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Research Article

METHOD DEVELOPMENT AND VALIDATION OF UV- VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF SOFOSBUVIR AND LEDIPASVIR IN PHARMACEUTICAL API AND ITS FORMULATION

N. Mohana Vamsi [1] *, Dr. Y. Padmanabha Reddy [2], Dr. R. Vijay Kumar [3]

*[1] Rayalaseema University, Kurnool-518007, Andhra Pradesh, INDIA; [2] RIPER College, Anantapur, Andhra Pradesh, INDIA.

[3] APPCO Pharma LLC, Somerset, New Jersey - 08873, USA.*

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ABSTRACT

Objective: *The objective of the present work is to develop a simple, efficient, and reproducible spectrophotometric method for the quantitative estimation of hepatitis-C drugs - Sofosbuvir and Ledipasvir in its active pharmaceutical ingredient (API) form and its formulation.* ***Methods:*** *The developed ultraviolet spectrophotometric method for the quantitative estimation of hepatitis-C drugs - Sofosbuvir and Ledipasvir is based on measurement of absorption at a wavelength maximum (λmax) of 260 and 338 nm using water and acetonitrile as solvent.* ***Results:*** *The method was validated in terms of specificity, precision, linearity, accuracy, and LOD & LOQ as per the ICH guidelines. The method was found to be linear in the range of 25-150% for Sofosbuvir and Ledipasvir. Relative standard deviation for precision results were found to be <2%.The correlation coefficient value observed for Sofosbuvir and Ledipasvir drug substances was more than >0.99, Results obtained from the validation experiments prove that the developed method is quantified for the estimation of Sofosbuvir and Ledipasvir drug substances.* ***Conclusion:*** *The developed method can be successfully applied for routine analysis, quality control analysis, and also suitable for stability analysis of Sofosbuvir and Ledipasvir in API form and its formulation as per the regulatory requirements.*

KEYWORDS: *Sofosbuvir, Ledipasvir, Method development, Validation, Ultraviolet-visible Spectrophotometry.*

INTRODUCTION

Sofosbuvir, a nucleotide analogue, is used in the treatment of chronic Hepatitis-C Virus (HCV)incombination with other drugs. It is a direct acting antiviral agent;Sofosbuvir inhibits the RNA polymerase that the HCV uses to replicate its RNA. It was discovered at Pharmasset and developed by Gilead Sciences [1].

Chemically, it is (S)-Isopropyl-2-((S)-(((2R,3R,4R,5R)-5(2,4-dioxo-3,4-dihydropyrimidin - 1(2H) - yl) - 4 - fluoro - 3 – hydroxy - 4 – methyltetrahydrofuran-2-yl) methoxy) - (phenoxy) phosphorylamino) propanoate as shown in the figure-1. Sofosbuvir, on the other hand, is metabolized to a uridine triphosphate mimic, which acts as a RNA chain terminator when incorporated into the RNA via the NS5B polymerase.

It is an orally administrable potent antiviral agent with fewer side effects and drug interactions along with favorable pharmacokinetic profile and excellent tolerability. Sofosbuvir is a prodrug and is converted to pharmacologically active uridine analog triphosphate (GS-461203), which acts as chain terminator when introduced into Ribose Nucleic Acid (RNA) of HCV by NS5B RNA-dependent RNA polymerase (RdRp).Thus, it inhibits viral RNA replication and acts as anti-viral agent.

Sofosbuvir is a white to off-white crystalline solid and is slightly soluble in water and acetonitrile [2] with a solubility of ≥2 mg/mL across the pH range of 2- 7.7 at 37°C [3]. Water solubility of 0.824 mg/mL and pKa value of 9.3 was reported [4].

***Corresponding author:**

N. Mohana Vamsi

Rayalaseema University,

Kurnool-518007, Andhra Pradesh, INDIA.

** E-Mail: vamsiriper@gmail.com*

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Analytical methods are not available in USP [5] and European Pharmacopoeia [6] for the quantitative determination of Sofosbuvir and Ledipasvir drugs.The present research work describes the estimation of assay content of Sofosbuvir and Ledipasvir in active pharmaceutical ingredient (API) and formulation by using ultraviolet-visible (UV-vis) spectrophotometry technique. The work gives a sensitive, specific, and economical method for the determination of Sofosbuvir and Ledipasvir in very short time by the UV-vis spectrophotometer. Water and acetonitrile is used as a solvent for diluent preparation based on the drug solubility properties of both Sofosbuvir and Ledipasvir. Developed UV-vis spectrophotometric method was validated with respect to specificity, linearity, precision, accuracy, LOD & LOQ.

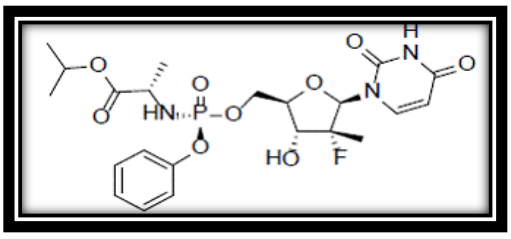

Fig. 1: Chemical Structure of Sofosbuvir

Ledipasvir is a drug for the treatment of hepatitis C [7-9] Ledipasvir inhibits an important viral phosphor protein, NS5A, which is involved in viral replication, assembly and secretion. Sofosbuvir, on the other hand, is metabolized to a uridine triphosphate mimic, which acts as a RNA chain terminator when incorporated into the RNA via the NS5B polymerase. It has a molecular formula of C49H54F2N8O6 and a molecular weight of 889.00. Ledipasvir is practically insoluble (<0.1 mg/ml) across the pH range of 3.0-7.5 and is slightly soluble below pH 2.3 (1.1 mg/ml). The partition coefficient (log P) for Ledipasvir is 3.8 and the pKa1 is 4.0 and pKa2 is 5.0.

The chemical name of Ledipasvir is Methyl [(2S)-1-{(6S)-6-[5-(9,9-difluoro-7{2-[(1R,3S,4S)-2-{(2S)-2-[(methoxycarbonyl) amino] - 3-methylbutanoyl}-2azabicyclo[2.2.1] hept-3-yl] - 1H-benzimidazol-6-yl}-9H-fluoren-2-yl) - 1H-imidazol-2-yl]-5azaspiro[2.4]hept-5-yl}-3-methyl-1-oxobutan-2-yl]carbamate as shown in the figure-2.

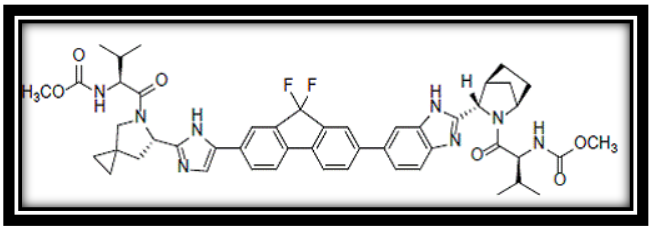

Fig. 2: Chemical Structure of Ledipasvir

From the literature survey, it is evident that very few research articles are available for sofosbuvir and ledipasvir. Ashok chakravarthy published an article on development of - daclatasvir and sofosbuvir in API [10]. P. Durga nithya, developed and validated the sofosbuvir by UV [11]. Mohamed el-kassem developed the simultaneous determination assay and dissolution for sofosbuvir and ledipasvir by RP-HPLC [12]. B*uyyashyam sunder developed and validated method on* ledipasvir and sofosbuvir drugs in human plasma by RP-HPLC method [13]. S. Naazneen developed the assay method and forced degradation study of ledipasvir and sofosbuvir by RP-HPLC in tablet formulation [14]. Benzildudekula developed and validated the simultaneous estimation of sofosbuvir and daclatasvir drug product by RP-HPLC method [15].

MATERIALS AND METHODS

Reagents: UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz was be used for measuring absorbance. Methanol, Acetonitrile and water were used as solvent and were HPLC grade.

Drug: Sofosbuvir & Ledipasvir (Sofosbuvir 400mg & Ledipasvir 90 mg) was received as a kind gift from Spectrum pharma labs and formulation was purchased from local market Radhakishan Pharmaceuticals

Preparation of Stock and Standard Solutions:

Preparation of solutions:

Preparation of diluent solution: Mixture of HPLC grade water and Acetonitrile taken in the ratio 1:1 v/v and sonicated for 15min.

Preparation of Standard stock solutions (500µg/mL & 112.5µg/mL): Accurately Weighed and transferred 50mg of Sofosbuvir and 11.25mg of Ledipasvir working Standards into a 100 ml&100ml clean dry volumetric flasks, add 7ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents.

Preparation of Standard working solutions (20µg/mL&4.5µg/mL) (100% solution): 0.4ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml Sofosbuvir of and 4.5µg/ml of Ledipasvir)

Sample Preparation:

Preparation of Sample stock solutions (800µg/mL&180µg/mL): 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 500mL volumetric flask, 250mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered.

Preparation of Sample working solutions (20µg/mL&4.5µg/mL): From the filtered solution 0.25ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Simultaneous Equation Method Development:

Solutions of both drugs were scanned in the UV range 200–400 nm. The overlay spectra of both drugs were recorded. From overlain spectra, wavelengths 260 nm of (Sofosbuvir) and 338 nm of (Ledipasvir) were selected for analysis of both drugs using simultaneous equation method (260 nm for Sofosbuvir and 338 nm for Ledipasvir). Consequently, it may be possible to determine both drugs by the technique of from method or simultaneous equation method.

Method Validation:

Linearity:

Linearity is the ability of the method to obtain results which are either directly or after mathematical transformation proportional to the concentration of the analyte with in a given range. The linearity of response for Sofosbuvir and Ledipasvir was determined in the range of 25% to 150 %. The six concentrations of each component were subjected to regression analysis by least square method to calculate correlation coefficient and calibration equation. The method of linear regression was used for the data evaluation.

Preparation of Standard stock solutions (500µg/mL&112.5µg/mL):

Accurately Weighed and transferred 50mg of Sofosbuvir and 11.25mg of Ledipasvir working Standards into a 100 ml&100ml clean dry volumetric flasks, add 7ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents.

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (5µg/ml of Sofosbuvir and 1.125µg/ml of Ledipasvir)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (10µg/ml of Sofosbuvir and 2.25µg/ml of Ledipasvir)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Sofosbuvir and 3.375µg/ml of Ledipasvir)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (20µg/ml of Sofosbuvir and 4.5µg/ml of Ledipasvir)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Sofosbuvir and 5.625µg/ml of Ledipasvir)

150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml (30µg/ml of Sofosbuvir and 6.75µg/ml of Ledipasvir)

Precision:

Precision is a measure of the reproducibility of the whole analytical method under normal operating conditions. The precision was expressed as the relative standard deviation (RSD).

%RSD = (standard deviation/average)x 100

The precision of the developed method was carried out by six determinations (preparations) of the test solution by measuring the absorbance of test solution and calculated the % RSD for estimation of drug content.

Repeatability:

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.211% and 0.34% respectively for Sofosbuvir and Ledipasvir. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy:

Accuracy or trueness was determined by applying the method to samples, in which known amounts of analyte have been added. These should be analyzed against standard and blank solutions to ensure that

no interference exists. The accuracy was calculated for the test results as a percentage of the analyte recovered by the assay.

The accuracy of the present method was carried out using the drug substance spiked solution at three different concentration levels of 50%, 100% and 150% in triplicate determinations. Percentage recovery and the mean percentage recovery were calculated for Sofosbuvir and Ledipasvir drug substances.

Preparation of Standard stock solutions: Accurately Weighed and transferred 50mg of Sofosbuvir and 11.25mg of Ledipasvir working Standards into a 100 ml&100ml clean dry volumetric flasks, add 7ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents.

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Limit of Detection (LOD) And Limit of Quantitation (LOQ):

Detection limit:

The lowest amount of analyte that can be detected but not necessarily quantitated as an exact value is called DL. It was measured based on the Standard Deviation (SD) of the response and the slope. SD of response can be estimated from SD of y-intercepts of regression lines and slope can be estimated from the calibration curve.

$$DL = 3.3\ \sigma / S$$

where, σ = SD of the response and S = Slope of the calibration curve.

Quantitation limit:

QL of an analytical procedure is defined as the lowest amount of analyte that can be quantitatively determined with precision and accuracy. It was measured based on SD of the response and the slope. SD of response can be estimated from SD of y-intercepts of regression lines and slope can be estimated from the calibration curve.

$$QL = 10\ \sigma / S$$

where, σ = SD of the response and S = Slope of the calibration curve

RESULTSAND DICUSSION

Optimization of UV-Visible Spectrophotometric Method Conditions:

The main purpose of the current method is to develop simple, sensitive, and precise UV-visible spectrophotometric method for the estimation of Sofosbuvirand Ledipasvir for the routine quantitative determination of samples which will reduce tedious sample preparations, cost materials and manpower required to perform the analysis.

Sofosbuvir and Ledipasvir are UV- absorbing molecules with specific chromophores in the structure that absorb at a particular wavelength, and this absorbance was successfully employed for their quantitative determination using the UV spectroscopic method. The spectral analysis showed that the λmax of Sofosbuvir and Ledipasvir are 260nm and 338 nm respectively. Simple mixture of HPLC grade water and Acetonitriletaken in the ratio 1:1 v/vwas used for the standard and sample solutions of Sofosbuvir and Ledipasvir drug substances. Thus the developed UV-visible spectroscopic method for the analysis of Sofosbuvir and Ledipasvir in its API form enables analysis of several samples at the same time due to its simplicity in performing the analysis.

The UV-visible spectra of Sofosbuvir and Ledipasvir as shown in the figure 3, 4 & 5.

Method Validation:

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended use. The described UV-vis spectrophotometric method for the estimation of Sofosbuvir and Ledipasvirhas been extensively validated for identification and quantification of its drug substances as per ICH guidelines. After successful completion of method development, method validation was performed to ensure that the developed method was capable of giving reproducible and reliable results when used by different operators employed on the same equipment of the same laboratory or different laboratories. The developed UV-vis spectrophotometric method was validated to quantify Sofosbuvir and Ledipasvirin its API form by determining the parameters including precision, linearity, accuracy, and LOD & LOQ according to the ICH guidelines.

Assay:

Working solutions of both drugs were scanned in the UV range 200–400 nm. The overlay spectra of both drugs were recorded. From overlain spectra, wavelengths 260 nm (of Sofosbuvir) and 338 nm (of Ledipasvir) were selected for analysis of both drugs using simultaneous equation method (260 nm for Sofosbuvir and 338 nm for Ledipasvir). Consequently, it may be possible to determine both drugs by the technique of from method or simultaneous equation method.

The concentration of drugs x (Sofosbuvir) and y (Ledipasvir) in sample solutions were determined by the SE method using the following formula:

$$Cx = \frac{A2\ aY1 - A1\ aY2}{a\ X2\ aY1 - a\ X1aY2}$$

$$Cy = \frac{A1\ a\ X2 - A2\ a\ X1}{a\ X2\ aY1 - a\ X1aY2}$$

whereCx and Cy are the concentration of Sofosbuvir and Ledipasvir
A1and A2 are the absorbance of sample solution at 260 nm and 338 nm, respectively
aX1 and aX2 are absorptivity of Sofosbuvir at 260 nm and 344.5 nm
aY1 and aY2 are absorptivity of Ledipasvir at 260 nm and 338 nm, respectively.

Determination of Absorptivity Value:

$$\text{Absorptivity} = \frac{\text{Absorbance}}{\text{Concentration}} \times 100$$

Table No. 1: Absorbance values for Sofosbuvir standard at 260 nm and 338 nm

S. No.	260 nm	338 nm
	aX1	aX2
1	0.588	0.013
2	0.588	0.013
3	0.589	0.012
4	0.588	0.013
5	0.589	0.012
6	0.589	0.013

Table No. 2: Absorbance values for Ledipasvir standard at 260 nm and 338 nm

S. No.	260 nm	338 nm
	aX1	aX2
1	0.0141	0.272
2	0.0139	0.271
3	0.0138	0.273
4	0.0140	0.275
5	0.0139	0.271
6	0.0138	0.275

Table No. 3: Absorbance values for sample Sofosbuvir & Ledipasvir 260 nm and 338 nm

S. No.	260 nm	338 nm
	A1	A2
1	0.597	0.284
2	0.595	0.282
3	0.594	0.283
4	0.592	0.287
5	0.593	0.283
6	0.595	0.285

Table No. 4: % of Assay for Sofosbuvir

S. No.	A2	aY1	(A2)x (aY1)	A1	aY2	(A1)x (aY2)	aX2	(aX2)x (aY1)	aX1	(aX1)x (aY2)	(A2 aY1)-(A1aY2)	(aX2 aY1)-(aX1 aY2)	CX	% Assay
1	0.284	0.0141	0.00400	0.597	0.272	0.162384	0.013	0.0002	0.588	0.15994	-0.1583	-0.159	0.99139	99.139
2	0.282	0.0139	0.00392	0.595	0.271	0.16125	0.013	0.0002	0.588	0.15935	-0.1573	-0.159	0.98838	98.838
3	0.283	0.0138	0.003905	0.594	0.273	0.162162	0.012	0.0002	0.589	0.16080	-0.1582	-0.160	0.98525	98.525
4	0.287	0.0138	0.003961	0.592	0.275	0.1628	0.013	0.0002	0.588	0.16170	-0.1588	-0.161	0.98336	98.336
5	0.283	0.0139	0.003934	0.593	0.271	0.16070	0.012	0.0002	0.589	0.15962	-0.1567	-0.159	0.98321	98.321
6	0.285	0.0138	0.003933	0.595	0.275	0.163625	0.013	0.0002	0.589	0.16198	-0.1596	-0.161	0.98696	98.696
							Avg.							98.643
							SD							0.316
							%RSD							0.320206

Table No. 5: % of assay for Ledipasvir

S. No.	A2	aY1	(A2)x (aY1)	A1	aY2	(A1)x (aY2)	aX2	(aX2)x (aY1)	aX1	(aX1)x (aY2)	(A2 aY1)-(A1aY2)	(aX2 aY1)-(aX1 aY2)	CX	% Assay
1	0.597	0.013	0.00764	0.284	0.588	0.166992	0.0141	0.0002	0.272	0.15994	-0.1593	-0.159	0.99746	99.746
2	0.595	0.013	0.00744	0.282	0.588	0.16582	0.0139	0.0002	0.271	0.15935	-0.1583	-0.159	0.99500	99.500
3	0.594	0.012	0.00737	0.283	0.589	0.166687	0.0138	0.0002	0.273	0.16080	-0.1593	-0.160	0.99188	99.188
4	0.592	0.013	0.00740	0.287	0.588	0.168756	0.0140	0.0002	0.275	0.16170	-0.1613	-0.161	0.99895	99.895
5	0.593	0.012	0.00735	0.283	0.589	0.16669	0.0139	0.0002	0.271	0.15962	-0.1593	-0.159	0.99929	99.929
6	0.595	0.013	0.00750	0.285	0.589	0.167865	0.0138	0.0002	0.275	0.16198	-0.1603	-0.161	0.99114	99.114
							Avg.							99.562
							SD							0.353
							%RSD							0.3549

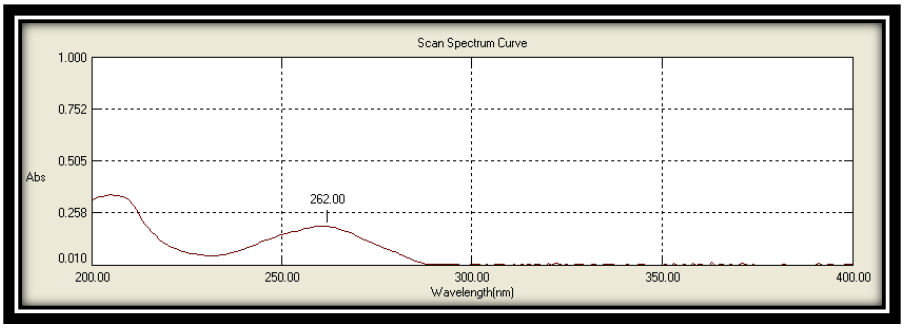

Fig. 3: Spectrum of Sofosbuvir

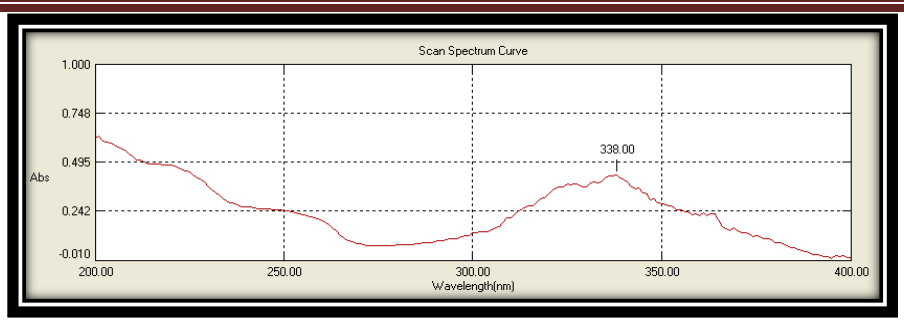

Fig. 4: Spectrum of Ledipasvir

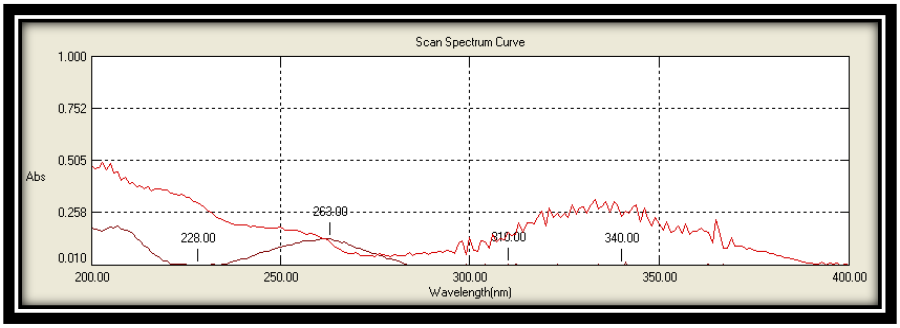

Fig. 5: Spectrum of Sofosbuvir & Ledipasvir

Specificity:

Specificity of the developed method was performed by scanning the UV-vis spectra of diluent, standard, and sample solutions of Sofosbuvir and Ledipasvirfrom 200 to 400 nm. Furthermore, spectral homogeneity of Sofosbuvir and Ledipasvircontrol samples found to be similar with those obtained for the standard solutions of Sofosbuvir and Ledipasvir.

Precision:

Method precision was determined by analyzing the test solution of six determinations, and the observed values of % RSD were shown in the below Table.Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.160% and 0.49% respectively for Sofosbuvir and Ledipasvir. % RSD for Sofosbuvir and Ledipasvir compounds in test solution for six determinations was observed less than 2.0% and the limit was less than 2.0 %.

Table No. 6: Precision for Sofosbuvir

S. No	Amount present in mg	Amount present in %
1.	19.73	98.64
2.	19.66	98.30
3.	19.69	98.47
4.	19.66	98.30
5.	19.69	98.47
6.	19.73	98.64
Mean	19.69	98.47
S.D	0.031	0.152
%RSD	0.160	0.154

Table No. 7: Precision for Ledipasvir

S. No	Amount present in mg	Amount present in %
1.	4.47	99.27
2.	4.43	98.53
3.	4.42	98.17
4.	4.43	98.53
5.	4.45	98.90
6.	4.47	99.27
Mean	4.45	98.78
S.D	0.02	0.445
%RSD	0.49	0.451

Linearity:

The linearity graphs were plotted between the absorbance versus concentration to obtain the calibration curve. Linearity graphs for Sofosbuvir and Ledipasvirwere shown in Figure-1 & 2. The response obtained for Sofosbuvir and Ledipasvirwas found to be linear from 25% to 150%. The correlation coefficient observed for Sofosbuvir and Ledipasvircompounds was greater than>0.99 and also statistical values of all compounds were shown in Table 2. Results demonstrate that an excellent correlation between the absorbanceand concentration of Sofosbuvir and Ledipasvirdrug substances.

Table No. 8: Absorbance values for Sofosbuvir

S. No	Concentration in ppm	Absorbance values
1.	5	0.148
2.	10	0.297
3.	15	0.441
4.	20	0.588
5.	25	0.723
6.	30	0.868

Table No. 9: Linearity table for Sofosbuvir

S. No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm (Sofosbuvir)	% Linearity Level
1	0.1	10	5	25
2	0.2	10	10	50
3	0.3	10	15	75
4	0.4	10	20	100
5	0.5	10	25	125
6	0.6	10	30	150

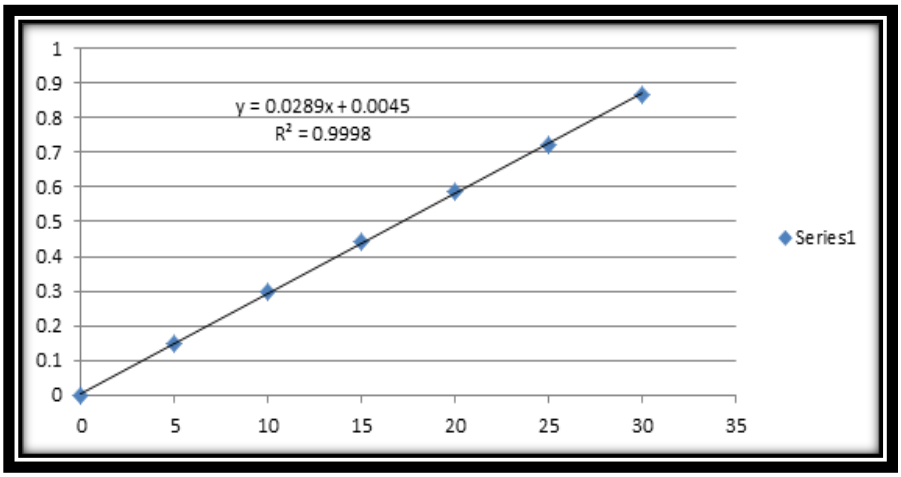

Fig. 6: Linearity plot for Sofosbuvir

Table No. 10: Absorbance values for Ledipasvir

S. No	Concentration in ppm	Absorbance values
1.	1.125	0.067
2.	2.250	0.139
3.	3.375	0.205
4.	4.500	0.276
5.	5.625	0.343
6.	6.750	0.395

Table No. 11: Linearity table for Ledipasvir

S. No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm (Ledipasvir)	% Linearity Level
1	0.1	10	1.125	25
2	0.2	10	2.25	50
3	0.3	10	3.375	75
4	0.4	10	4.5	100
5	0.5	10	5.625	125
6	0.6	10	6.75	150

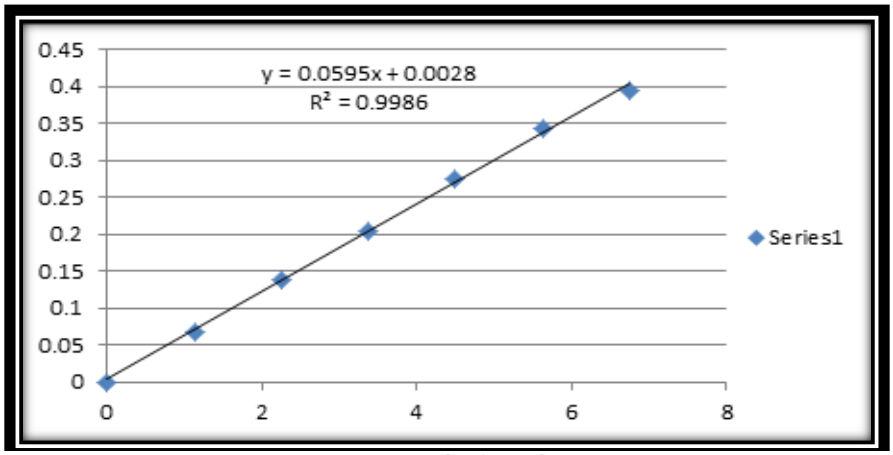

Fig. 7: Linearity plot for Ledipasvir

Accuracy:

The percentage recovery results for Sofosbuvir and Ledipasvirwere varied from 99.64% to 101.67 % and 98.93% to 101.69 % at three different concentration levels, and the results were shown in Table 3. Based on the % recovery data, it was concluded that the developed method is capable for the estimation of Sofosbuvir and Ledipasvirdrug substances and is adequate for routine analysis.

Table No. 12: Accuracy for Sofosbuvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	10.14	101.43	101.16%
	10	10.0	100.00	
	10	9.964	99.64	
100%	20	20.25	101.25	
	20	20.322	101.61	
	20	20.179	100.89	
150%	30	30.50	101.67	
	30	30.42	101.43	
	30	30.465	101.55	

Table No. 13: Accuracy for Ledipasvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	2.25	2.237	99.44	100.17%
	2.25	2.2547	100.19	
	2.25	2.237	99.44	
100%	4.5	4.57	101.69	
	4.5	4.55	101.32	
	4.5	4.57	101.69	
150%	6.75	6.72	99.69	
	6.75	6.67	98.93	
	6.75	6.69	99.18	

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

LOD for Sofosbuvir:

The lowest amount of analyte that can be detected but not necessarily quantitated as an exact value is called DL.

$$DL = 3.3\ \sigma / S$$

Where, σ = SD of the response and S = Slope of the calibration curve.

$$DL=3.3*0.004/0.028 = 0.471429$$

LOQ for Sofosbuvir:

QL of an analytical procedure is defined as the lowest amount of analyte that can be quantitatively determined with precision and accuracy.

$$QL = 10\ \sigma / S$$

Where, σ = SD of the response and S = Slope of the calibration curve

$$QL =10*0.004/0.028 = 1.4$$

LOD for Ledipasvir:

The lowest amount of analyte that can be detected but not necessarily quantitated as an exact value is called DL.

$$DL = 3.3\ \sigma / S$$

Where, σ = SD of the response and S = Slope of the calibration curve.

$$DL=3.3*0.002/0.059 = 0.11$$

LOQ for Ledipasvir:

QL of an analytical procedure is defined as the lowest amount of analyte that can be quantitatively determined with precision and accuracy.

QL = 10 σ / S

where, σ = SD of the response and S = Slope of the calibration curve

QL =10*0.002/0.059 = 0.3

CONCLUSION

Simple, precise and economical UV-visible spectrophotometric method has been developed for the quantitative estimation of Sofosbuvir and Ledipasvir in its API and formulation. Method is validated as per the ICH guidelines and also the developed method is robust with respect to wavelength. The developed method can be used for the quantification of Sofosbuvir and Ledipasvir in API and its formulation.

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