir tablets (SOVI HEP 400 mg). The results of Sofosbuvir were compared with the label amounts and reported. The amount of Sofosbuvir – 400mg was obtained at 398mg. The percentage assay found was 99.50% for 400mg. The amount obtained was within limits. The % RSD was obtained < 2

Table 6: Assay of Sofosbuvir tablets (400mg) by spectrofluorimetric method

Label claim (mg)	AM ± SD (mg) (n=3)	% Assay	% RSD
400	398 ± 2	99.50	0.502

CONCLUSION

As mentioned in the objectives of the present work, efforts were made to develop an analytical method for the estimation of Sofosbuvir using MBTH with Ferric chloride by the spectrofluorimetric method. The results obtained and the discussion aroused were described in the earlier contents. The following conclusions were made.

- Suitable spectrofluorimetric method was developed for Sofosbuvir using methanol, MBTH, Ferric chloride, and water which showed fluorescence intensity at λ_{ems} 385nm.

- The linearity was observed in the range of 1-5µg/mL with regression equation Y=19.645x+101.86 and correlation coefficient R^2 = 0.999.

- The developed method was validated as per the guidelines of ICH.

- The parameters such as precision, linearity, accuracy, and assay were satisfactory as indicated by low% RSD (<2).

- Hence the developed fluorometric method for the quantification of Sofosbuvir in the API and marketed dosage forms.

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