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ESTIMATION OF LEDIPASVIR AND SOFOSBUVIR BY VIERDOT'S METHOD IN BULK AND DOSAGE FORMS

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ABSTRACT

A simple, precise, accurate, rapid and specific UV spectroscopic method was developed for the simultaneous estimation of Ledipasvir and Sofosbuvir in bulk and dosage form (tablet). The present study is based on Vierdot's method, in which 296 and 260 nm were selected for measuring absorbance of Ledipasvir and Sofosbuvir respectively. The developed method was validated as per ICH guidelines and the results were statistically validated. The method was linear in the range of 5-25µg/ml with r^2 value of 0.999 for both the drugs. Good recovery results were obtained between 94 to 99%. The relative standard deviation for precision and ruggedness was less than 2.0%. The detection limit and quantification limit were found to be 0.07041µg/ml and 0.136502 µg/ml for Sofosbuvir and 0.02112 and 0.00818 µg for Ledipasvir respectively. The method was successfully applied to the assay of Ledipasvir and Sofosbuvir in tablet dosage form.

KEY WORDS

Ledipasvir, Sofosbuvir, simultaneous estimation, vierdot's method, ICH guidelines.

INTRODUCTION:

Hepatitis C is chronic viral disease of liver. Inhibitors of hepatitis C virus (HCV) limit the progression of infection by HCV. Ledipasvir and sofosbuvir are novel, potent anti-viral agents indicated for hepatitis C infection [1]. Ledipasvir(LPS) is a selective inhibitor of non-structural protein 5A (NS5A), which is involved in viral replication [2]. The chemical name of LPS is (1- { 3-[6-(9,9-difluoro – 7- { 2-[5-(2-methoxy carbonyl amino-3-methyl-butyryl) -5-aza-spiro [2.4] hept-6-yl] -3H-imidazol-4-yl}-9H-fluoren-2-yl)-1H-benzoimidazol-2-yl]-2-aza-bicyclol-[2.2.1]heptanes-2-carbonyl}-2-methyl-propyl)-carbamic acid methyl ester. LPS is off-white to yellow coloured powder and exists in amorphous form [3-6]. The structure is shown in figure 1. Sofosbuvir(SOFO) is a potent inhibitor of non-structural protein 5B (NS5B), RNA dependent RNA polymerase required for viral replication. SOFO is chemically known as (S)-isopropyl 2-(((2R, 3R, 4R, 5R) – 5-(2,4-dioxo-3,4-dihydropyrimdin-1(2H) -yl) - 4-fluoro3 hydroxy – 4 – methyltetrahydrofuran – 2 –yl) methoxy) (phenoxy) phosphoryl) amino) propanoate. SOFO is white to off-white powder and is in crystalline form[7-10]. The structure of SOFO is shown in figure 2. The fixed dose combination of LPS (90mg) and SOFO (400mg) was approved by USFDA in 2014 for the treatment of chronic infection of HCV genotype 1[10].

Various methods are reported in literature for estimation of Ledipasvir and sofosbuvir individually and in combination using different analytical techniques such as UPLC-MS/MS [11,12], LC-MS/MS[13,14], RP-HPLC[15-19]. Review of Literature revealed that there was no UV Spectroscopic, vierdot's method reported or available for simultaneous estimation of LPS and SOFO in tablet dosage form. The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes. A survey of literature revealed that, the simultaneous analytical method are not available for the drug combination LPS and SOFO. Even though very few methods of individual estimation of these drugs are available. Hence it is proposed to develop new method for the assay of LPS and SOFO in pharmaceutical dosage forms adopting a UV spectrophotometry. The objective of the study was to develop a simple and accurate method for the determination of LPS and SOFO simultaneously using VIERDOTS method by UV spectrophotometry in pharmaceutical dosage forms.

MATERIALS AND METHODS:

LPS and SOFO obtained from Hetero laboratory (Visakhapatnam, India) were of analytical grade. Our optimized formulation was used within their shelf life period. Potassium di hydrogen orthophosphate, di-potassium hydrogen orthophosphate, (S D fine chemicals limited, Mumbai) and methanol (Merck, Mumbai) were of analytical grades.

Quantitative estimation was performed on Lab India UV 3000+ (Maharashtra, India)and Elico SL 210 (Andhra Pradesh, India) double beam UV visible spectrophotometers with matched 1cm path- length Quartz cells. Absorption spectra was recorded on a fast scan speed, setting slit width to be 1nm and sampling interval to be auto. Lab India UV win (Maharashtra, India) software was used along with quartz cuvettes for the λmax prediction to develop a suitable and robust simultaneous estimation by VIERDOTS method for the determination of the LPS and SOFO. The different diluents like methanol, 10 mM Phosphate buffer at pH 6 were tried based on solubility and functional groups present in the compound. Finally 0.33 ml of 1M K_2HPO_4, 2.17 ml of 1M KH_2PO_4 to a 250 ml volumetric flask and making upto the mark with distilled water, was selected due to its reproducible results. Absorbance's were measured at selected λ max (260 nm and 296nm) based on overlap spectrum of both drugs. The data were collected and analyzed with a software (Lab India UV win, Maharashtra, India) in a computer system.

PREPARATIONS:

The Stock solution of LPS (1000 µg/ml) was prepared by dissolving 10mg of drug in a 10ml volumetric flask containing 10ml of methanol. The solution was sonicated to about 15 min and then made upto volume with the solvent. From the stock solution, 1ml was pipetted out and transferred in to the 10ml of volumetric flask to get (100 µg/ml) concentration using 10mM Phosphate Buffer. From the second solution 1 ml was pipetted out and transferred into the 10ml volumetric flask to get (10 µg/ml) concentration using 10 mM Phosphate Buffer. The same procedure was followed for SOFO standard. The final solution of both standard drug solutions were scanned and the spectra obtained were overlapped. From the overlapping spectra, two wavelengths were selected. Among the two, 296 nm is the λ max of LPS and 260 nm is the λ max of SOFO. Then the absorbance was measured at 296 nm and 260 nm, the absorptivity is calculated from the formula: $\boldsymbol{a} = \frac{\boldsymbol{A}}{\boldsymbol{bc}}$ where A is Absorbance, c is Concentration, b is the path length.

PREPARATION OF STANDARD MIXTURE:

From the solution of 100 µg/ml, 0.5 ml, 1ml, 1.5ml, 2 ml, 2.5ml of LPS and SOFO was pipetted out individually and mixed in 10 ml volumetric flask and was made up to the mark with 10 mM Phosphate buffer at pH 6. Absorbance were measured at selected λ max (296nm and 260 nm).

PREPARATIONS OF TABLET MIXTURES:

10 tablets were weighed and powdered. The amount of powder equivalent to 400 mg of SOFO and 90mg of LPS were weighed and transferred into the 100 ml volumetric flask containing methanol. The solution was sonicated for about 20 min and then made up to volume with methanol.

The solution was filtered using 0.25 microns filter paper and vacuum-associated filtration unit. From the

filtrate, 1ml was pipetted out and transferred into the 10 ml volumetric flask and made up to the mark with 10 mM Phosphate buffer at pH 6. From the second solution, 0.45 ml of SOFO and 2ml of LPS was pipetted out and transferred into the 10 ml volumetric flask and made up to the mark with 10 mM Phosphate buffer at pH 6. The amount of drug present in pharmaceutical formulation was calculated using the formula.

$$Cx = \frac{A_2 aY_1 - A_1 aY_2}{(ax_2 ay_1) - (ax_1 ay_{2)}}$$

$$Cy = \frac{A_1 ax_2 - A_{12} ax_1}{(ax_2 ay_1) - (ax_1 ay_{2)}}$$

The absorptivity's of x at λ_1 and λ_2, ax_1 and ax_2 respectively,

The absorptivity's of y at λ_1 and λ_2, ay_1 and ay_2 respectively,

The absorbance's of the diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively.

Let Cx and Cy be the concentration of x and y respectively in the diluted sample.

The equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbance's of x and y.

VALIDATION:

The described method has been validated for the assay of LPS and SOFO using the following parameters [International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) 1995]. Linearity was studied to find out the relationship of concentration with absorbance. Five different concentrations of LPS and SOFO drug mixtures (5 to 25µg/mL of each drug in the mixture) were employed i.e., 5,10,15,20 and 25 µg/mL. All solutions were scanned and absorbance measured at 260 nm and 296 nm. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (µg/mL) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of LPS and SOFO (15 µg/mL) on the same day. On different days, the same solutions were scanned using different Instruments (Elico SL 210, Lab India UV 3000+) and ruggedness is determined. The precision of each method was ascertained separately from the absorbance obtained by actual determination of five replicates of a fixed amount of drug (15 µg/mL). Precision and ruggedness were done on the same day and the different day respectively, and the percentage of relative standard deviation (% RSD) was calculated for each. The accuracy of the method was shown by analyzing the model mixtures containing 10, 15 and 20 µg/mL of SOFO and LPS mixture and 10 µg/mL of placebo. After the measurement, the amount found, amount added for LPS and SOFO and individual recoveries were calculated. Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope.

RESULTS:

A simultaneous equation (Vierodts) method procedure was proposed as a suitable method for the analysis of the drugs LPS and SOFO in dosage forms. A typical overlap spectrogram of standard LPS and SOFO and their mixture is shown in Figure 3(data in Table 1). The λmax was found to be 296 nm and 260 nm. The regression equation for the method at 296 nm was found to be y = 0.00009x - 0.000095 (r=0.999) and linear over Beer's range 5–25 µg/mL. The regression equation for the method at 260 nm was found to be y = 0.000079x + 0.000096 (r=0.999) and linear over Beer's range 5–25µg/mL. The linearity graph of LPS and SOFO mixtures is shown in Figure 4. A typical overlap spectrogram of different concentration of mixture of LPS and SOFO is shown in Figure 5.

Figure 1: Structure of Ledipasvir

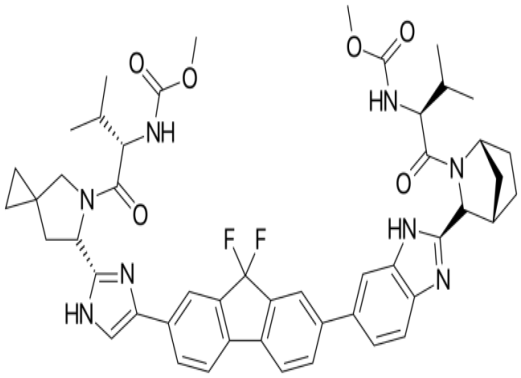


Figure 2: Structure of Sofosbuvir

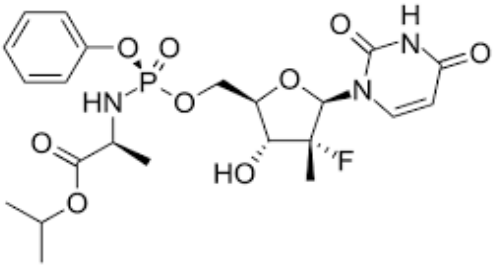


Figure 3: UV absorbance spectra of LPS+SOFO mixture (a), LPS (b) and SOFO (c).

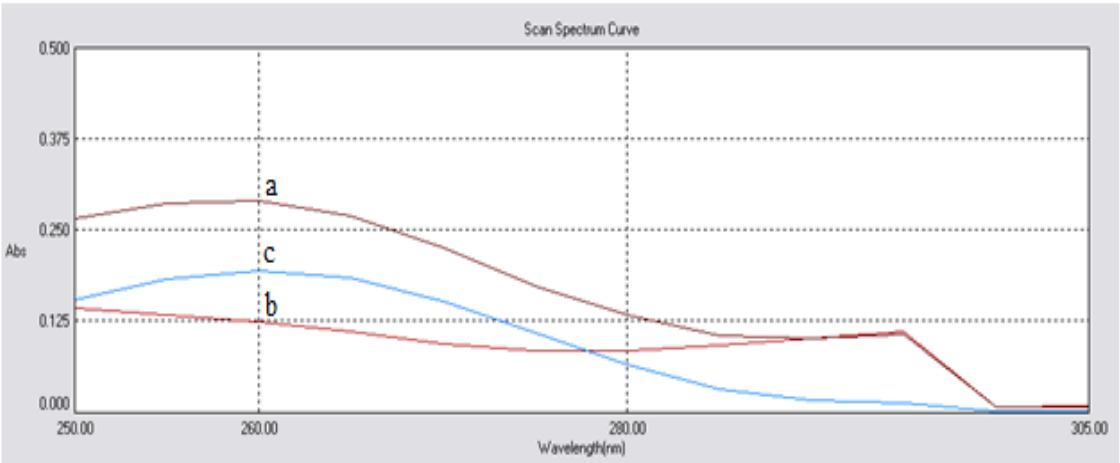


Figure 4: Linearity Curve of SOFO and LPS

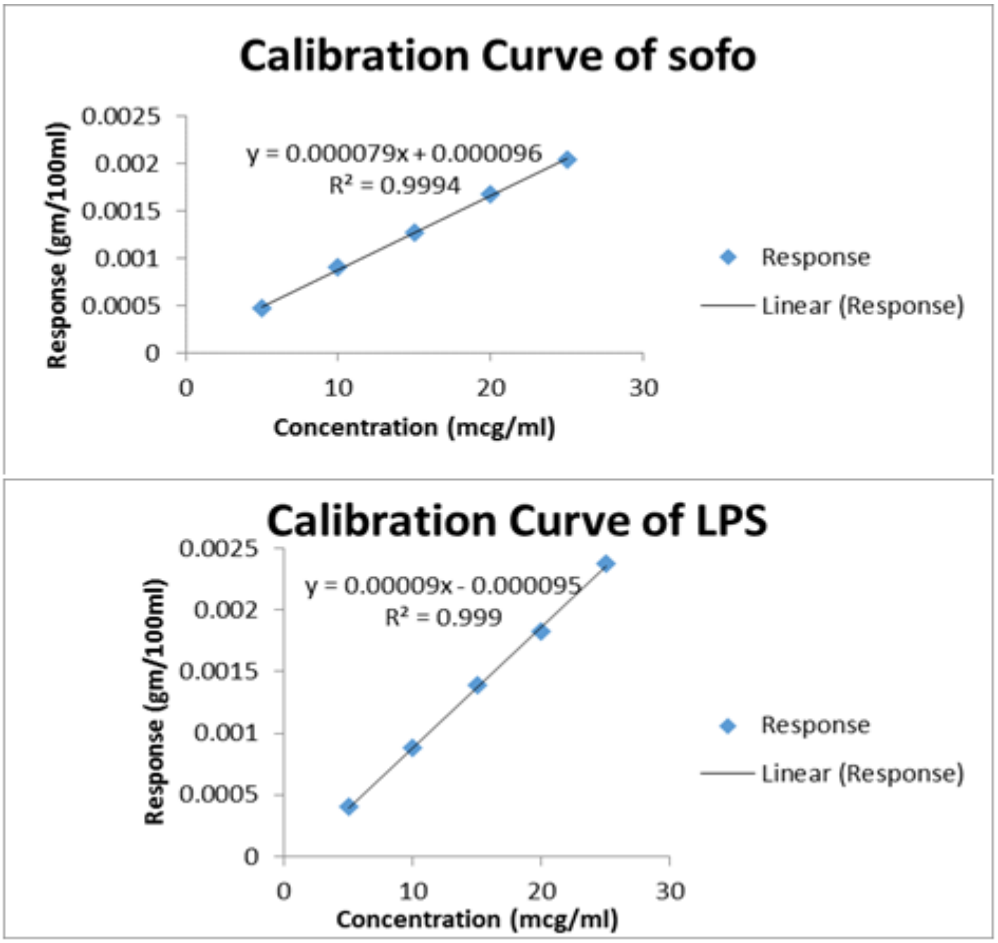
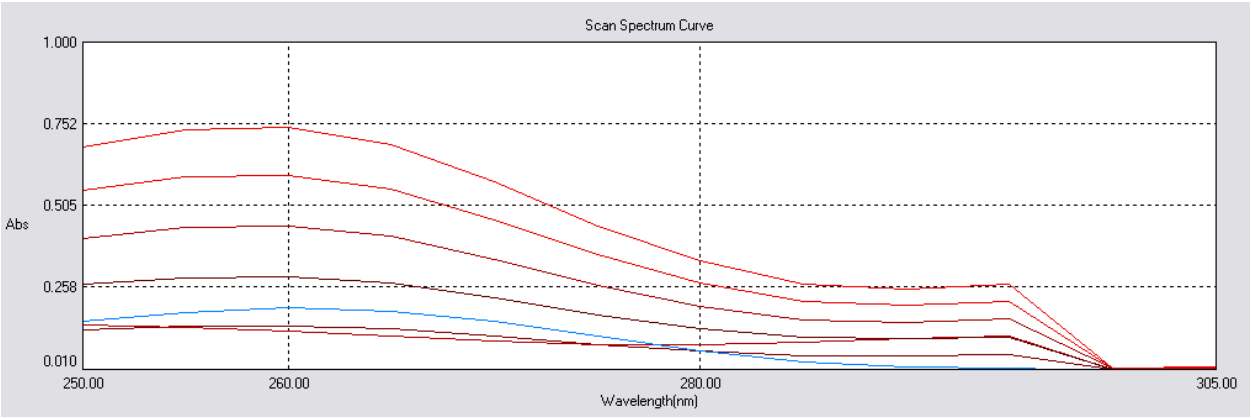


Figure 5: Overlap Linearity UV spectra of LPS+SOFO mixture



The percentage of purity of LPS and SOFO in tablet dosage form was 98.59% and 109.50% respectively. The precision of the spectrophotometer system was determined using the %RSD of the absorbance for five replicate preparations of the drug. The % RSD was less than 2. Precision data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures containing 50%, 100% and 150% of sample solution of drug of LPS and drug SOFO along with 10 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be for LPS 94.46%, 94.24% and 101.35% w/w and for SOFO 99.8%, 100.49% and 99.01%w/w for 50%, 100% and 150% respectively. The mean percentage Accuracy data are presented in Table 3. LOD for LPS and SOFO was found to be 0.0211 µg/ml and 0.0409 µg/ml respectively. LOQ

for LPS and SOFO was found to be 0.0704 µg/ml and 0.1365 µg/ml respectively. Stability of the solution were determined by bench top stability. Five replicate of optimized concentrations were prepared and its absorbance were measured on 1st day at room temperature. % RSD were calculated. The results of bench top stability shown that there is no changes in the solutions even after 2 days at room temperature. The % RSD was found to be less than 2. Robustness were performed by changing buffer pH (±0.2) using the same procedure. Absorbance of triplicate solutions using 10mM Phosphate buffer pH 5.8 and another triplicate solution using 10mM Phosphate buffer pH 6.2 were measured and %RSD were calculated. The results shows that %RSD was in within the criteria limit.

Table 1: Linearity Regression Data

Parameters	LPS	SOFO
Linearity range(µg/ml)	5-25	5-25
Slope ± SD	0.000098 ± 0.000012	0.000079 ± 0.000013
Intercept ± SD	-0.000095 ± 0.000069	0.000096 ± 0.000107
r^2 ± SD	0.9995 ± 0.00555	0.9994 ± 0.001839

Table 2: Results of Recovery Studies.

Concentration(µg/ml)	LPS	SOFO
	% Recovery (mean ± SD)	% Recovery (mean ± SD)
10	94.96522 ± 1.19736	99.86405 ± 3.63242
15	94.24694 ± 1.91548	98.49472 ± 2.57414
20	101.35953 ± 0.86456	99.01661 ± 3.38455

Table 3: Data for Precision of LPS and SOFO

Concentration	LPS absorbance at 296nm	SOFO absorbance at 260nm
Mixture 1	0.122	0.488
Mixture 2	0.121	0.494
Mixture 3	0.117	0.476
Mixture 4	0.120	0.486
Mixture 5	0.123	0.471
Average	0.12060	0.48300
SD	0.00230	0.00930
% RSD	1.90893	1.93113

SD = Standard Deviation; %RSD = Percentage Relative Standard Deviation.

Table 4: Summary of Validation Parameters.

Parameter	LPS	SOFO
Linearity range (µg/ml)	5 - 25	5 - 25
Correlation coefficient ± SD	0.9995 ± 0.00555	0.9994 ± 0.001839
LOD (µg/ml)	0.02112	0.00818
LOQ (µg/ml)	0.07041	0.136502
% Recovery	94 – 101	99 – 100
Precision (%RSD)		
Intraday	1.90	1.93
Interday (Ruggedness)	1.80	1.81
Robustness	Robust	Robust
Assay (%W/W)	98.59	109.5
Stability (%RSD)		0.33 1.2

DISCUSSION:

The developed method can be used for routine analysis because the linearity found in LPS and SOFO is nearing 1 that is 0.9995 and 0.9994 respectively which shows the good regression for linearity. Maximum recovery is obtained by this developed method and the mean percentage recovery for each component is nearing 100%. Therefore this method can be used for the routine analysis and one most important reason is that the developed method does not involve the use of expensive reagents. The spectrophotometric assay methods employed in our study indicated less interference from excipients used in formulation by the percent recoveries values. Most of the existing methods consumed expensive reagents for individual drug analysis. But the method we developed involves chemicals like Methanol, Phosphate buffer and distilled water, which are very simple, economical and also easily available. Also, our proposed method requires less time for the determination of LPS and SOFO simultaneously compared to other methods and these methods also required reagents which are costly and time consuming.

CONCLUSION:

The presented method was found to be precise, sensitive and accurate. This method has simple sample preparation. The good recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of LPS and SOFO in pharmaceuticals.

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