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Research Article

Development And Validation Of Three Novel UV Spectrophotometric Methods For Determination Of Newly Discovered Combination For The Treatment Of Hepatitis C And Their Comparison Using ANOVA

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Abstract

Sofosbuvir and Velpatasvir combination is used for hepatits C and newly introduce in market. It is necessary to develop suitable quality control methods for rapid and accurate determination of these drugs. Three simple, accurate, sensitive, precise and economical UV spectrophotometric methods (A, B & C) have been developed for simultaneous estimation of Sofosbuvir and Velpatasvir in pharmaceutical dosage form and their comparision using ANOVA. Method (A) is based on the first order derivative spectrophotometric method at zero crossing wavelength. In this method the zero crossing point of Sofosbuvir is 260 nm and for Velpatasvir is 250 nm. The linearity was obtained in the concentration range of 4-24µg/ml for Sofosbuvir and 1-6µg/ml for Velpatasvir using methanol as a solvent. Method (B) is based on principle of absorbance correction, it was performed at 260 nm for Sofosbuvir and at 302 nm for Velpatasvir. Method (C) is based on principle of dual wavelength method developed using absorbance difference at 250 nm and 268.26 nm for Sofosbuvir and 280 nm and 259.28 nm for Velpatasvir. The accuracy and precision of the methods were determined and validated statistically. All the methods showed good reproducibility and recovery with % RSD less than 2. The three methods were compared using one - way ANOVA and the f_{cal} value was found to be less than f_{tab} value indicating that there is no significant difference in the assay results by the three methods. All methods were found to be rapid, specific, precise and accurate and these methods require no preliminary separation and found no interferences from the tablet excipients so it can be used for routine analysis of both drugs in quality control laboratories.

Keywords: Sofosbuvir, Velpatasvir, First order derivative, Absorbance correction, Dual wavelength, Validation.

Introduction

Sofosbuvir (SOFO) is chemically known as 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 (Figure 1) [1-3]. Velpatasvir (VELP) is chemically known as Methyl {(2*S*)-1-[(2*S*,5*S*)-2-(9-{2-[(2*S*,4*S*)-1-{(2*R*)-2-[(methoxycarbonyl)amino]-2-phenylacetyl}-4-(methoxymethyl)-2-pyrrolidinyl]-1H imidazol-4-yl}-1,11-dihydroisochromeno[4',3':6,7]naphtho[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2 butanyl}carbamate (Figure 2)[4]. Sofosbuvir and Velpatasvir are used in combination in treatment of hepatitis C. Sofosbuvir and Velpatasvir acting as a NS5B and NS5A inhibitor respectively[5-8].The deep literature survey reveals

that various Spectrophotometric and chromatographic methods are available for the estimation of SOFO[9-13] and VELP alone and in combination with other drugs like daclatasvir[14] and ledipasvir[15-18]. Combination of SOFO and VELP is not official in any pharmacopoeias and hence no official method available for analysis of both drugs in combination. Literature survey also reveals that there are no reported spectrophotometric methods available for simultaneous estimation of SOFO and VELP in combined dosage form. Therefore, simple, rapid, and reliable method for simultaneous estimation of these drugs in combined dosage form seemed to be necessary. Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. The purpose of this study was to determine and validate both drugs concurrently by simple, accurate, rapid and precise first derivative spectrophotometric, absorbance correction and dual wavelength assays for routine analysis [19].

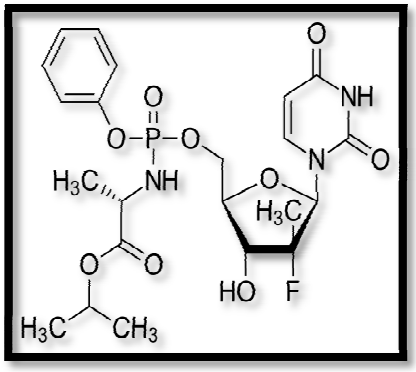

Figure 1: Chemical structure of Sofosbuvir

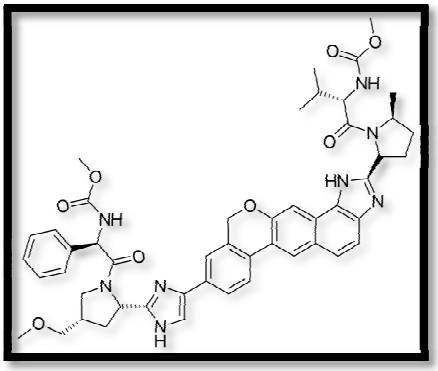

Figure 2: Chemical structure of Velpatasvir

MATERIALS

Reagent and chemicals

SOFO (Zydus health care pvt. Ltd., Ahmadabad) and VELP (Hetero drugs pvt. Ltd., Hyderabad) were received as a gift sample. Marketed formulation containing 400 mg of SOFO and 100 mg of VELP was purchased from local market. Methanol (AR Grade) and other reagent were provided by Department of Quality Assurance, Pioneer Pharmacy Degree College, Vadodara, Gujarat, India.

Instruments and Apparatus

UV visible double beam spectrophotometer (Lab India 3000+) with software UV –win 5 and spectral slit width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1cm matched quartz cells and digital balance Shimadzu ATX 224, Japan and ultrasonicator were used. Volumetric flasks and pipettes of borosilicate glasses were used in the study.

Selection of common solvent

Methanol of analytical reagent grade was selected as a common solvent for developing spectral characteristics of both drugs. The selection was made after assessing the solubility of both drugs in different solvents like water, chloroform, ether etc.

Preparation of standard stock solution (SOFO and VELP 100µg/ml)

Accurately weighed quantities of SOFO (10 mg) and VELP (10 mg) transferred to separate volumetric flasks (100 ml), dissolved in methanol (small quantity) and diluted up to mark with methanol (100 µg/ml of SOFO and VELP).

METHODOLOGY

Method A : First order derivative spectroscopic method

For first derivative spectrophotometric method, accurate aliquots of SOFO equivalent to 4-24 µg/ml were transferred from its stock solution (100 µg/ml) into a series of 10 ml volumetric flasks and diluted to mark with methanol and mixed well. Accurate aliquots of VELP equivalent to 1-6 µg/ml were transferred from its working solution (100 µg/ml) into a series of 10 ml volumetric flasks and diluted to mark with methanol and mixed well. Considering all the derivative order spectra of SOFO and VELP from first to fourth derivative, the first derivative order spectra with coefficient 100 and no of point 21 found suitable. The zero crossing point on the first derivative spectra of one drug, the other drug shows substantial absorbance,

these two wavelengths can be employed for the estimation of SOFO and VELP without any interference from other drug in combined formulations. From the derivatised spectra of prepared mixtures the absorbances were measured at 250 nm for SOFO (ZCP of VELP) and 260 nm for VELP (ZCP of SOFO). These absorbances Vs concentration were plotted in the quantitative mode to obtain the working curves from which by extrapolating the value of absorbances of the sample solution, the concentration of the corresponding drugs were determined. Both the drugs obeyed Beer's Law.

Method B: Absorbance correction method

This method is modification of simultaneous equation method. This method uses the absorbances at two selected wavelengths, one at λmax of one drug where other drug also shows considerable absorbance (λ2) and other being the wavelength at which the first drug has practically nil absorbance (λ1).For this method, it was observed that SOFO (4-24 µg/ml) has zero absorbance at 302 nm, where as SOFO has substantial absorbance. Therefore, VELP (1-6 µg/ml) estimated at 302nm with no interference from SOFO. To estimate SOFO, absorbance of VELP measured at 260nm. The contribution of VELP was deducted from the total absorbance of sample mixture at 260nm. The calculated absorbance for SOFO was called as 'Corrected Absorbance' for SOFO. The concentration of SOFO was determined from calibration curve at 260nm using corrected absorbance

Corrected Absorbance = Total Absorbance – Interfering Absorbance

The concentration of two drugs(X and Y) in the mixture can be calculated using following equations:

Cy = A2 / ay2…… …… (1)

Cx = A1-ay1* Cy/ax1..... ..... (2)

Where, A1 and A2 are the absorbances of mixture at λ1 and λ2 respectively, ay1 and ay2 are absorptivities of y at λ1 and λ2 respectively, ax1 is absorptivity of X at λ2, CX is concentration of X, CY is concentration of Y.

Method C: Dual wavelength method

The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components.The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration. The solutions were prepared for SOFO from 8-28µg/ml concentration and for VELP 2-7µg/ml using methanol as a solvent. The absorbance difference for SOFO was measured at 250nm and 268.26nm and for VELP absorbance difference taken at 259.28nm and 280nm. Calibration curves were constructed for SOFO and VELP by plotting absorbance difference versus concentrations at both wavelengths. Each reading was average of three determinations.

ANALYSIS OF SOFO AND VELP IN TABLETS

Marketed tablets formulations containing SOFO (400 mg) and VELP (100 mg) were analyzed using these three methods. From the triturate of 20 tablets, an equivalent to 40 mg of SOFO and 10 mg of VELP was weighed and dissolved in 10 ml of methanol in 100 ml volumetric flask by sonication for 15 mins. Then final volume of the solution was made upto 100 ml with methanol to get final concentration of 400 µg/mL of SOFO and 100 µg/mL of VELP. The solution was filtered through whatmann filter paper no.41 and filtrate was appropriately diluted to get approximate concentration of 8 µg/mL of SOFO and 2 µg/mL VELP of for method A & B and 12 µg/mL of SOFO and 3 µg/mL of VELP for method C . The concentration of each analyte was determined with the equations generated from calibration curve of respective drugs (Method A, B and C).

VALIDATION PARAMETERS

Validation was carried out according to ICH guideline (ICH Q2 (R1), 2005).

Accuracy

For studying the accuracy of the proposed methods, and for checking the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition

method. This study was performed by addition of known amounts of SOFO and VELP to a known concentration of sample solution. The amounts of standard recovered were calculated in terms of mean recovery with the upper and lower limits of % R.S.D.

***Precision* /Repeatability**

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for SOFO and VELP without changing the parameter of the proposed spectrophotometry methods.

Intermediate Precicion

Intra-day precision and inter-day precision for the developed methods were measured in terms of % R.S.D. The experiments were repeated three times a day for intra-day precision and on 3 different days for inter-day precision. The concentration values for both intra-day precision and inter-day precision were calculated three times separately and % R.S.D. were calculated.

Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the $3s/m$ and $10s/m$ criterions, respectively, where s is the standard deviation of intercept (n =6) of the sample and m is the slope of the corresponding calibration curve.

ANOVA

Statistical analysis was performed to assess the effect of three methods in simultaneous estimation of SOFO and VELP using one-way analysis of variance ($P < 0.05$).

RESULT AND DISCUSSION

Method A: First order derivative Spectroscopic method

In contrast to zero-order spectra, first derivative spectra show more resolution in terms of zero crossing points shown in Figure 3 and 4 explains overlain first order derivative spectra for SOFO and VELP. At 250 nm, VELP having zero crossing point and SOFO can be determined. At 260 nm, SOFO having zero crossing point and VELP can be determined.

Method B: Absorbance correction method

Figure 5 & 6 explains overlain spectra of SOFO and VELP which absorb at 260 nm common wavelength and VELP absorbs at 302 nm wavelength where SOFO shows zero absorbance so these wavelengths were selected and equation 1 and 2 were directly utilized for determination of SOFO and VELP in sample solution.

Method C: Dual wavelength method

Figure 7 explains in dual wavelength method four wavelengths were selected where single drug shows zero absorbance difference. These wavelengths were used for each other drugs and absorbance difference measured and calibration curves were prepared for both the drugs.

Table 1, Table 2 and Table 3 exhibits results of assay, results of accuracy studies and summary of various validation parameters of all methods respectively.

Statistical comparison of the results of the developed three methods:

Method A, B and C were compared using one-way ANOVA and no significant difference was found between them as the F_{cal} value is less than F_{tab}. The results of one-way ANOVA are shown in table 4 and 5.

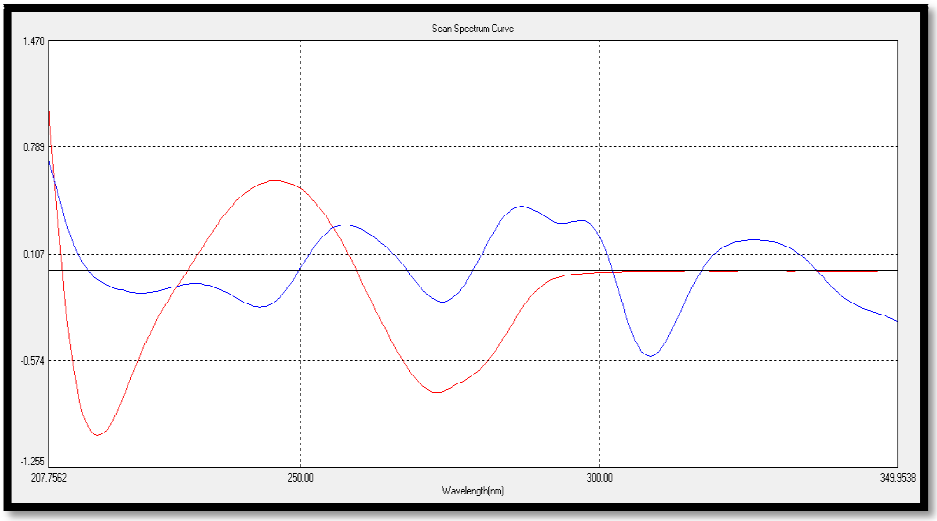

Figure 3: Overlain First order spectra of Sofosbuvir (8µg/ml) and Velpatasvir (2 µg/ml)

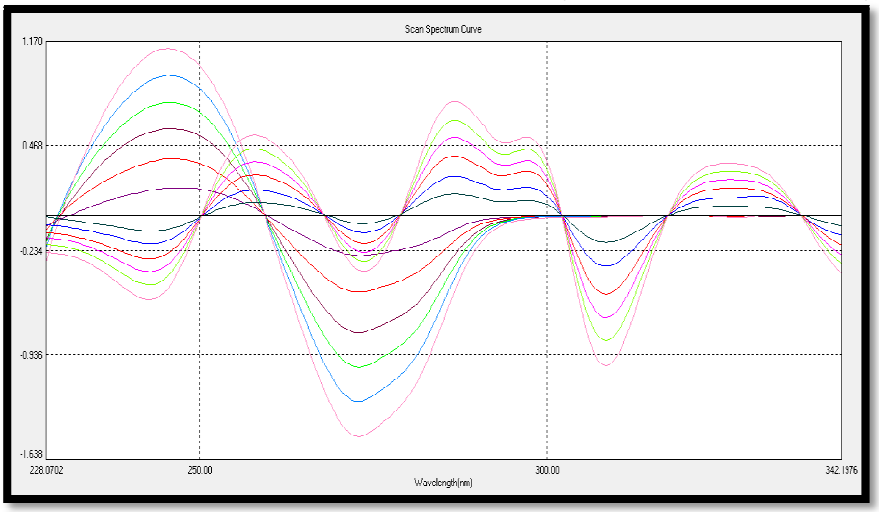

Figure 4: Overlain First order Derivative Spectra of Standard Sofosbuvir (4-24µg/ml) and Velpatasvir (1-6µg/ml)

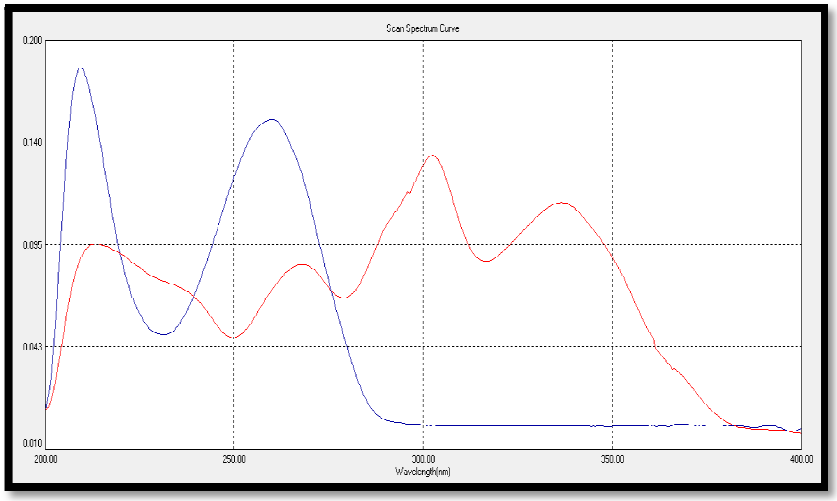

Figure 5: Overlain spectra of SOFO (8 µg/ml) and VELP (2µg/ml)

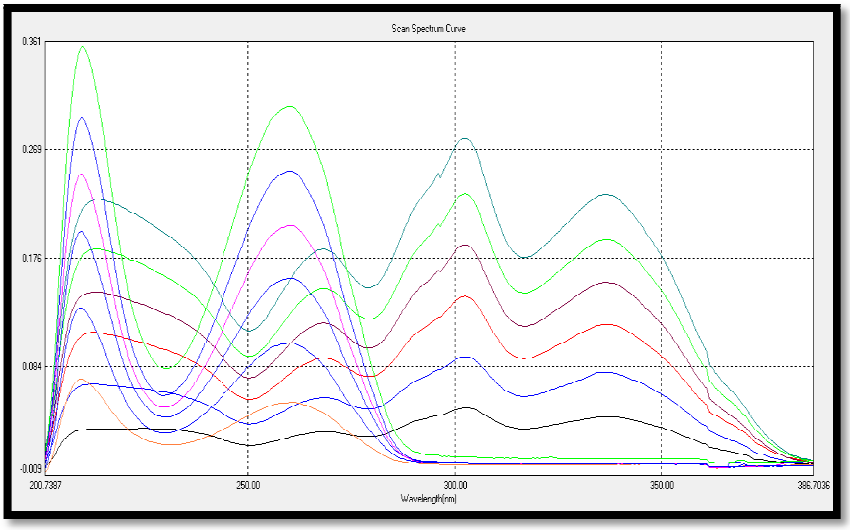

Figure 6: Overlain spectra of SOFO (4-24µg/ml) and VELP (1-6µg/ml)

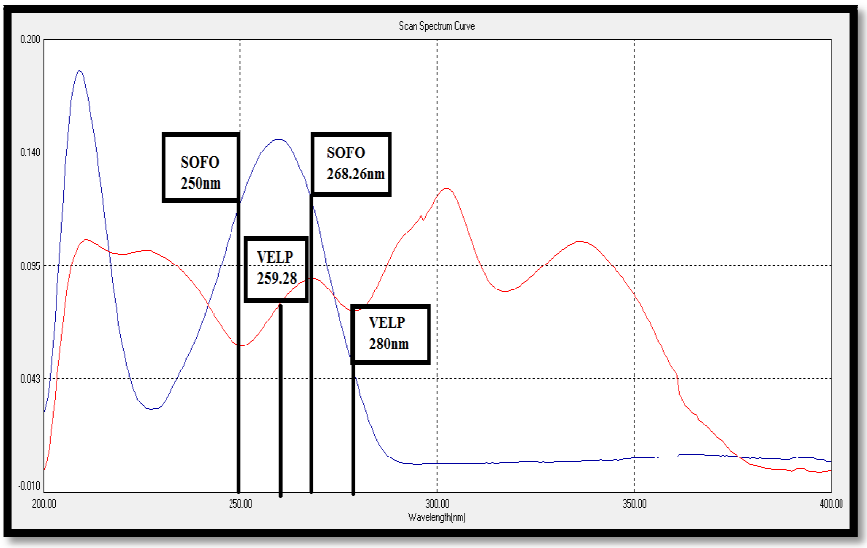

Figure 7: Overlain spectra of SOFO (12µg/ml) and VELP (3µg/ml)

Table 1: Assay results for tablets using the proposed methods

Formulation	Proposed methods	Label Claim (mg)		Amount of drug found (mg)		% Label Claim Assay (n^a=3) ± SD^b	
		SOFO	VELP	SOFO	VELP	SOFO	VELP
Tablets	METHOD A	400	100	405.28	101.93	101.32 ±0.51	101.92 ±0.45
	METHOD B	400	100	404.56	101.81	101.14±0.27	101.81±0.98
	METHOD C	400	100	405.88	100.27	101.47 ±0.34	101.61±1.14

Table 2: Application of the standard addition technique to the analysis of SOFO and VELP in tablets by the proposed methods

Method	Drugs	Amount present (µg/ml)	Amount added (µg/ml)	Total amount of drug (µg/ml)	Amount found (µg/ml)	%Recovery ± SD(n = 3)	%RSD
Method A	SOFO	8	4	12	11.90	99.18 ± 1.2693	1.27
			8	16	16.04	100.30 ± 1.0670	1.06
			12	20	19.93	99.67 ± 0.8119	0.81
	VELP	2	1	3	2.97	99.32 ± 1.0230	1.02
			2	4	3.98	99.59 ± 1.2693	1.27
			3	5	4.95	99.18 ± 0.8565	0.86
Method B	SOFO	8	4	12	11.86	98.84 ± 0.4009	0.40
			8	16	15.83	98.95 ± 0.5208	0.52
			12	20	19.83	99.16 ± 0.4166	0.42
	VELP	2	1	3	2.99	99.72 ± 1.2418	1.24
			2	4	3.97	99.39 ± 1.0561	1.06
			3	5	4.98	99.67 ± 0.5632	0.56
Method C	SOFO	12	6	18	17.77	98.76 ± 0.6172	0.62
			12	24	24.22	100.92 ± 1.2248	1.21
			18	30	29.85	99.50 ± 1.1315	1.13
	VELP	3	1.5	4.5	4.51	100.37 ± 0.6415	0.63
			3	6	5.93	98.88 ± 0.9622	0.97
			4.5	7.5	7.43	99.11 ± 0.7698	0.77

Table 3: Summary of validation parameter by developed method

Parameters	Method-I		Method-II		Method-III	
	SOFO	VELP	SOFO	VELP	SOFO	VELP
Working wave-length(nm)	250nm	260nm	260nm	302nm	Absorbance difference at 280nm & 259.28nm	Absorbance difference at 250nm & 268.26nm
Concentration range(µg/ml)	4-24	1-6	4-24	1-6	8-28	2-7
Sandell's sensitivity (µg/cm2/0.001A.U)	0.042	0.014	0.189	0.020	0.20	0.11
Regression equation	y= 0.041x +0.018	y= 0.082x -0.007	y= 0.012x + 0.003	y= 0.041x +0.005	y= 0.009x -0.007	y= 0.010x -0.000
Correlation coeffi-cient(r^2)	0.999	0.997	0.999	0.999	0.998	0.998
SD of slope	0.041	0.081	0.012	0.041	0.009	0.0096
SD of intercept	0.002	0.001	0.0005	0.0005	0.0005	0.0005
LOD(µg/ml)	0.16	0.06	0.15	0.04	0.21	0.19
LOQ(µg/ml)	0.48	0.18	0.47	0.14	0.64	0.60

Precision						
Repeatability(n=6) %RSD	0.33	0.69	1.46	1.84	0.87	1.73
Intraday(n=3) %RSD	0.08-0.18	0.48-0.91	0.38-1.14	0.65-1.30	0.93-1.56	1.13-1.84
Intrday(n=3) %RSD	0.21-0.84	0.48-1.03	0.68-1.52	0.43-1.12	0.67-0.90	1.12-1.14

Table 4: One way ANOVA for SOFO

Source of Variation	Sum of Square	Degree of freedom	Mean Squares	F_{cal}	P-value	F_{tab}
Between Groups	0.32	2	0.16	1.1562	0.34	3.68232
Within Groups	2.09	15	0.13			
Total	2.42	17				

Table 5: One way ANOVA for VELP

Source of Variation	Sum of Square	Degree of freedom	Mean Squares	F_{cal}	P-value	F_{tab}
Between Groups	0.29	2	0.14	0.96	0.40	3.68232
Within Groups	2.28	15	0.15			
Total	2.57	17				

CONCLUSION

Three spectrophotometric methods (first derivative spectroscopic, absorbance correction and dual wavelength) were developed for simultaneous estimation of SOFO and VELP in their combined pharmaceutical formulation without prior separation. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of SOFO and VELP in formulation. The one-way ANOVA results show that there is no significant difference between assay results obtained from these three methods. So the proposed methods can be used in routine analysis of SOFO and VELP with relatively less expensive and simple to operate instrumentation.

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