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R E S E A E C H A R T I C L E

New Simple Spectrophotometric Method for Simultaneous Determination of Binary Mixture of Sofusbuvir and Velpatasivir in Bulk and Dosage Form

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A B S T R A C T

Two simple accurate reproducible and non-sophisticated Spectrophotometric methods were developed and validated for simultaneous determination of sofusbuvir and velpatasivir without prior separation. The first method was based on employing simultaneous equation method for analysis of both drugs. sofusbuvir and velpatasivir have shown absorbance maxima at 260 and 296 nm in methanol, respectively. Method (II) involves the formation of Q-absorbance equation using the respective absorptivity values at 271nm (isoabsorptive point) and 296 nm (ʎmax of VEL).The drugs obey Beer's Lambert's law in the concentration range of 1–60µg/mL for both SOF and VEL (for Method I) and in the range of 1–60 and 2–60 µg/mL for SOF and VEL, respectively (for Method II). The accuracy and precision were determined and recovery studies confirmed the accuracy of the developed methods that were carried out following the International Conference on Harmonization (ICH) guidelines. Statistical comparison of the suggested methods with the reported spectrophotometric one using F and t tests showed no significant difference regarding both accuracy and precision.

Keywords: Simultaneous equation method, Binary mixture, Q-absorbance method, Velpatasvir, Sofosbuvir, Isosbiestic point.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Hepatitis C infection is a major issue concerned to global health. According to the centers for disease control and prevention estimates, of the people infected with liver diseases, about 3 million deaths occur worldwide each year due to hepatitis C virus (HCV) related causes.Despite the rapid development of new therapies, including interferon-free regimens, there remains an unmet medical need for certain groups of patients with hepatitis C virus infection, in particular for those with genotype 2 and 3 and severe liver disease.Epclusa is a combination product containing sofosbuvir 400mg and velpatasvir100 mg. Epclusa is a prescription medicine used to treat adults with chronic hepatitis C infection. Sofosbuvir is (S)-isopropyl 2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H) -yl) -4-fluoro-3-hydroxy-4-methyltetra hydrofuran-2-yl) methoxy) -(phenoxy) phosphoryl amino)propanoate(figure 1) corresponding to the molecular formula $C_{22}H_{29}FN_3O_9P$ and has a relative molecular mass of 529 g/mol. is a nucleotide prodrug.[1-3] used for treatment of patients with genotypes 1 to6 HCV infections with or without use of Ribavarin.[5,6,8]Velpatasvir chemically designted as Methyl {(1R)-2-[(2S,4S)-2-(5-{2-[(2S,5S)-1-{(2S)-2-[(methoxy carbonyl)amino]-3-methylbutanoyl}-5-methy lpyrrolidin-2-yl]-1,11-dihydro[2] benzopyrano [4',3':6,7] naphtho[1,2-d]imidazol-9-yl}-1H-imidazol-2-yl) -4-(methox ymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl} carbamate (Figure 2) is a novel HCV nonstructural protein 5A (NS5A)[4] inhibitor that is being developed in combination with sofosbuvir and other direct acting antivirals for the treatment of HCV infection[4,7]. Due to the additive antiviral interaction and lack of cross-resistance observed in vitro between Sofosbuvir and Velpatasvir, the administration of these 2 drugs as a film-coated tablet is expected to provide significant antiviral activity and a favourable resistance profile[9,10,11]. Literature survey revealed thatfew RP-HPLC methods[13-19]was reported for determination of the binary mixture in tabletdosage form. But so far one spectrophotometric method[20,21] and one bio analytical method[12] has been reported for simultaneous determination ofSOF and VEL in combination; hence an attempt has been madeto develop simple, sensitive, rapid, precise, accurate and economic methods to analyze the studied drugs simultaneouslyby two spectrophotometric methods, simultaneous equation and Qanalysismethods. The proposed methods have been optimizedand validated as per the International Conference on Harmonization(ICH) guidelines and were found to complywith the acceptance criteria.

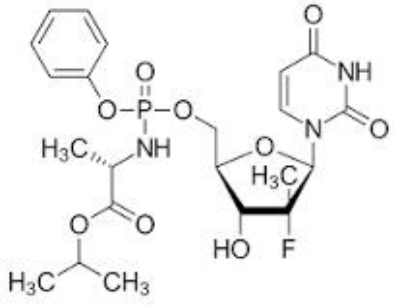

Figure 1: Chemical Structure of Sofusbuvir

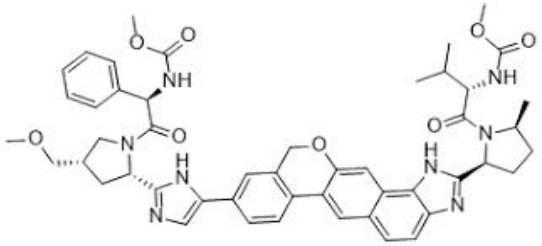

Figure 2: Chemical structure of Velpatasivir

2. Experimental

Apparatus

Spectrophotometer:

SYSTRONICS UV-2202 PC, dual beam UV–visible spectrophotometer with two matched 1 cm quartz cells, connected to an HP compatible personal computer (PC) and an HP-600 inkjet printer. Bundled UV-PC personal spectroscopy software version (3.7) was used to process the absorption and the derivative spectra. The spectral band width was 2nm with wavelength scanning speed of 2800 nm min_1.

Materials

Pure samples: Sofusbuvir and Velpatasivirwere kindly supplied by Hetero drugs pvt ltd Hyderabad.Their purity wasfound to be 99.84 ±1.26 and 100.50 ± 0.71, respectively,according to the manufacturer's direct spectrophotometric method (personal communication).

Market samples:

Epclusa tablets- It was labelled to contain 400 and 100 mg Sofusbuvir and Velpatasivir respectively, per tablet.

Chemicals and reagents:

Methanol (AR Grade) was purchased from Merck(India) Ltd., Mumbai, India. AR grade chemicals and distilled water were used during experimentation.

Stock solutions

Standard stock solutions each containing 1000µg/mL of SOFand VEL were prepared separately in methanol and water 1:1. Working standard solutions of these drugs (100 µg/mL) were obtained by dilution of the respective stock solutions in distilled water.

Procedure:

Spectral characteristics and wavelength selection:

The absorption spectra of 10µg/mL each of SOF,VEL and their1:1 mixture (containing 5µg/mL of each) in distilled water wererecorded over the range 200–350 nm using Methanol:Water (1;1) as blank.The overlain spectra were observed for selection of the suitablewavelengths for each of the developed methods, Fig. 3.

Method I: simultaneous equation method:

Appropriate volume, 1 mL of SOF and VEL standardstock solution was transferred to two separate 10 mL volumetricflasks and the volume was adjusted to mark withwater to get concentration 10µg/mL, respectively.The solutions were scanned separately in the UV-region i.e.400-200 nm. From the overlain spectra (Fig. 3) two wavelengths,260nm (ʎmaxof SOF) and 296nm (ʎmaxofVEL) wereselected for the formation ofsimultaneous equation. The A(1%, 1 cm) was determined at both the wavelengths selectedfor each drug. A set of two simultaneous equations was formed as:

$$Cx = \frac{[(A2ay1 - A1ay2)]}{ax2ay1 - ax1ay2}$$

$$Cy = \frac{[(A1ax2 - A2ax1)]}{ax2ay1 - ax1ay2}$$

Where,

A1 and A2 are the absorbance of sample solutions at 260and 296 nm, respectively. ax1 and ax2 (393,35) are E (1%, 1 cm) of SOF at 260and 296 nm. ay1 and ay2 (58,290.9) are E (1%, 1 cm) of VEL at 260and 296 nm.

Cx and Cy are concentrations of Sofusbuvir andVelpatasivir in mg/mL in sample solution. The values of Cxand Cy were calculated by putting the values of A1 and A2 tosolve the simultaneous Eqs. 1 and 2[22].

Method II (Q-analysis method):

Standard solutions containing 1–10µg/mL each of SOF and VEL were prepared separately using distilled water. The absorptionspectra of the prepared solutions were recorded in therange of 200–350 nm and the absorbance values at 271nm(ʎiso) and 296 nm (ʎmax of VEL) were measured from whichthe absorptivity values for both drugs at the selected wavelengthswere calculated. The method employs Q values andthe concentrations of the studied drugs in the prepared solutionswere determined by using the following equations:

$$Cx = \frac{[Qm - Qy]}{[Qx - Qy]} * \frac{A}{Ax}$$

$$Cy = \frac{[Qm - Qx]}{[Qy - Qx]} * \frac{A}{Ay}$$

Where,

Cx and Cy are the concentrations of SOFandVEL in µg/mL, respectively; Qm is the absorbance of sample at ʎ271/absorbance of sample at ʎ296; Qx is the absorptivity of SOF at ʎ296/absorptivity of SOF at ʎ271; Qy is the absorptivityof VEL at ʎ296 /absorptivity of VEL at ʎ271; Ax is the absorptivityof SOF at ʎ271; Ay is the absorptivity of VEL at ʎ271;and A is the absorbance of the sample at ʎ271.

Analysis of laboratory prepared mixtures:

Different laboratories prepared mixtures containing different ratios of SOF and VEL were prepared. Zero order absorption spectra of these mixtures were recorded using Methanol:Water (1:1) as a blank and then the absorbance at 260, and 296 (for Method I) were measured, also the absorbance values at 271 and 296 nm (for Method II) were recorded.From the calculated regression equations, concentrations ofSOF and VEL in the prepared mixtures were calculated [23].

Analysis of the marketed formulation: Ten EPCLUSA tablets after removing the coating were weighed and crushed to obtain a fine powder. An accurately weighed tablets powder equivalent to 100 mg of tablet was transferred into 100-mLcalibrated measuring flask, 10 mL methanol was added. The prepared solution was sonicated for 45 mins; the volume wascompleted with methanol:water(1:1) and the solution was then filtered.The filtrate was appropriately diluted with methanol and Water (1:1) to prepare a working solution equivalent to 10 µg/mL. The prepared mixture was analyzed and the absorbance values at the selected wavelengths were determined and the methods given under analysis of laboratory prepared mixtures were followed.

Validation methods

Recovery studies: To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at different levels (80%, 100% and 150%). Known amounts of the studied drugs were separately added to the pre-analyzed tablets powder and the percentage recoveries were calculated.

Linearity: Under the optimized experimental conditions calibration curve for SOF and VEL was constructed by analyzing a series of standard solutions of the drug.The regression equation for the results were derived using least square method.

Precision:

Precision was assessed as RSD% at different levels; repeatability was evaluated by the analysis of three different concentrations of pure drugs (2, 6 and 8µg/mL) for each in triplicates on the same day and intermediate precision by repeating analysis of the same concentrations of each seven times on four consecutive days.

LOD and LOQ: They were calculated from the standard deviation (d) of the response and the slope of the calibration curve (S) in accordance to the following equations: LOD = 3.3 (σ/S) and LOQ =10(σ /S).

3. Results and Discussions

Development of simple, rapid, sensitive and accurate analyticalmethods for routine quantitative determination of sampleswill reduce unnecessary tedious sample preparations cost,materials and laboratories. UV-spectrophotometric methodsof analysis offer cost effective and time saving alternative toHPLC method of analysis[24].As shown in Fig. 3, zero order absorption spectra of SOF and VEL show strong spectral overlap which interfere with directspectrophotometric analysis of the studied drugs withoutderivatization. On the other hand, the simultaneous equation method and Q-analysis methods provide a simple, rapid, convenientand accurate way for simultaneous analysis of SOF and VEL in their combined dosage form without derivatization procedure. The main step in the development and validation of an analyticalmethod of analysis is to improve the conditions andparameters which should be followed in the development andvalidation [25]. Different solvents were studied(methanol, ethanol, acetonitrile, water, 0.1N HCl and 0.1NNaOH) to develop suitable methods of analysis, the criteriaemployed were the sensitivity of the method, availability andtoxicity of the solvent. From a solvent effect studies spectralbehaviors of SOF and VEL, Methanol and Water(1:1) was selected as a solventfor the two suggested methods.

Simultaneous equation method:

As the overlay spectrum of SOF and VEL (Fig. 3) shows thatthere was interference in quantitation of individual drug attheir ʎmax due to absorption of another drug at that particularwavelength. So, the simultaneous equation

method was developedfor estimation of drugsfrom the pharmaceuticaldosage form.

Q-analysis (graphical absorbance ratio) method:

This method depends on the property that for the substancethat obeys Beer's Lambert's law at all wavelengths, the ratioof absorptivity (or absorbance) values at any two wavelengthsare constant, independent of the concentration or path length.This ratio is referred as Q-ratio.[26] One of the two selected wavelengths is an isoabsorptivepoint and the other is the wavelength of maximumabsorption of one of the two components.[26-29]. From the overlain spectra of the two drugs and their mixture,Fig. 3, it is evident that SOF and VEL show isoabsorptivepoints at 271and 246 nm, SOF has ʎmaxat 260 nm while VEL hasʎmaxat 296 nm. Using the absorbance values at 271nm (ʎiso) and 296 nm (ʎmax for VEL) gave the best resultsregarding selectivity. The absorbance values at 271 and296nm for SOF in the range of 1–10µg/mL were obtainedand similarly for VEL absorbance values in the range of 2–10µg/mL were measured, absorptivity coefficients were determinedfor both drugs and the average values were taken. Thevalues and the absorbance ratio were used to develop the followingsets of equations from which the concentration of eachcomponent in the sample can be calculated.

CSOF=(Qm -1.37/0.157-1.37)*A/0:022

CVEL=(Qm -0.157/1.37-0.157)*A/0:0217

Where

CSOF is the concentrations of SOF in µg/mL; CVEL is theconcentrations of VEL in µg/mL; Qm is the absorbance ofsample at ʎ 271/absorbance of sample at ʎ 296; and A is the absorbance of the sample at ʎ271.To test the selectivity of developing methods, they were applied for analysis of number oflaboratory prepared mixtures containing SOF and VEL in differentratios. The good percentage recoveries and low SD valuesshown in Table 2, confirming the high selectivity of thesuggested methods. The proposed methods have been success-fully applied for determination of the studied drugs in bulkpowder as well as in their combined dosage form. The resultsobtained upon using the suggested methods for analysis of SOF and VEL in EPCLUSA tablets, Table 2, showed good agreementbetween the amounts estimated and those claimed by themanufacturer. Moreover, results obtained by the suggestedmethods showed no significant difference when compared withthose obtained by applying the reported spectrophotometricone [21] as confirmed from F and t values presentedin Table 2. The developing methods have advantages over thereported one on being more simple, rapid, economic and canbe used for simultaneous determination of the two studieddrugs without derivatization or sample pre-treatment.

Methods validation: Methods validation has been performed as per the InternationalConference on Harmonization (ICH) guidelines [30] and USP requirements[31].

Linearity:

The linearity of the developed methods was evaluated by analyzingdifferent concentrations of standard solutions of SOFand VEL in triplicates. For Simultaneous method, Beer's Lambert's concentration range was found to be 1–10µg/Mlfor both SOF and VEL. On the other hand, for Q-analysismethod the range of SOF was found to be 1–10µg/mL while for VEL was found to be 2–10µg/mL. The values of correlationcoefficients were close to unity indicating good linearity,the characteristic parameters for the constructed equations are summarized in Table 1.

Specificity:

The specificity of the proposed methods was assessed by theirapplication to the analysis of laboratory prepared mixturescontaining different ratios of intact SOF and VEL. Satisfactory results were obtained and presented in Table 2, confirming thateach of the cited drugs could be successfully determined withoutinterference from the other.

Accuracy:

Accuracy was calculated as the percentage recoveries of blindsamples of pure SOF and VEL and it indicated the agreementbetween obtained results and those accepted as true, detailed results are presented in Table 1. Percentage recoveries for SOF and VEL byboth the two methods were found to be acceptable, Table 2.

Precision

The results of intra-day and inter-day precisionconfirmed the precision of the proposed methods, Table 1.

Limits of detection (LOD) and quantitation (LOQ):

Results presented in Table 1, indicated that the methodis sensitive for determination of the studied drugs.

Comparison with the reported method:

The results of developed methods were compared with the reportedmethod and expressed in terms of t value and F value(Table2). The calculated F value was less than the critical value 6.39 for variance at a 0.05%. The calculated t value wasalso less than theoretical critical value 2.776 for the two optimizedspectroscopic methods. The differences between means were considered insignificant. The comparison with the reported method shows that the developed methods are accurate and precise.

Table 1: Optical characteristics and validation of proposed method

Parameters	Method I		Method II	
	SOF	VEL	SOF	VEL
ʎmax	260	296	271(ʎiso)	296
Linearity range	1-60µg/ml	1-50 µg/ml	2-50 µg/ml	1-50µg/ml
Regression Equation	Y=0.03915X+0.002	Y=0.02855X+0.0059		
Correlation coefficient	0.9996	0.9998	0.994	0.9998
Slope	0.03915	0.02855	0.013	0.02855
Limit of detection(µg/ml)	0.119	0.097	0.35	0.097
Limit of	0.36	0.29	1.08	0.29

Quantification(µg/ml)				
Interday precision (%RSD)	1.545	1.30	0.89	1.1
Intraday precision	1.6	1.5	1.16	1.32

Table 2: Determination of studied drugs in Lab prepared mixtures Pharmaceutical preparation by the proposed method and statistical comparision with reported spectrophotometric method

Parameters	SEM Method		Q Analysis Method		Reported Method	
	SOF	VEL	SOF	VEL	SOF	VEL
Accuracy	101.54±1.06	100.41±1.05	102.58±0.97	99.97±1.80	102.33±1.12	99.98±1.05[a]
LP Mixtures[b]	102.4±0.376	100.12±1.093	102.32±0.446	100.23±0.840		
Tablets[c]	96.84±0.77	96.45±0.59	92.75±0.797	98.96±0.862		
Std addition[b]	101.2±0.58	99.68±0.72	101.59±0.344	100.04±0.586	99.5±1.093	98.4±1.035
F test(6.388)[d]	1.124	1.000	1.324	2.491		
t test(2.306)[d]	1.147	0.642	0.376	0.006		

a First derivative spectrophotometric determination of SOF at 250 nm and VEL at 260 nm using CH3OH as a solvent.[20]
b Average of three determinations., c Average of six determinations, d The values in the parenthesis are the corresponding theoretical values at p = 0.05.

4. Conclusion

The developed methods have been successfully applied for simultaneous determination of SOF and VEL in combined sample solution, they were found to be rapid, simple, sensitive and accurate. Once the equations were constructed, analysis required only measuring the absorbance values of the sample solution at the selected wavelengths followed by few simple Calculations. The suggested methods were completely validated showing satisfactory data for all the method validation parameters tested. Recovery studies indicated that practically there was no interference from tablet additives, so these method scan be easily and conveniently adopted for routine quality control analysis of SOF and VEL.

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