IJPSR (2021), Volume 12, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 July 2020; received in revised form, 15 December 2020; accepted, 14 June 2021; published 01 July 2021

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR BY UV-VISIBLE SPECTROPHOTOMETRY

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Keywords:

Sofosbuvir, Velpatasvir, Simultaneous estimation, Maximum Absorbance, UV spectroscopy

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ABSTRACT: A simple, linear, precise, and economical UV spectrophotometric method has been developed for the simultaneous estimation of sofosbuvir and velpatasvir in the bulk and pharmaceutical dosage form. The standard stock solutions were prepared by using methanol as solvent. Estimation of sofosbuvir and velpatasvir was conducted by using a simultaneous equation method based on the measurement of Maximum absorbance at two wavelengths. The maximum absorbance was found at 261 nm for sofosbuvir and velpatasvir at 303 nm, respectively. The linearity was found in the concentration range of (5.0 µg/mL- 30.0 µg/mL) for sofosbuvir and (2.5 µg/mL-20.0 µg/mL) for velpatasvir with correlation coefficient of 0.996 for sofosbuvir and 0.994 for velpatasvir, respectively. Regression equation for sofosbuvir and velpatasvir were y = 0.0198x - 0.0061, y = 0.0225x + 0.029. The % accuracy was within the range of 98.04- 101.70%, and precision (%RSD) was found to be < 2. The developed method was validated according to ICH guidelines and successfully applied for the assay of marketing formulations.

INTRODUCTION: Sofosbuvir (SOF) belongs to the class of antiviral drugs. It is used for the treatment of Hepatitis C virus [1]. The drug directly interferes with the HCV2 RNA by NS5B polymerase and acts as a chain terminator. The drug is available for oral administration in the form of 400 mg tablets. The IUPAC Name of SOF is Isopropyl (2S)-2-[[[(2R, 3R, 4R, 5R)-5-(2,4-dioxopyrimidin-1-yl) -4-fluoro-3- hydroxy-4-methyl-tetrahydrofuran-2-yl] methoxy-phenoxy-phosphoryl] amino] propionate [3]. Its molecular formula is $C_{22}H_{29}FN_3O_9$ Pandmolecular weight is 529.4 g/moL **Fig. 1**.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(7).3969-74

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(7).3969-74

Velpatasvir (VEL) is also an antiviral drug. It acts as an NS5A inhibitor, which is used along with SOF in the treatment of the Hepatitis C virus. Upon oral administration and intracellular uptake, it appears to bind to domain I of the NS5A protein.

This inhibits the activity of the NS5A protein and results in the disruption of the viral RNA replication complex, blockage of viral HCV RNA production, and inhibition of viral replication. The drug is available in the form of 100 mg tablets. The IUPAC name of VEL is Methyl {(2S) - 1 -[(2S, 5S)-2- (9 - { 2 -[(2S ,4S) – 1 - {(2R) - 2 -[(methoxy carbonyl) amino] -2 - phenyl acetyl} - 4-(methoxymethyl) -2 - pyrrolidinyl] -1 H - imidazol-4-yl} -1, 11 – dihydroisochromeno [4', 3': 6,7] naphtha [1, 2-d] imidazol-2-yl) - 5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate [4]. Its molecular formula is $C_{49}H_{54}N_8O_8$ and molecular weight is 883.02 g/mol. **Fig. 2**.

The literature survey revealed that the methods reported [5-7] for the estimation of SOF and VEL individually or in their combined dosage forms by UV Spectroscopy [8-10] and RP-HPLC method [11-14]. The proposed method is highly recommended in routine analysis where method is more economical, rapid, precise, and accurate. The proposed method was validated as per the International Conference on harmonization (ICH) guidelines [15].

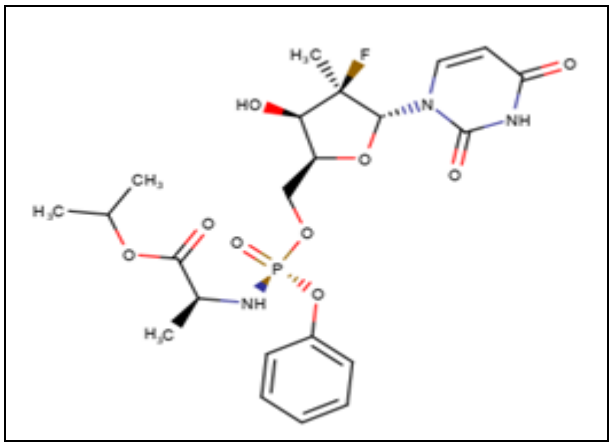

FIG. 1: STRUCTURE OF SOFOSBUVIR

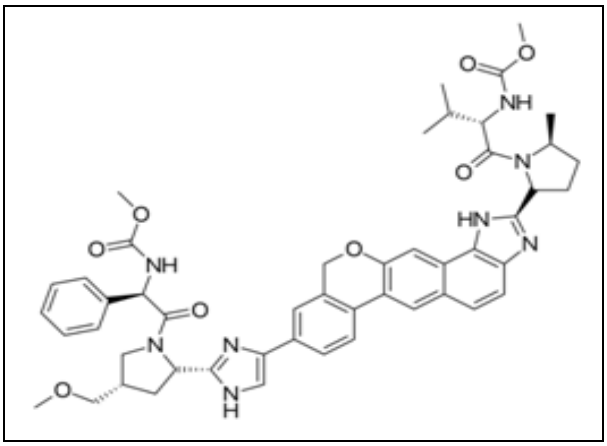

FIG. 2: STRUCTURE OF VELPATSVIR

MATERIALS AND METHODS:

Materials and Instruments: Pure drug sample of Sofosbuvir and Velpatasvir were kindly supplied by Hetero Drugs Pvt. Ltd., Hyderabad. The tablet used for analysis was EPCLUSA which contains 400 mg of SOF and 100 mg of VE. Methanol of AR Grade was supplied by Research lab fine Chem industries. UV - Visible double beam Spectro-photometer (UV 1800S) with software UV probe was used for the study.

Selection of Solvent: Methanol of Analytical reagent grade was selected as a solvent for developing spectral characteristics of both drugs. The selection was made by assessing the solubility of both drugs in different solvents like water, acetonitrile, chloroform.

Preparation of Standard Stock Solution: Accurately weighed quantity of SOF (10 mg) and VEL (10 mg) were transferred into two separate 10 ml volumetric flasks, dissolved in methanol, and made up to the volume with the same solvent to get the concentration of 100 µg/mL of each. From the above stock solutions, 1 mL each was transferred into another 10 mL volumetric flask and made up to the volume (10 µg/mL).

Simultaneous Equation (Method Development):

Selection of Wavelength: Working standard solutions of both the drugs were scanned in the UV range of 200-400 nm. The overlay spectra of both the drugs were recorded **Fig. 3**. The maximum absorbance was found at 261 nm for SOF and 303 nm for VEL for analysis using the simultaneous equation method from overlay spectra. This developed method obeys Beer's law [16].

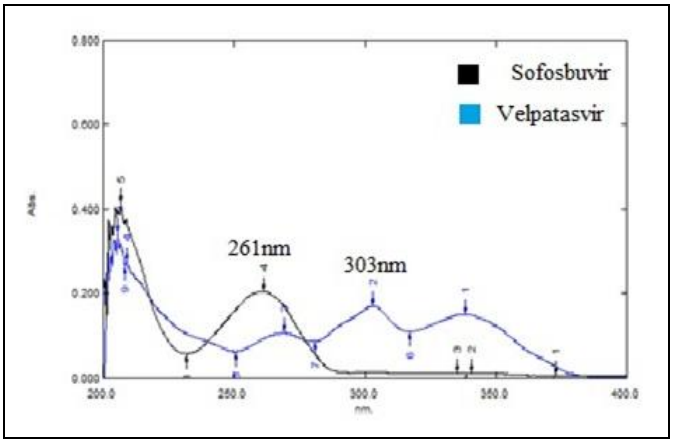

FIG. 3: OVERLAY SPECTRA OF SOFOSBUVIR AND VELPATASVIR

A standard concentration of 10µg/ml of each drug SOF and VEL was prepared using methanol, and their respective absorbance's were measured at 261 nm and 303 nm.

The concentration of drugs SOF and VEL in sample solutions were determined by the simultaneous equation method [17]using the following formula:

$$Cx = A2\ ay1 - A1ay2 / ax2ay1 - ax1ay2 \quad \ldots\ldots (1)$$

$$Cy = A1\ ax2 - A2ax1 / ax2ay1 - ax1ay2 \quad \ldots\ldots (2)$$

Where CX and Cy are the concentrations of SOF and VEL, A1 andA2 is the absorbance of standard solution at 261 nm and 303 nm, respectively, ax1and ax2 is absorptivity of SOF at 261 nm and 303 nm and ay1 and ay2 are absorptivity of VEL at 261 nm and 303 nm, respectively.

Determination of Beer's Law: The mathematical relationship between concentration and absorbance is called the Beers law; the results are presented in **Table 1**.

$$A = €CL \rightarrow € = E_{1cm}^{1\%} \times (\text{Molecular weight}) /10$$

A = absorbance, € = molar absorptivity, C= molar concentration, L = Path length (1 cm)

The developed method was validated according to ICH guidelines.

TABLE 1: SIMULTANEOUS ESTIMATION RESULTS OF SOFOSBUVIR AND VELPATASVIR

Working standards	Absorbance λ_1 -261nm	Absorptivity λ_1 -261nm	Absorbance λ_2 -303nm	Absorptivity λ_2 -303nm
SOF	0.171	0.0171	0.037	0.0037
VEL	0.185	0.0185	0.341	0.0341
Sample	0.278	-	0.288	-

RESULTS AND DISCUSSION:

Method Validation:

Specificity: The Specificity of the method was determined by measuring the absorbance of SOF and VEL individually at 261 nm, and 303 nm against the blank and synthetic excipients, and their absorbance were compared with the blank and synthetic excipients. No interference was observed at 261 nm and 303 nm, indicating that the method is specific

Linearity: The calibration curves **Fig. 4** and **5**were constructed by plotting the absorbance versus the concentration ranges from 5to 30 µg/ml and 2.5to 20 µg/ml for SOF and VEL, respectively.

It was found that the calibration curves **Fig. 6** and **7** were linear in these concentration ranges with their correlation coefficient values (R2) 0.984 and 0.991 for SOF and VEL, respectively.

Results revealed that a good correlation exists between the concentration of the drug and their corresponding absorbance values. The summary of the calibration plot is given in **Table 2**.

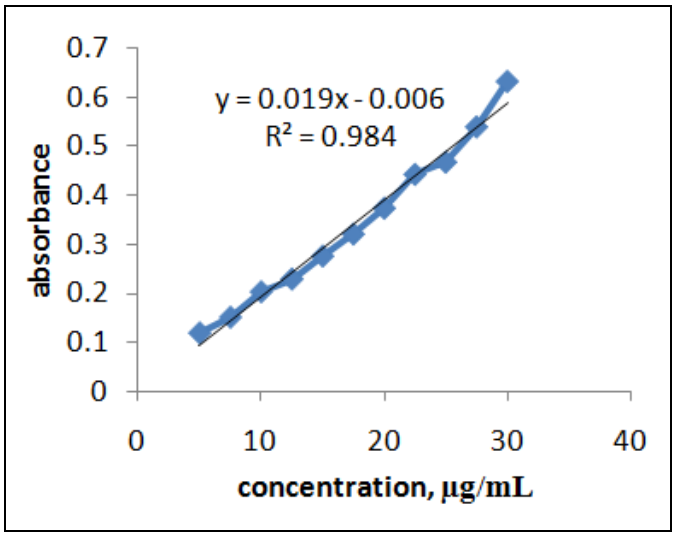

FIG. 4: A) CALIBRATION GRAPH OF SOFOSBUVIR

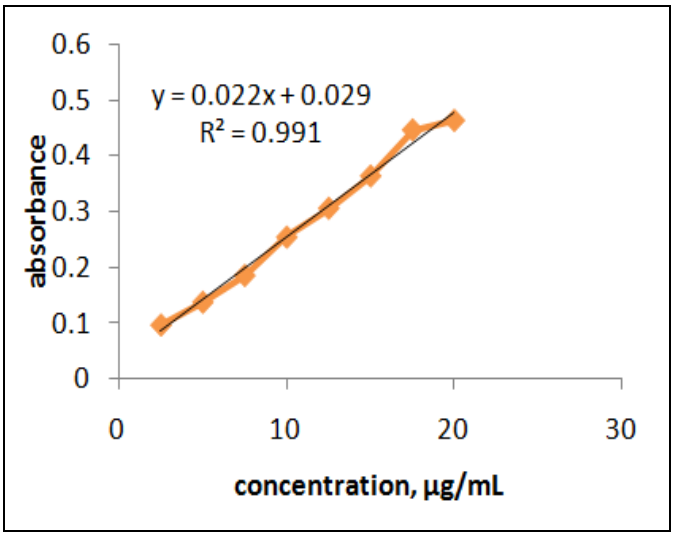

FIG. 5: B) CALIBRATION CHART OF VELPATASVIR

TABLE 2: SUMMARY OF VALIDATION PARAMETERS BY DEVELOPED METHOD

Parameters	Sofosbuvir	Velpatasvir
Working wavelength	**261nm**	**303nm**
Concentration range(µg/mL)	5.0-30.0	2.5-25.0
LOD(µg/ml)	1.51	0.76
LOQ(µg/ml)	5.0	2.5

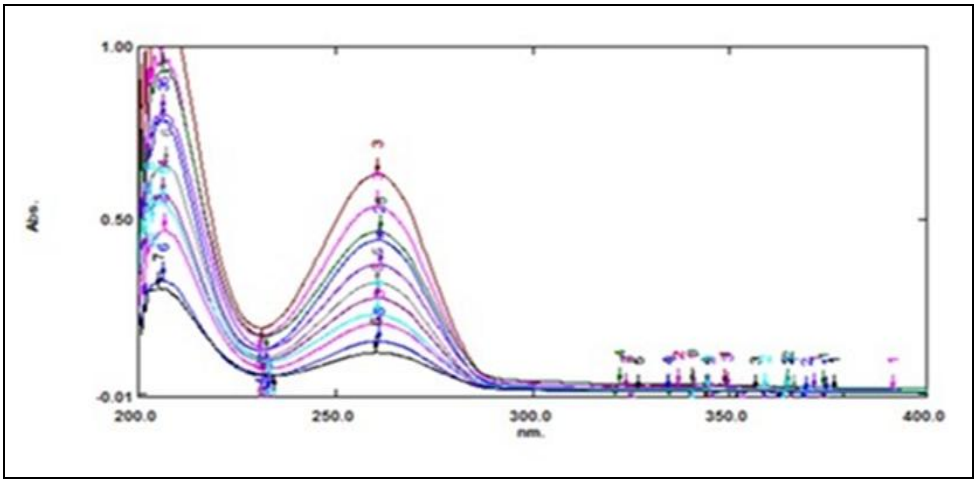

FIG. 6A: OVERLAY SPECTRA OF SOF

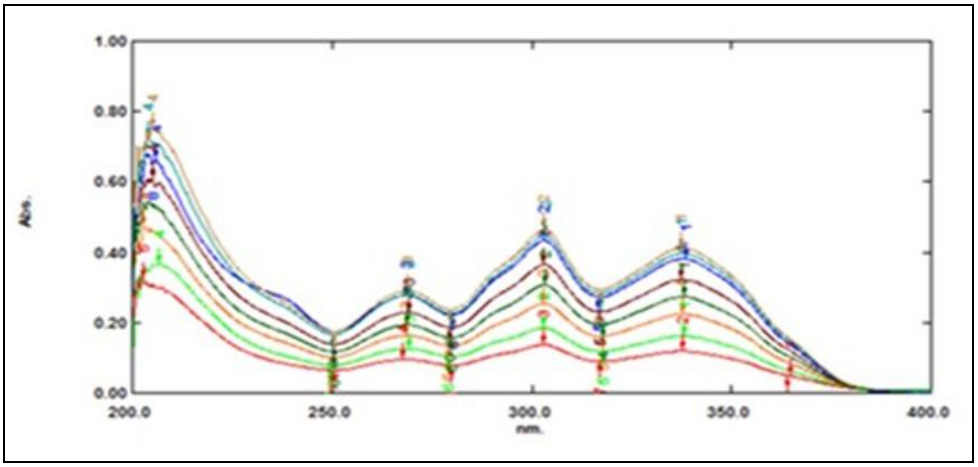

FIG. 6B: OVERLAY SPECTRA OF VEL

Accuracy: For studying the accuracy of the developed method, and for measuring the interference of excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by the addition of known amounts of each drug corresponding to 80%, 100%, and 120% of the label claim of SOF and VEL to a known concentration of sample solution. The amounts of standard recovered were calculated in terms of mean recovery at each level, and the results are presented in **Table 2**. The percentage recovery varied from 98.0 - 102.02% for SOF and VEL, indicating good accuracy of the method.

TABLE 3: ACCURACY RESULTS OF SOFOSBUVIR AND VELPATASVIR

Drug	Levels (%)	Amount added (mg/mL)	Amount recovered (mg/mL)	% Recovered
	80	18	17.8	98.80
SOF	100	20	19.89	99.40
	120	22	21.86	99.50
	80	4.5	4.49	99.71
VEL	100	5.0	4.96	99.20
	120	5.5	5.48	99.63

Precision: A precision study was established by measuring the absorbance of sample solution without changing the assay procedure, and the results are presented in **Table 3**.

Intraday Precision (Repeatability): This study was performed with a minimum of six replicate measurements of absorbance of sample solution at 0hr, 1hr, 2hr, and 3hr within the same day.

Interday Precision: Interday precision was performed after 24 h time-lapse, and % RSD of SOF and VEL were found to be < 2 %.

TABLE 4: PRECISION STUDY RESULTS OF SOF AND VEL

Working standard	Precision	Average	Standard Deviation	% RSD
SOF	Intra-day	0.286	0.000122	0.20
	Inter-day	0.284	0.000266	0.21
VEL	Intra-day	0.107	0.000132	0.12
	Inter-day	0.104	0.000298	0.58

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were calculated depending on the response of standard deviation (50% concentration solution) and the slope of the calibration graph. LOD and LOQ values were found to be 1.51 and 5.0 µg/ml for SOF and 0.76 and 2.5 µg/ml for VEL, respectively. The LOD and LOQ were calculated as

$$LOD = 3.3\sigma/ S \text{ and } LOQ = 10\sigma/ S$$

Where σ is the standard deviation of the lowest standard concentration and S is the standard curve slope.

Application of Developed Method to Marketed Dosage Forms: Accurately weighed 20 tablets of marketing combinational dosage form were made into a fine powder. Powder weight equivalent to10 mg of SOF was transferred into a 100 ml volumetric flask, and methanol was added and then sonicated for 20min. Then the final volume was made up to the mark with the solvent and filtered. Transferred the 1ml from primary stock solution into another 10ml volumetric flask and made up with solvent to get the concentration of 10 µg/ml. The absorbance of the resulting solution was measured at 261 nm and 303 nm and the amount of SOF and VEL present in each tablet was calculated. The assay results are presented in **Table 5**.

TABLE 5: ASSAY RESULTS OF TABLET DOSAGE FORM

Working standards	Label claim (mg)	Amount found (mg)	% Assay (%)
Sofosbuvir	400	396.9	99.2
Velpatasvir	100	98.9	98.9

CONCLUSION: The spectrophotometric method (simultaneous equation method) was developed to simultaneously estimate SOF and VEL in the combined pharmaceutical formulation without prior separation. This developed method was simple, precise, and accurate, which can be reflected from validating data. The proposed method can be successfully applied for simultaneous estimation of Sofosbuvir and Velpatasvir in routine analysis.

ACKNOWLEDGEMENT: The authors are thankful to the Anurag Group of Institutions for providing the necessary facilities.

CONFLICTS OF INTEREST: The Author(s) declare(s) that there is no conflict of interest.

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How to cite this article:
Srawanthi P, Sireesha D, Kiran G, Haque MA and Bakshi V: Method development and validation for simultaneous estimation of sofosbuvir and velpatasvir by UV-visible spectrophotometry. Int J Pharm Sci & Res 2021; 12(7): 3969-74. doi: 10.13040/IJPSR.0975-8232.12(7).3969-74.