Research Article

Development of Spectrophotometric Procedures for the Simultaneous Determination of Voxilaprevir, Sofosbuvir and Velpatasvir in Pure Form and in Tablets Ion-Association Complex Method

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ABSTRACT

Two spectrophotometric procedures are developed for the estimation of three antihepatitic drugs namely Voxilaprevir (VOXL), Sofosbuvir (SOFOS) and Velpatasvir (VELP) in pure form and in different pharmaceutical formulations. The first method is based on the oxidation of velpatasvir and sofosbuvir with potassium iodate in an acidic medium followed by extracting the liberated iodine with cyclohexane and measurement at λ= 525 nm. Beer's law is valid in the concentration ranges from 10–400 and 5–400 µg/ml for velpatasvir and sofosbuvir, respectively. The apparent molar absorptivity of the resulting-colored complex was found to be 1.34 × 10 for velpatasvir and sofosbuvir, respectively. The second method is based on the interaction of the antithepatatis drugs, Voxilaprevir, Velpatasvir and sofosbuvir in 1,2-dichloroethane with bromophenol blue (BPB), bromocresol green (BCG) and bromocresol purple (BCP) in the same solvent to produce stable colored ion pairs with maximum absorbance at 385–405 nm. Regression analysis of Beer's plots showed good correlation in the concentration ranges 10–60, 10–60 and 5–40 µg/mL for voxilaprevir and 1.64 × 103 l.mol−1.cm−1 for velpatasvir, 10–150, 10–150 and 10–60 µg/mL–for sofosbuvir with BPB, BCG and BCP reagents, respectively. The limit of detection is 0.46–7.69 µgml11 for sofosbuvir and 10–250, 10–150 and 10–100 µgml−1 and limits of quantitation range between 1.52–8.53 µgml1. The optimum assay conditions were investigated and the recovery of the drugs from their dosage forms ranged from 99.33% to 100.5%. Intraday relative standard deviations (RSD) were 0.029–1.397% and the correlation coefficients ranged from 0.9992 to 1. The two methods can be applied successfully for the determination of these drugs in tablets. The results of analysis were validated statistically through recovery studies.

Keywords: Voxilaprevir, Sofosbuvir, Velpatasvir, BCG, Vosevi®, Epclusa®, BCG, BPB, KIO_3

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INTRODUCTION

VOSEVI is a fixed-dose combination tablet containing sofosbuvir, velpatasvir, and voxilaprevir for oral administration[1]. VOSEVI is indicated for the treatment of adult patients with chronic hepatitis C virus (HCV) infection without cirrhosis or with compensated cirrhosis.[2] Sofosbuvir is a nucleotide analog HCV NS5B polymerase inhibitor, velpatasvir is an NS5A inhibitor, and voxilaprevir is an NS3/4A protease inhibitor. Sofosbuvir is a pan-genotypic inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is required for viral replication. Sofosbuvir has a molecular formula of C22H29FN3O9P and a molecular weight of 529.4. Velpatasvir, has a molecular formula of C49H54N8O8 and a molecular weight of 883.0. Sofosbuvir is a white to off-white crystalline solid with a solubility of at least 2 mg/mL across the pH range of 2–7.7 at 37 oC and is slightly soluble in water. Velpatasvir is a pan-genotypic HCV inhibitor targeting the HCV NS5A protein, which is required for viral replication[3,4]. It is practically insoluble (less than 0.1 mg/mL) above pH 5, slightly soluble (3.6 mg/mL) at pH 2, and soluble (greater than 36 mg/mL) at pH 1.2. Voxilaprevir is a pan-genotypic inhibitor of the HCV NS3/4A protease[5-7]. Voxilaprevir acts as a noncovalent, reversible inhibitor of the NS3/4A protease. It has a molecular formula of C40H52F4N6O9S and a molecular weight of 868.9. Voxilaprevir is a white to light brown solid. It is slightly hygroscopic to hygroscopic. Voxilaprevir is practically insoluble (less than 0.1 mg/mL) below pH 6.8. The purpose of the current study was to develop spectrophotometric procedures for the determination of Voxilaprevir (VXLP), Sofosbuvir (SOFS) and Velpatasvir (VELP) in pure form and tablets. Different experimental conditions were optimized, and then Beer's law was applied. The methods were successfully applied for the determination of these drugs in tablets, with high percentage recovery values. The data obtained using the proposed methods are compared well with those obtained by the official method.

MATERIALS AND METHODS

Apparatus

Shimadzu Model UV-1601, UV-Visible double-beam spectrophotometer with 1.0 cm quartz cells incorporated with a PC computer was used. Small volumes were taken using automatic pipettes Socorex Swiss (50–200µl).

Materials

Pure samples

Sofosbuvir (SOFS) and Velpatasvir (VELP) was kindly supplied by Mylan laboratories. The purity of the sample was checked by thin-layer chromatography (TLC) and melting point. Voxilaprevir (VXLP), was supplied from Hetero Drugs, Hyderabad. Bromocresol purple (BCP), bromophenol blue (BPB) and bromocresol green (BCG), and KIO3 are obtained from Sigma-Aldrich. Ethanol, Cyclohexane and chloroform are acquired from Rankem, Mumbai. All the chemical were used in this study were of AR grade.

Market samples

EPCLUSA® is a fixed-dose combination tablet containing Sofosbuvir and Velpatasvir for oral administration. Each tablet contains 400 mg Sofosbuvir and 100 mg Velpatasvir. VOSEVI® is a fixed-dose combination tablet containing Sofosbuvir, Velpatasvir, and Voxilaprevir for oral administration. Each tablet contains 400 mg Sofosbuvir, 100 mg Velpatasvir, and 100 mg of Voxilaprevir.

Reagents

All reagents and chemicals used were of analytical grade and solvents were of spectroscopic grade. Potassium iodate solution (1% w/v) and sulphuric acid (30% v/v) were prepared. Stock solutions of VXLP, VELP and SOFOS drugs containing 1 mg ml^{-1} in 10% ethanol were prepared. The dyestuffs were used as 0.02% solutions of bromocresol purple (BCP), bromophenol blue (BPB) and bromocresol green (BCG), all in ethanol. A series of buffer solutions in the pH range from 2–12 were prepared as recommended by Indian Pharmacopoeia.

Application to dosage forms

The contents of 20 tablets were weighed, finely ground and mixed well. An amount of the fine powder equivalent to 50 mg VXLP was weighed and transferred to a 50 ml volumetric flask. It was dissolved in about 40 ml ethanol by shaking for 15 min and completed to volume with ethanol, then filtered. The clear filtrate purportedly containing 1 mg ml^{-1} VXLP was analysed. Twenty tablets each of Epclusa® and Vosevi® tablets were weighed and finely ground. An amount of the powder equivalent to 100 mg Sofosbuvir and an amount equivalent to 100 mg VELP was weighed, dissolved in about 40 ml ethanol and shaken for 10 min. It was completed to volume then filtered. The clear aqueous filtrate labeled as containing 1 mg ml−1 of Sofosbuvir and that containing VELP was analysed. All solutions were protected from light by keeping them in dark-colored quick fit bottles throughout the whole procedure.

General procedure using KIO_3 reagent

A portion of tablet powder equivalent to100mg of Velpatasvir and Sofosbuvir was prepared in 100ml of 10% (v/v) ethanol. Aliquots containing drug solutions of VELP and SOFOS of each drug (concentrations50– 200µ g ml−1) were added separately, in a 100 ml separating funnel, to 1ml of 30% (v/v) H_2SO_4, 1 ml of 1% (w/v) KIO3 and 10 ml cyclohexane. The reaction mixture was shaken well for one minute, followed by separation of the cyclohexane layer and measurement of its absorbance at 520nm against cyclohexane as reagent blank.

General procedure using ion-pair reagent as chromogenic agent

Aliquots containing VXLP, VELP or SOFOS in the working concentration range of (50–200 µg ml^{-1}) were prepared using 1–2 ml of 1mg ml^{-1} of BPB, BCG or BCP reagents. This was allowed by the addition of 4ml universal buffer of pH 4.6 and the resulting solution was made up to 10ml with bi-distilled water. The reaction mixture was left for 20–40 minutes at 40◦C. The ion-pairs were collected in 10ml measuring flasks using 1,2-dichloroethane.The absorbance of each was measured at its λ max against 1,2-dichloroethane as a reagent blank.

Procedure for the assay of dosage forms

An amount of the tablet powder equivalent to 50mg of the drug under investigation was weighed accurately and extracted into 50ml chloroform with shaking. Filtration through whatmanNo.42 filter paper was performed. The filtrate was evaporated to dryness under vacuum and the residue was dissolved in ethanol and transferred to a 50ml standard volumetric flask, diluting to volume. The assay was completed using the procedure described above.

RESULTS AND DISCUSSION

Spectrophotometric determination of VELP and SOFOS drugs using KIO_3

The absorption spectra of the cyclohexane extract of the reaction of VELP and SOFOS drugs with KIO3 in an acidic medium are represented graphically in Figure 1. The measured absorbance of the liberated iodine in cyclohexane has a maximum wavelength at 520 nm. Different experimental conditions affecting the development of the colored product were taken into consideration and carefully controlled. An investigation of the effect of sulphuric acid solution on the formation and extraction of iodine in cyclohexane is shown in Figure 2A. It shows that 1 ml of 30% (v/v) sulphuric acid solution, in the presence of 1 mg ml^{-1} of either drug, is sufficient for complete oxidation of the drugs with KIO3. The excess of H_2SO_4 concentration has no effect and the absorbance is nearly constant. In order to study the effect of time, samples are assayed and the absorbance are determined after varying the time intervals at room temperature as

shown in Figure 2B. The results indicate that 25 minutes is the time required for completion of the oxidation process for both drugs. Figure 2C shows the effect of temperature on the color reaction between VELP and Sofosbuvir drugs with KIO_3 in acidic medium. The absorbance increased with the increase in temperature and became nearly constant at 25–30 ◦C. At higher temperatures the absorbance decreased, which may be attributed to loss of iodine at elevated temperatures. Figure 2D shows that 1 ml of 1% (w/v) KIO_3 is found to be the optimum reagent concentration. In order to prove the validity and applicability of the proposed method and the reproducibility of the results obtained, five replicate experiments were carried out at four concentrations of where RSO= VELP or Sofosbuvir drugs, i.e., each mole of IO_3^- oxidizes one mole of either drug producing one mole of liberated iodine under selected optimal conditions. Under these conditions a linear correlation is obtained between absorbance (A) and concentration (C) of VELP and Sofosbuvir over the concentration range from 10 to 400 (r2 = 0.9998) and 5 to 400 µg ml−1 (r2 = 0.9999) for VELP and SOFOS, respectively. The apparent molar absorptivity's, Sandell sensitivities and the regression line equations for each drug are tabulated inTable2. The mean recovery values are ranged between 99.72–100.0% and 99.80–100% for VELP and SOFOS respectively. The low values of the calculated standard deviation (SD = 0.017–0.446 and 0.019–0.129 for VELP and SOFOS, respectively) and relative Standard deviation (RSD% = 0.006–0.559 and 0.007–0.501% for VELP and Sofosbuvir drugs, respectively) indicate the high accuracy and precision of the proposed method. The limits of detection (LOD) and limits of quantification (LOQ) were also calculated and their low values indicate the sensitivity of the proposed method. The proposed method can be applied successfully to the pharmaceutical preparations of the drugs that were studied.Table-3 shows the results obtained during the determination of VELP and SOFS drugs in the dosage forms. The results are compared with those obtained by applying the official methods for SOFS and VELP drugs. The results obtained were compared statistically by their percentage recovery with those obtained by official method on samples of the same batch. It is clear from the data listed in Table 3 that the percentage recovery values obtained using the proposed method (99.51–99.90 and 99.95–100.0) are higher than those obtained using the official method (98.50 and 98.94%) for VELP and SOFS respectively. There is no significant difference between accuracy and precision of the proposed and the official methods.

Spectrophotometric determination of Voxilaprevir, Velpatasvir and Sofosbuvir by ion-pair formation

Voxilaprevir, Velpatasvir and Sofosbuvir form ion-pair complexes in an acidic buffer with dye stuffs such as bromocresol purple (BCP), bromophenol blue (BPB) and bromocresol green (BCG) and these complexes are quantitatively extracted into 1,2–dichloroethane. The absorption spectra of the ion-pair complexes extracted into 1,2–dichloroethane are shown in Figures 3A–C. These figures show that the ion–pairs of Voxialprevir attain their maxima at 385 and 570 nm with BPB, 400 and 570 nm with BCG and 400 nm with BCP. As for VELP, λmax is found to be at 405 and 570 nm with BPB, 405 and 560 nm with BCG and at 405 and 520 nm with BCP. Ion-pairs of SOFOS show maximum absorbance at 570 nm with BPB, 405 and 580 nm with BCG and at 395 nm with BCP reagent. The reagent blank under similar conditions showed no absorption. The effect of solvents on the extraction and absorbance of the ion pairs formed was studied using different solvents. The results indicated that 1, 2-dichloroethane, methylene chloride and chloroform can be used for the extraction of the ion pairs formed and 1,2-dichloroethane was chosen for having the highest molar absorptivity. Figure 4A shows the increase in absorbance with time up to 40 minutes for Voxilaprevir with BPB, BCG and BCP reagents. The optimum time for the completion of the reaction of VELP with BPB or BCP was 20 minutes and 40 minutes with BCG (Figure 4A). For Sofosbuvir, it was 40 minutes with BCG or BCP and 30 minutes with BPB (Figure 4A). The absorbance values remained almost unchanged with increasing time. The results indicate that ion pairs needed these time intervals for their complete formation. The absorbance of the extracted ion–pairs was measured at different temperatures in the range from 0 to 60°C. The results show that the absorbance generally increased with an increase in temperature and attained maximum value at 40°C for Voxilaprevir, Velpatasvir and Sofosbuvir drugs using BPB, BCG or BCP reagents. (Figure 4B). The concentrations of VXLP, VELP and SOFS were kept constant (20 µg ml−1) and the concentrations of BPB, BCG and BCP reagents were varied from 0.1–2.5 ml (0.02% w/v). It was found that 1.75, 1.5 and 1.0; 1.5 and 1.5 and 1.0, and 1.0, 1.0 and 1.0 ml of BPB, BCG or BCP reagents, respectively, were sufficient for complete reaction with VXLP, VELP and SOFS (Table 4). The ion–pairs were found to be formed in an acidic medium in the pH range from 4–5 and specifically at pH = 4.6 for VXLP, VELP and SOFS with BPB, BCG or BCP reagents (Table 4). In order to establish the molar ratio between drug and the dyestuffs used, the continuous variation and molar ratio methods were applied. In these methods, solutions of drugs and dyestuff with identical molar concentrations were mixed in varying volume ratios or in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the molar ratio of the drugs, [drugs]:[dyestuff] (Figure 4C). These measurements showed that 1:1 complexes were formed with each dyestuff. VXLP, VELP and SOFS contained a tertiary amino group, which is protonated in an acid medium, while a sulfonic acid group is present in BTB; that was the only group undergoing dissociation in the pH range 1–5. Bromophenol blue, BCG and BCP are examples of sulfone phthalein types of dye. The color of such dyes is due to the opening of the lactoid ring and the subsequent formation of the quinoid group. It is supposed that the two tautomers are present in equilibrium but, due to the strongly acidic nature of the sulfonic acid group, the quinoid body predominates.

Finally the protonated drugs, VXLP+, VELP+ or SOFS+, form ion pairs with the dyestuffs, which are quantitatively extracted into 1,2-dichloroethane. Possible reaction mechanisms are given in Scheme 2.

Validity of Beer's law

Under the above conditions of the proposed method, Beer's law was obeyed over the concentration range from 10–50, 10–150 and 10–250 µg ml−1 for VXLP, VELP and SOFS, respectively, using the BPB reagent. Using the BCG reagent, the concentration ranges were found to be 10–60 µg ml−1 for VXLP and 10–150 µg ml−1 for both VELP and SOFS drugs. Finally, using the BCP reagent, the concentration ranges were found to be 5–40, 10–60 and 10–100 µg ml−1 for VXLP, VELP and SOFS drugs, respectively (Table 4). The linear regression equations, Sandell's sensitivity and the correlation coefficient are tabulated in Table 4. In order to prove the validity and applicability of the proposed method and the reproducibility of the results obtained, five replicate experiments were carried out at two concentrations of VXLP, VELP and SOFS. It was found that the between–day relative standard deviations for different concentrations of the drugs obtained from experiments carried out over a period of four days were less than 1%, (Table 5), which indicates that the proposed method is highly reproducible and can be successfully applied to determine the studied drugs via ion pair formation. The limit of detection (LOD) does not exceed 7.96 µg ml^{-1} for all the proposed chelates whereas the limit of quantization (LOQ) was between 1.52 and 8.53 µg ml^{-1} (Table 4).

Application

The validity of the proposed method was tested for the determination of VXLP, VELP and SOFS in dosage forms manufactured by local companies[9-12]. The concentration of the drugs in the dosage forms was calculated from the appropriate calibration graphs. The determination of drugs in the dosage forms was compared with those obtained by applying the official methods for VXLP, SOFS and VELP (Table 6). The results obtained were compared statistically for percentage recovery with those obtained by official method on samples of the same batches and by means of F-tests and *t*-tests at the 95% confidence level. In all cases, the average results obtained by the proposed methods and the reference method were statistically identical, as the difference between the average values had no significance at 95% confidence level. The proposed methods are simple, sensitive and reproducible and can be used for routine analysis of VXLP, VELP and SOFS drugs in pure form and in formulations.

CONCLUSION

Only VXLP can be determined by non-selective titrimetric official method with standard NaOH in the British Pharmacopoeia. Most of the reported methods are HPLC, which requires complicated instrumentation. The electrochemical methods are less sensitive. In addition the conventional UV methods suffer from interference due to UV absorbing compounds in the determination of the drugs being studied. The few reported visible spectrophotometric methods are mainly concerned with charge transfer complexation with different electron acceptors, which give similar reactions with all basic compounds, or are concerned with the reducing activity of VXLP or metal chelate formation, where the linear range is narrow compared with the proposed methods. As a general conclusion, the two spectrophotometric methods can readily be used for routine analysis in bulk form and in pharmaceutical formulations as they offer simple systems with short analytical time, good reproducibility and accuracy. It is obvious from the results that VELP and SOFS drugs can be determined in a wider concentration range via an oxidation reaction with KIO3 reagent (10–400 and 5–400 µg ml−1 for VELP and SOFS, respectively) rather than using the ion-pair formation reaction. So the oxidation process using KIO_3 is more sensitive to low concentration of the drugs than the ion-pair process

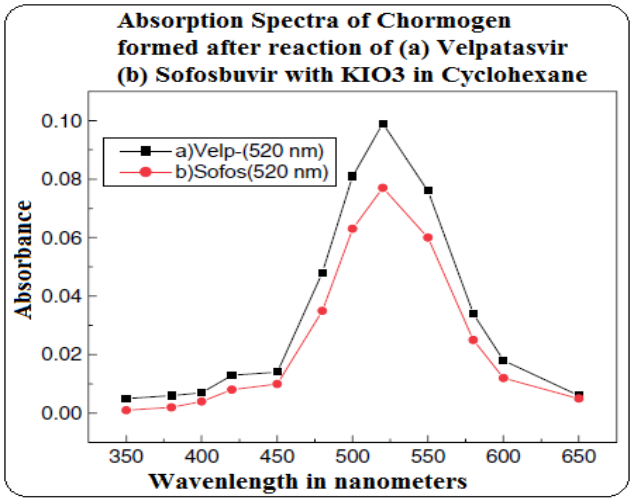


Figure 1: Absorption Spectra maximum of (a) Velpatasvir and (b) Sofosbuvir with KIO_3 in Cyclohexane

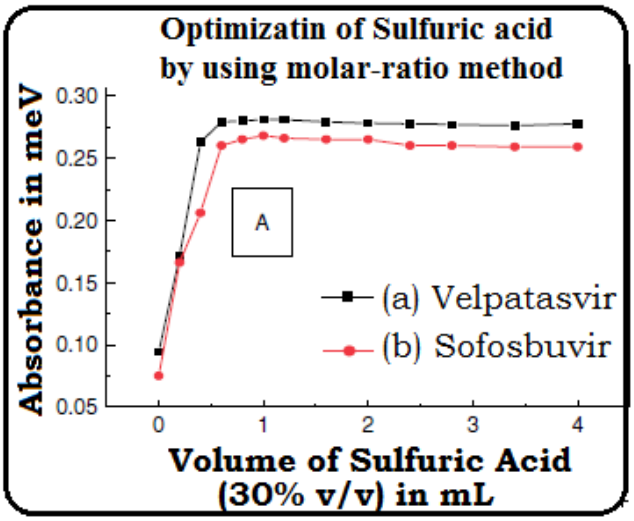


Figure 2(a): Optimizing the quantity of sulfuric acid in determination of Velpatasvir (VELP) and Sofosbuvir (SOFOS) using KIO_3 reagent.

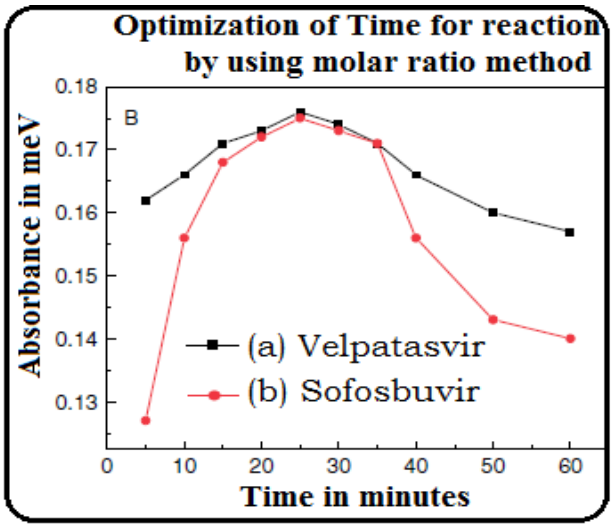


Figure 2(b): Figure-2(a): Optimizing the reaction time in determination of Velpatasvir (VELP) and Sofosbuvir (SOFOS) using KIO_3 reagent

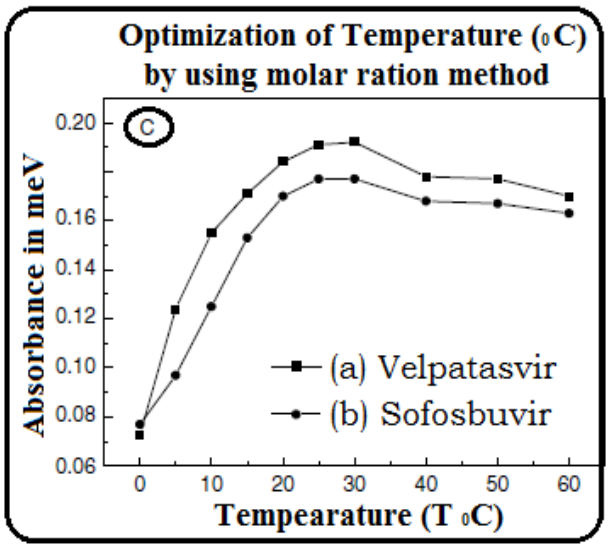


Figure 2(c): Optimizing the reaction temperature in determination of Velpatasvir (VELP) and Sofosbuvir (SOFOS) using KIO_3 reagent

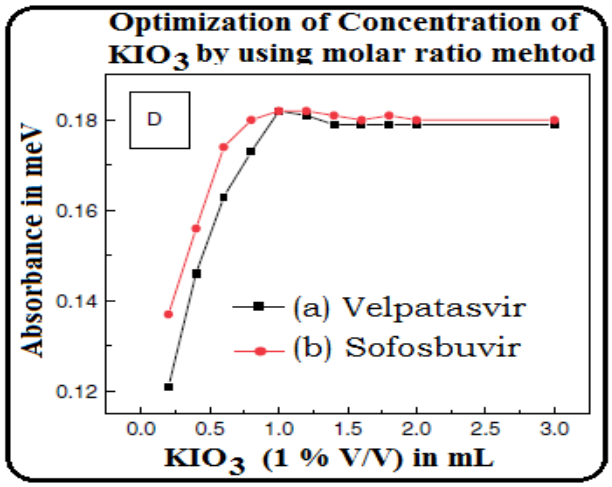


Figure 2(d): Optimizing the volume of KIO_3 determination of Velpatasvir (VELP) and Sofosbuvir (SOFOS) using KIO_3 reagent

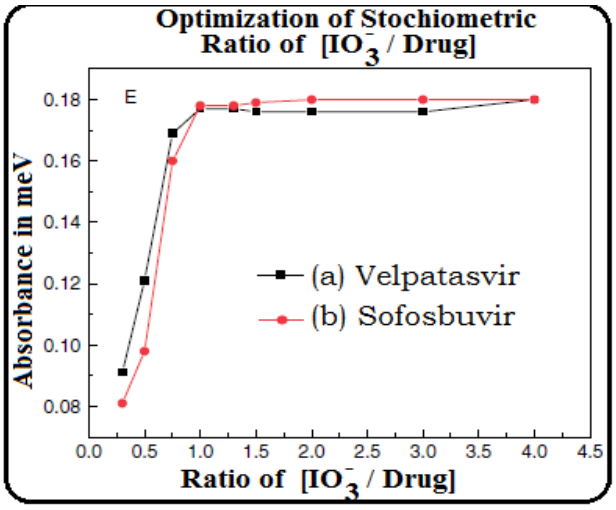


Figure 2(e): Optimization of stoichiometric ratio of Drug and reagent in determination of Velpatasvir (VELP) and Sofosbuvir (SOFOS) using KIO_3 reagent

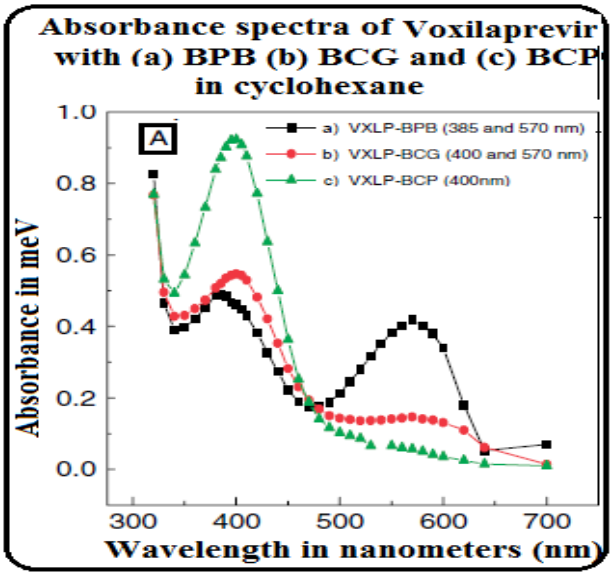


Figure 3(a): Absorption spectra of Voxilaprevir by using BPB/BCG/BCP-Chromogenic reagents

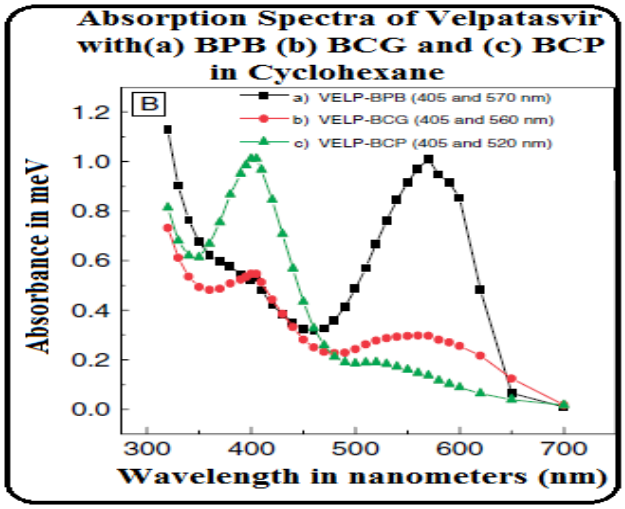


Figure 3(b): Absorption spectra of Velpatasvir by using BPB/BCG/BCP-Chromogenic reagents

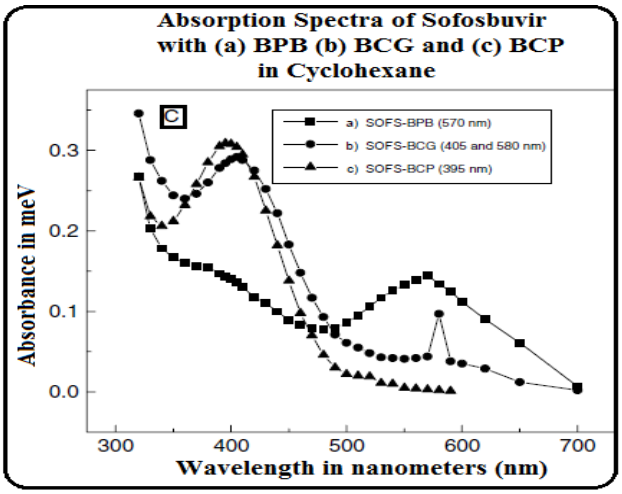


Figure 3(c): Absorption spectra of Sofosbuvir by using BPB/BCG/BCP-Chromogenic reagents

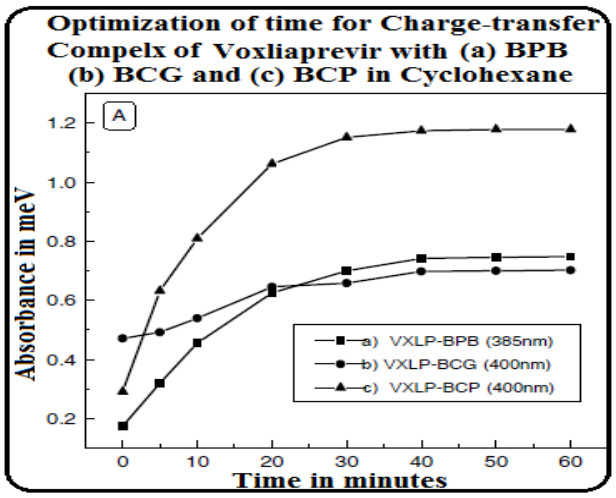


Figure 4 (a): Optimization of reaction time for charge transfer complex between Voxilaprevir and BPB/BCG/BCP-Chromogenic reagents

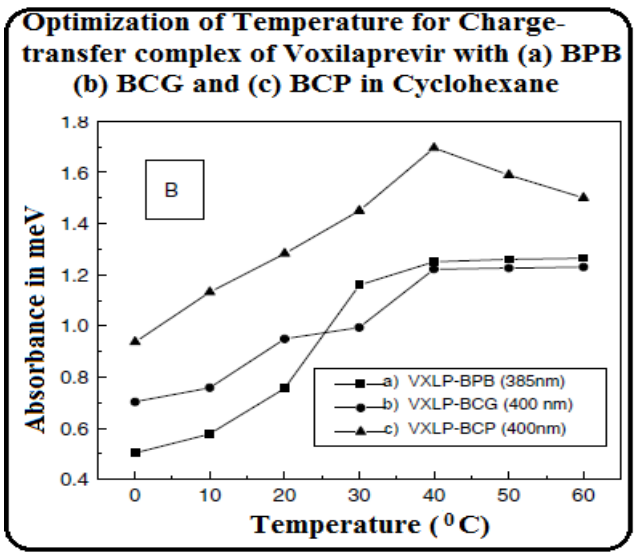
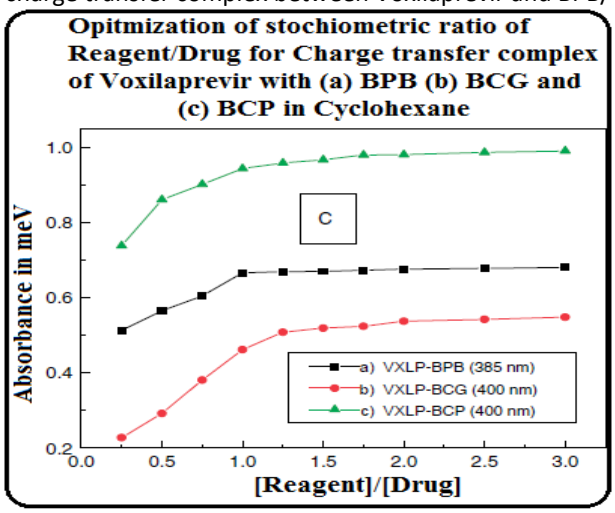


Figure 4(b): Optimization of reaction temperature for charge transfer complex between Voxilaprevir and BPB/



BCG/BCP-Chromogenic reagents

Figure 4-(c): Optimization of reaction stoichiometric ratio between drug/reagent for charge transfer complex between Voxilaprevir and BPB/BCG/BCP-Chromogenic reagents

Table 2: Analytical Parameters for the determination of Velpatasvir and Sofosbuvir using KIO_3.

Parameter	Velpatasvir	Sofosbuvir
Absorption maxima (λmax)	525nm	525nm
Concentration Range	10-400	5-400
(ε) [Liter/mole/cm]	$1.45x10^3$	$1.75x10^3$
Stability Time in minutes	40	40
Temperature (^{0}C)	25	25
Volume of KIO_3 reagent	1 ml	1 ml
Sandell's Sensitivity	0.00354	0.00452
Slope	0.007	0.008
Intercept	0.0325	0.0365
Correlation Coefficient ($r^{2)}$	0.9995	0.9998
Standard deviation	0.022-0.528	0.020-0.145
Relative Std. deviation (%RSD)	0.07-0.772	0.009-0.685
Limit of detection (LOD)	3.5 µg/mL	4.5 µg/mL
Limit of quantification (LOQ)	7.8 µg/mL	9.5 µg/mL

Table 1: Inter-day Precision for the determination of Velpatasvir (VELP) and Sofosbuvir (SOFOS) using KIO_3 reagent.

Compound	Drug taken in µg/mL	Drug found in µg/mL	% Recovery	% RSD
Velpatasvir	50	49.98	99.96	0.178
	100	99.76	99.76	0.076
	150	149.8	99.86	0.075
	200	199.5	99.75	0.032
Sofosbuvir	50	49.72	99.44	0.130
	100	99.48	99.48	0.121
	150	149.7	99.8	0.045
	200	199.8	99.9	0.048

Table 3: Estimation of Velpatasvir and Sofosbuvir using KIO_3 in commercial formulations

Marketed Product	Active Ingredients	Amount of the drug taken	Amount of the drug found	% Recovery
Epclusa ®-Each 400 mg/100 mg tablet contains 400 mg Sofosbuvir and 100 mg Velpatasvir.	Velpatasivr	100 µg/mL	99.36 µg/mL	99.36
		50 µg/mL	49.65 µg/mL	99.3
	Sofosbuvir	400 µg/mL	399.54 µg/mL	99.88
		200 µg/mL	199.25 µg/mL	99.62

Table 4: Analytical parameters for the estimation of Voxilaprevir /Velpatasvir and Sofosbuvir by using BCG/BPB/BCP reagents

Analytical Parameters	Bromophenol Blue method			Bromocresol green method			Bromocresol Purple method		
	VOXL	VELP	SOFOS	VOXL	VELP	SOFOS	VOXL	VELP	SOFOS
Ʌmax-	390	410	565	405	410	410	400	395	400
Drug-Range (µg/ mL)	10-60	10-150	10-250	10-60	10-150	10-250	5-40	10-60	10-100
(ε) [Liter/mole/cm]	$4.78x10^3$	$4.28 x10^3$	$1.85x10^3$	$6.1x10^3$	$3.2x10^3$	$2.8x10^3$	$8.2x10^3$	$11.5x10^3$	$4.5 x10^3$
Stability Time	60	45	60	60	60	60	45	60	60
Temperature (^{0}C)	30	30	30	30	30	30	30	30	30
pH	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Volume of Reagent	1.5 mL	1.5 mL	1.5 mL	2 mL	2 mL	2 mL	1.5 ml	1.5 ml	1.5 ml
Sandell's Sensitivity	0.0084 µg/cm^2	0.0096 µg/cm^2	0.0044 µg/cm^2	0.0056 µg/cm^2	0.00135 µg/cm^2	0.00158 µg/cm^2	0.0056 µg/cm^2	0.0038 µg/cm^2	0.0092 µg/cm^2
Slope	0.022	0.095	0.009	0.022	0.0088	0.009	0.032	0.0312	0.011
Intercept	0.012	0.029	0.0056	0.007	0.009	-0.0008	-0.00318	0.021	0.0074
Corr. Coeff ($r^{2)}$)	0.999	0.994	0.996	0.995	0.996	0.998	0.993	0.992	0.993
Standard deviation	0.053	0.065	0.118	0.027	0.025	0.077	0.115	0.029	0.034
(%RSD)	0.69	0.58	0.45	0.82	0.09	0.63	0.72	0.43	0.79
LOD (µg/ mL)	0.95	1.25	6.5	1.4	1.8	2.8	1.2	2.5	3.6
LOQ (µg/ mL)	2.98	3.96	12.4	4.6	5.4	6.2	3.8	7.8	9.6

Table 5: Intra-day precision study for the estimation of Voxilaprevir /Velpatasvir and Sofosbuvir by using BCG/BPB/BCP reagents.

Intra-Day precision Analytical parameters	Bromophenol Blue method			Bromocresol green method			Bromocresol Purple method		
	VOXL	VELP	SOFOS	VOXL	VELP	SOFOS	VOXL	VELP	SOFOS
Amount of the drug found (µg/ mL)	100	100	400	100	100	400	100	100	400
Amount of the drug found (µg/ mL)	99.25	99.45	395	99.20	97.5	396.8	99.4	99.65	398.5
% Recovery	99.25	99.45	98.75	99.2	97.5	99.2	99.4	99.6	99.62
% Relative Std.Deviation	0.272	0.35	0.65	0.85	0.28	0.64	0.95	0.84	0.48

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