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Chemometrically Assisted RP-HPLC and Spectroscopic Method Development for Simultaneous Multi-Component Analysis of Ledipasvir and Sofosbuvir in Pure and Pharmaceutical Formulation

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INTRODUCTION

Hepatitis C virus (HCV) is an RNA virus that chronically infects about 71 million individuals worldwide [1]. Approximately 80% of acutely infected HCV patients progress to chronic infection, 20% of whom develop cirrhosis within 25 years, with 25 % of patients with cirrhosis developing hepatocellular carcinoma and/or decompensated liver disease. There are six major HCV genotypes with many subtypes based on genomic sequence heterogeneity. Since the discovery of HCV in 1989, strategies to cure the infection have evolved dramatically. A cure is defined as a sustained virologic response and consists of undetectable levels of plasma HCV RNA 12 or 24 weeks after completion of therapy. Among the people who have chronic HCV infection, approximately 60% have the genotype 1 strain of the virus. The treatment of patients infected with HCV genotype 1 is rapidly evolving. HCV therapy has been recently revolutionized by the development and approval of direct-acting antiviral agents (DAAs), borne out of the intense study of the viral life cycle and the elucidation of the crystal structure of several critical viral proteins [2,3]. Ledipasvir and Sofosbuvir in combination are directly acting antivirals attacking at the former stage of replication, reducing the disease leading to its chronicity.

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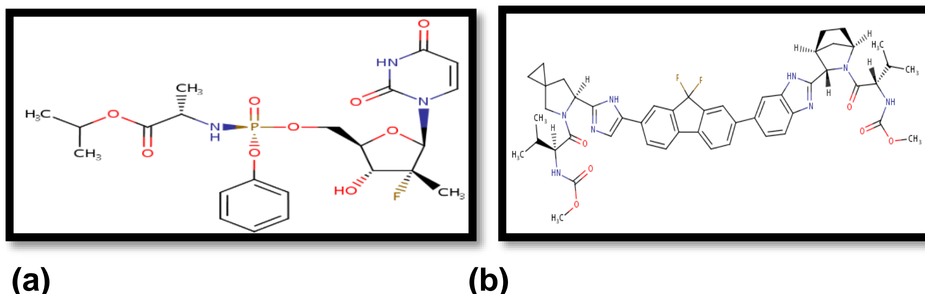
SOF is chemically known as (S)-Isopropyl 2-((S)-(((2R,3R,4R,5R)5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3hydroxy-4-methyl tetrahydrofuran-2-yl)methoxy)-(phenoxy) phosphorylamino) propanoate **(Fig.1a).** It has a molecular formula of $C_{22}H_{29}FN_3O_9P$ and a molecular weight of 529.54. Sofosbuvir is white to off-white powder with a solubility of ≥ 2 mg/mL across the pH range of 2-7.7 at 37°C. The partition coefficient (log P) for Sofosbuvir is 1.62 and the pKa is 9.3. Sofosbuvir is a pangenotypic inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is essential for viral replication [4,5].



(a) **(b)**

Fig.1 Chemical structure of (a) Sofosbuvir and (b) Ledipasvir

LEDI is chemically known as methyl N-[(2S)-1-[(6S)-6-[5-[9,9-difluoro-7-[2-[(1S,2S,4R)-3-[(2S)-2-(methoxy carbonylamino)-3-methylbutanoyl]-3-azabicyclo[2.2.1]heptan-2-yl]-3H-benzimidazol-5-yl]fluoren-2-yl]-1H-imidazol-2-yl]-5-azaspiro[2.4]heptan-5-yl]-3-methyl-1-oxobutan-2-yl]carbamate **(Fig.1b).** It has the molecular formula of $C_{49}H_{54}F_2N_8O_6$ and a molecular weight of 889.00. Ledipasvir is a white to tinted (off-white, tan, yellow, orange, or pink), slightly hygroscopic crystalline solid. Ledipasvir is practically insoluble (<0.1 mg/mL) across the pH range of 3.0-7.5 and is slightly soluble below pH 2.3 (1.1 mg/mL). The partition coefficient (log P) for LEDI is 3.8 and the pKa1 is 4.0 and pKa2 is 5.0. Ledipasvir is an HCV inhibitor targeting the HCV NS5A protein, which is essential for both RNA replication and the assembly of HCV virions [4,6]. The combination of these two drugs is not official in any pharmacopoeia.

Recently, a limited number of methods have been developed for the individual and simultaneous determination of both drugs. The degradation products of SOF under several stress conditions has been determined by HPLC [7,8]. SOF's deposition study into various in vivo cell [9], In human plasma by UPLC-MS/MS [10], SOF and its metabolite, GS-331007, in human plasma by UPLC-ESI-MS/MS [11], Simultaneous quantification of ribavirin, Sofosbuvir and its metabolite in rat plasma by UPLC-MS/MS [12], SOF in pure form [13], in bulk and tablet dosage form by RP-HPLC [14], determination of Daclatasvir (DAC) in human plasma using SOF as an internal standard (IS) by UPLC-MS/MS [15]. While for LEDI, UV spectrophotometry [16] and RP-HPLC [17] methods are reported.

Both SOF and LEDI were determined by UPLC-MS/MS [18] and LC-MS/MS method [19] and Chromatographic analysis in Human plasma [20]. Ledipasvir, Sofosbuvir and its metabolite in rat plasma were also, determined by UPLC-MS/MS [21]. LEDI, SOF and various directly acting antivirals quantification by A UHPLC-MS/MS [22].

According to the best of our knowledge, only two HPLC method [23,24], has been published, during the preparation of the present work for publishing. The present study aims to develop a simple, sensitive, short retention time and accurate RP-HPLC method and First-Order derivative UV-Spectroscopic method for the simultaneous determination of both SOF and LDV together in pure and tablet dosage forms with high sensitivity, selectivity. Validation of the developed methods was performed according to ICH Q2 (R1) guideline [25].

EXPERIMENTAL

Reagents and materials

LEDI and SOF pure API were received as a gratis sample from NATCO Pharma (Hyderabad) and MSN LABS (Hyderabad) and the marketed formulation used was HEPCINET-LP manufactured by NATCO Pharma. All employed Chemicals were AR and HPLC grade.

Instruments

The instruments were Double beam UV-Visible Spectrophotometer (Shimadzu 1800), HPLC (Analytical Technologies), S1122 series pump, 2203 UV-Visible detector, and Rheodyne injector (20 μl). Swisher electronic balance was used for weighing the samples.

Chromatographic conditions

A BDS-C_{18} (250 mm × 4.6 mm, 5μm) chromatographic column and mobile phase consisting of Methanol: Acetonitrile: (1 %) Ammonium acetate (50:20:30 % v/v/v) were used. Flow rate was maintained at 1 ml/min and effluents were monitored at 281 nm. The sample was injected using 20 μL Rheodyne injector. Freshly prepared samples were used at the time of use.

Test and Standard solution preparation for first-order derivative and RP-HPLC method

a) Preparation of standard stock solutions

A standard stock solution (100 μg/ml) each of LEDI and SOF was separately prepared by dissolving 10 mg each in 100 ml of volumetric flask, diluted upto the mark with methanol.

b) Preparation of combined standard solution of LEDI and SOF

Accurately weighed LEDI (9 mg) and SOF (40 mg) were transferred into 100 ml volumetric flask. Dissolved and diluted up to the mark with Methanol to give a combined stock solution (90 μg/ml) of LEDI and (400 μg/ml) of SOF. Stock solution (10 ml) was transferred in 100 ml volumetric flask and diluted up to mark with Methanol to obtain combined working standard solution (9 μg/ml) of LEDI and (40 μg/ml) of SOF. This solution was used to prepare standard solution for linearity in RP-HPLC.

c) Preparation of Tablet Sample solution

Twenty tablets were weighed accurately and powdered. The powder equivalent to 9 mg of LEDI or 40 mg of SOF was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with Methanol to get strength 90 μg/ml Ledipasvir or 400 μg/ml Sofosbuvir (Stock solution). The solution was filtered through Whatman filter paper no.41 and first few ml of filtrate were discarded. From stock solution, 10 ml solution was transferred to 100 ml volumetric flask and volume was adjusted to the mark with Methanol to get strength 9 μg/ml Ledipasvir and 40 μg/ml Sofosbuvir (Working solution).

VALIDATION PARAMETERS

First-order derivative and HPLC method was validated in terms of Specificity, linearity, accuracy, precision, LOD and LOQ, robustness in accordance with ICH Q2(R1) guideline and system suitability test as per USP [26].

a) System Suitability test (For RP-HPLC)

System suitability is established to prove that suitability and reproducibility of the chromatographic system are adequate to perform an analysis. Single set of mixed standard solution was prepared as mentioned in the test method and six replicate injection of mixed standard preparation were injected and chromatogram was taken. Various parameters like peak area, tailing factor, theoretical plates, and resolution and retention time were evaluated.

b) Specificity

Chromatograms of standard and sample solutions of LEDI and SOF were compared.

c) Linearity and Range

The linearity of an analytical method is its ability, within a given range, to provide results that are directly, or through a mathematical transformation, proportional to the concentration of the analyte. The

linearity response was determined by analyzing 6 independent levels of calibration curve in the selected interval between upper and lower quantitation level of LEDI and SOF, respectively. Plot the calibration curve of absorbance verses concentration and determine Regression co-efficient and Regression equations for Ledipasvir and Sofosbuvir.

d) Accuracy

Accuracy of the method was measured in terms of % recovery of standard. Recovery studies were performed by spiking standard drug solution at the level of 80 %, 100 % and 120 % to the pre-analyzed sample. In this method the known concentration standard drug was added to the assay sample. Recovery studies were performed in triplicate by calculating the recovery and % RSD for both the drugs.

e) Precision

The precision of an analytical method was expressed as the percent relative standard deviation and standard error of mean of the series of measurements. It was ascertained by the replicate estimation of standard drugs. It involves Repeatability, Intraday and Interday precision. In repeatability study, one concentration of both drugs was analysed six times. Intra-day precision was carried out by performing three replicates of three different concentrations on same day and peak area measured was expressed in terms of percent relative standard deviation (% RSD). In Interday precision, three replicates of three concentrations were analysed at three consecutive days and % RSD was calculated.

f) L O D and L O Q

Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were calculated from the standard deviation of the response and slope of the calibration curve of drugs using the formula as per ICH guideline,

L O D = 3.3 × (SD / Slope)

L O Q = 10 × (SD / Slope)

where SD = standard deviation of Y-intercept of 5 calibration curves.

Slope = mean slope of the 5 calibration curves.

g) Robustness (For RP-HPLC)

The robustness of an analytical method is the measure of its capacity to stay unaffected by small but deliberate variation in method parameters and provides an indication of its reliability throughout the normal usage. The determination of an robustness requires that method characteristics are assessed when single or more operating parameter is varied.

RESULT AND DISCUSSION

Spectroscopic Experimental condition

a) Selection of analytical wavelength

LEDI 10 µg/ml solution was prepared by diluting 1.0 ml of Standard stock solution (100 µg/ml) in 10 ml volumetric flask and Sofosbuvir 40 µg/ml prepared by diluting 4.0 ml of standard Stock solution (100 µg/ml) in 10 ml volumetric flask – and both were diluted up to mark with Methanol. Both solutions were scanned separately in the range of 200-400 nm. Convert these spectra into first order derivative spectra. It was observed that LEDI showed dA/dλ zero at 284.0 nm in contrast to SOF that has considerable dA/dλ at this wavelength. Further, SOF has zero dA/dλ at 257.0 nm while at this wavelength LEDI has significant dA/dλ. Therefore wavelengths 257.0 nm and 284.0 nm were employed for the determination of LEDI and SOF, respectively without any interference.

b) Construction of Calibration Curve

The solution of LEDI ranging from 2 - 12 µg/ml and SOF 8 - 48 µg/ml were prepared by pipetting out 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml and 0.8, 1.6, 2.4, 3.2, 4.0 and 4.8 ml of the working standard solution of LEDI (100 µg/ml) and SOF (100 µg/ml) into series of 10

ml volumetric flasks and the volume was adjusted to mark with Methanol. Absorbance of each solution was measured at 257.0 nm for LEDI and 284.0 nm for SOF using first order derivative spectrophotometry. The graph of absorbance at individual wavelength of 257.0 nm and 284.0 nm were plotted against their respective concentration.

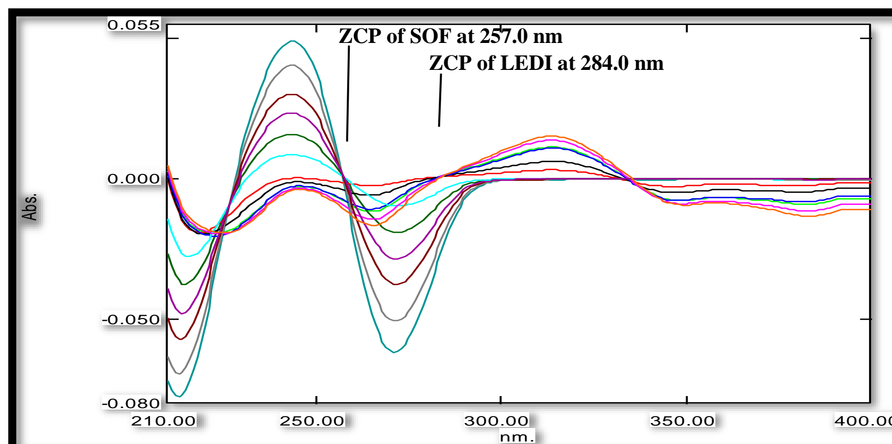


Fig. 3: First-order derivative overlain spectra of LEDI (2-12 µg/ml) and SOF (8-48 µg/ml)

c) Analysis of Tablet formulation (Assay)

The tablet sample solution of the final concentration of LEDI 9 µg/ml and SOF 40 µg/ml was analyzed by the first-order derivative spectroscopic method, and absorbance was measured at 257.0 nm for determination of LEDI and 284.0 nm for SOF respectively. The procedure was repeated five times for sample analysis. The concentrations of LEDI and SOF were calculated from the calibration graph.

Chromatographic Experimental condition

a) Method Development and Optimization of Chromatographic Conditions.

The optimization of chromatographic conditions were done with a view to develop HPLC method for the simultaneous determination of SOF and LEDI in bulk and in pharmaceutical dosage form**.** Various mobile phases comprising different ratios of water, acetonitrile, methanol, and ammonium acetate were tried. Finally, mobile phase comprising of Methanol: Acetonitrile: (1%) Ammonium acetate (50:20:30 % v/v/v) was found to be satisfactory and gave two symmetric and well-resolved peaks at an acceptable retention time of LEDI (5.381 ± 0.447 min) and SOF (8.078 0.3951 min) at 281.0 nm and 1.0 ml/min flow rate (**Fig.4)**. The injection volume to carry out chromatography was set at 20 µL.

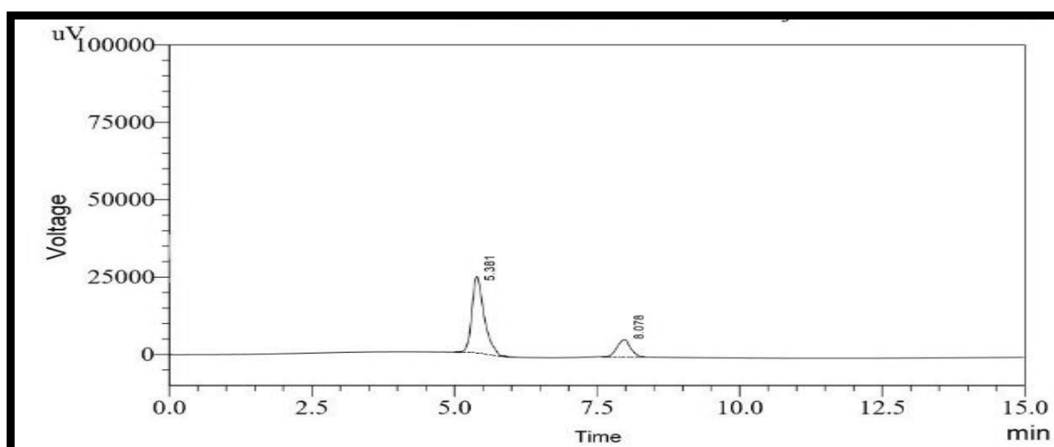


Fig.4: Chromatogram of LEDI (10 µg/ml) and SOF (40 µg/ml) in Methanol: Acetonitrile: ammonium acetate (1%) (50:20:30 %v/v/v)

b) Calibration curve for Ledipasvir and Sofosbuvir

The combined solution of LEDI and SOF ranging from 1-11 µg/ml and 4 – 44 µg/ml were prepared by pipetting out 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 ml of the combined working standard solution of Ledipasvir (90 µg/ml) and Sofosbuvir (400 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. Chromatogram of each solution was recorded. The graph of area verses respective concentration was plotted.

c) Analysis of Marketed formulation

The tablet sample solution of the final concentration of LEDI 9 µg/ml and SOF 40 µg/ml was analyzed by taking the Chromatogram of this solution at a detection wavelength of 281 nm. The procedure was repeated five times for sample analysis. The concentrations of LEDI and SOF were calculated using the regression equation.

Method Validation

The developed and optimized method was validated for system suitability as per USP, specificity, sensitivity [limit of detection (LOD) & limit of quantitation (LOQ), linearity, precision (repeatability, intraday precision, interday precision), accuracy and robustness as per ICH Q2 (R1) guideline.

a) System suitability parameters (For RP-HPLC)

The system suitability test was carried at a 9 µg/ml and 40 µg/ml standard solution of LEDI and SOF respectively by five replicate injections. Various parameters like peak area, tailing factor, theoretical plates, and resolution and retention time were evaluated.

Table 1. System suitability test parameters for SOF and LEDI by RP-HPLC method

Parameters	DRUGS	
	SOF	LEDI
Retention time[a]	5.388	8.085
Tailing factor[a]	1.446	1.336
Theoretical plates[a]	3147.926	4516.577
Resolution factor[a]	-	6.233
Peak area (%RSD)[a]	375676	29122
[a] mean of 6 determination		

b) Specificity (For RP-HPLC)

The specificity of the method was determined by comparing the chromatogram of the standard and sample solutions of LEDI and SOF. For HPLC peak purity index of each drug in the sample solution was found to be nearer to 1. Result obtained under optimized conditions has shown no interference from common Tablet excipients and impurities. Result demonstrates the specificity of the method **(Fig. 5).**

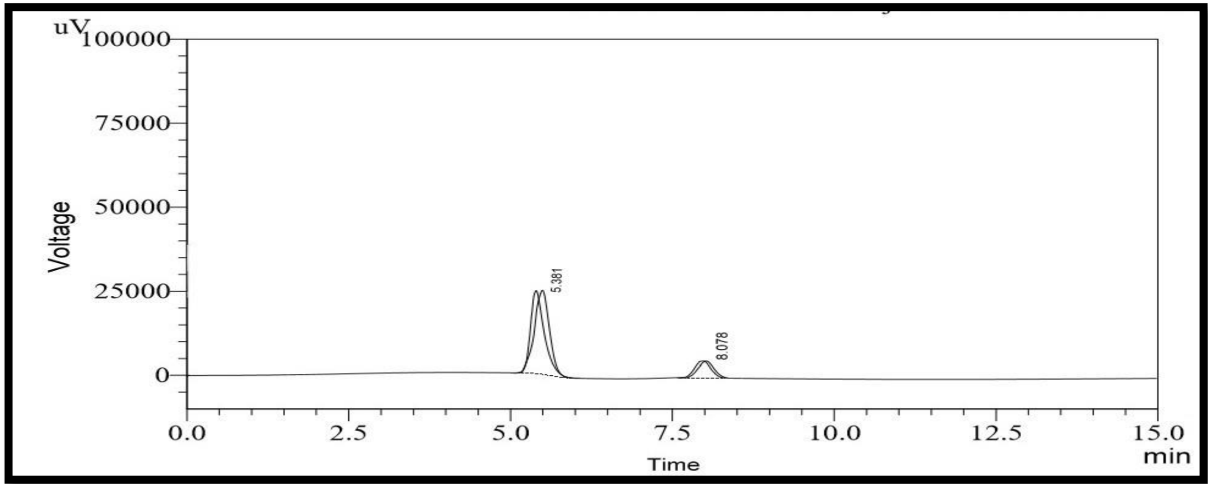


Fig. 5: Overlay chromatogram of Blank, Standard and Sample solutions of LEDI and SOF

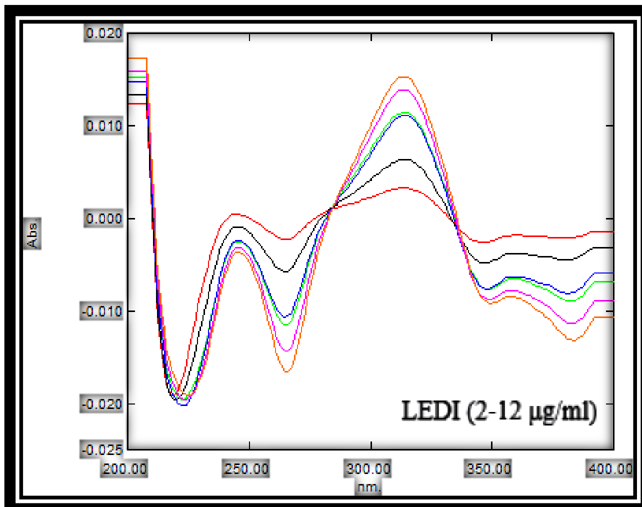
c) Linearity

Linearity was checked by diluting standard stock solution at six different concentrations. The linear regression analysis obtained by plotting the absorbance (for UV) and peak area (for RP-HPLC) of analyte vs. concentration shown correlation coefficients (r^2) greater than 0.995. The statistical results such as correlation coefficient (r^2), slope and intercept are reported in Table 2.

Table 2. Linear regression data for calibration curve

Parameters	UV		RP-HPLC	
	LEDI	**SOF**	**LEDI**	**SOF**
Wavelength	257.0 nm	284.0 nm	281.0 nm	
Concentration range (µg/ml)	2-12 µg/ml	8-48 µg/ml	1-11 µg/ml	4-44 µg/ml
correlation coefficient(r^2)	0.9984	0.9994	0.9987	0.997
Intercept	0.0425	0.008	27939	229904
slope	0.0517	0.0018	1135.8	5323.4

[a] mean of 6 determination



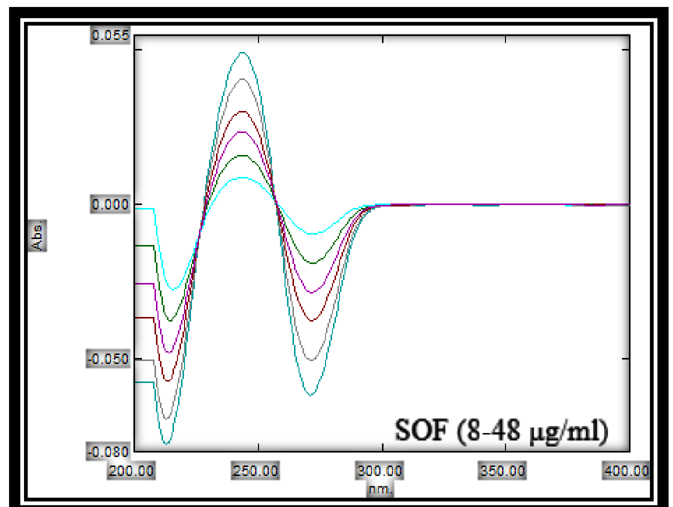


Fig. 6: Overlain spectra of Ledipasvir (2-12 µg/ml) and SOF (8-48 µg/ml)

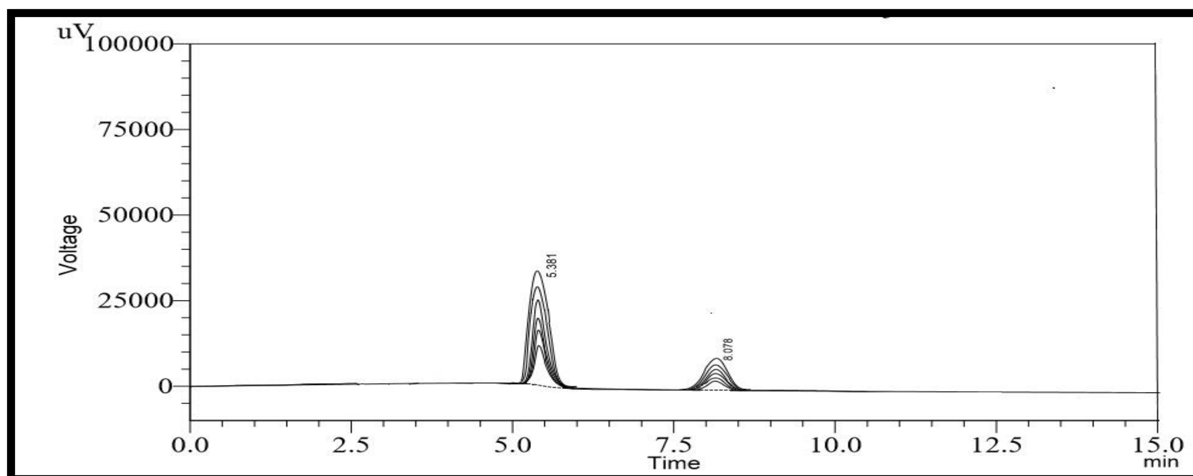


Fig. 7: Overlain chromatogram of Ledipasvir (1-11 µg/ml) and Sofosbuvir (4-44 µg/m)

d) Accuracy

The accuracy of the proposed method was determined by performing recovery study at 80, 100, and 120% level for LEDI and SOF. The recovery study was done by adding pure drug solution to the pre-analyzed tablet formulation, and concentrations of LEDI and SOF were determined by using the calibration graph. The values of percent relative standard deviation and recovery studies were showing satisfactory accuracy.

Table 3. Result of recovery studies of LEDI

Parameters	LEDIPASVIR					
	UV			RP-HPLC		
Level (%)	80	100	120	80	100	120
Sample concentration (µg/ml)	3	3	3	3	3	3
Amount of standard added (µg/ml)	2.4	3.	3.6	2.4	3	3.6
Total concentration (µg/ml)	5.4	6	6.6	5.4	6	6.6
Found concentration (µg/ml)[a]	5.41	6.017	6.62	5.38	6.057	6.23
% Recovery (mean ± sd)[a]	100.31 ± 0.7511	100.27 ± 1.2339	100.31 ± 1.1348	99.75 ± 1.3046	100.29 ± 1.2307	100.39 ± 1.0292
% RSD[a]	0.7487	1.2305	1.1313	1.3079	1.2271	1.0252
[a] mean of 3 determination						

Table 4. Result of recovery studies of SOF

Parameters	SOFOSBUVIR					
	UV			RP-HPLC		
Level (%)	80	100	120	80	100	120
Sample concentration (µg/ml)	20	20	20	12	12	12
Amount of standard added (µg/ml)	16	20	24	9.6	12	14.4
Total concentration (µg/ml)	36	40	44	21.6	24	26.4
Found concentration (µg/ml)[a]	36.06	40.14	44.24	21.66	24.07	26.29
% Recovery (mean ± sd)[a]	100.16 ± 0.7636	100.36 ± 1.0641	100.58 ± 0.6568	100.29 ± 0.7304	100.32 ± 0.8464	99.66 ± 0.7015
% RSD[a]	0.7623	1.0603	0.6532	0.7272	0.8437	0.7039
[a] mean of 3 determination						

e) Precision

The precision of both the method was confirmed by repeatability and intermediate precision. Repeatability expresses the precision under the same operating conditions over a short interval of time. The repeatability was performed by the analysis of the formulation was repeated for six times with the same concentration. The intermediate precision of the method was confirmed by intraday (variation of results within the same day) and interday (variation of results between days) analysis. The intraday and interday precision of the proposed methods were performed by analyzing the corresponding responses three times on the same day for intraday precision and over a period of three days for inter day with three different concentrations of standard tertiary mixture solutions. The results were reported in terms of percentage of relative standard deviation (% RSD). The precision studies comparison data are represented in Table 5, 6 and 7 for LEDI and SOF, respectively.

Table 5. Result of Repeatability studies of LEDI and SOF

Parameters	UV		RP-HPLC	
	LEDI	SOF	LEDI	SOF
Concentration (µg/ml)	6	24	5	20
Mean (Absorbance, Area)	0.2674	0.0355	33585	331942
SD[a]	0.0011	0.0003	185.813	230.493
% RSD[a]	0.3969	0.8046	0.553	0.069
[a] mean of 6 determination				

Table 6. Results of Intraday precision and Interday precision studies of LEDI

Parameters		UV			RP-HPLC		
Concentration		2	8	12	1	7	11

(µg/ml)							
Intra-day precision	Mean (Absorbance/ Area ± SD)[a]	0.0572 ± 0.0005	0.3596 ± 0.0013	0.5775 ± 0.0015	29122 ± 147.85	35695 ± 120.20	40655 ± 195.59
	% RSD[a]	0.874	0.374	0.262	0.51	0.34	0.48
Inter-day Precision	Mean (Absorbance/ Area ± SD)[a]	0.0577 ± 0.0009	0.3601 ± 0.0021	0.5785 ± 0.0027	29124 ± 234.16	35696 ± 211.14	40653 ± 290.50
	% RSD[a]	1.475	0.599	0.471	0.80	0.59	0.71
[a] mean of 3 determination							

Table 7. Results of Intraday precision and Interday precision studies of SOF

Parameters		**UV**			**RP-HPLC**		
Concentration (µg/ml)		8	32	48	4	28	40
Intra-day precision	Mean (Absorbance/ Area ± SD)[a]	0.0711± 0.0006	0.0493± 0.0004	0.0792± 0.0005	252376 ± 145.93	375676 ± 188.74	448538 ± 199.27
	% RSD[a]	0.847	0.819	0.636	0.06	0.05	0.04
Inter-day Precision	Mean (Absorbance/ Area ± SD)[a]	0.0715± 0.0010	0.0490± 0.0009	0.0795± 0.0010	252374 ± 226.24	375676 ± 252.04	448532 ± 275.27
	% RSD[a]	1.343	1.704	1.181	0.09	0.07	0.06
[a] mean of 3 determination							

f) Sensitivity

The sensitivity of UV and RP-HPLC method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were calculated on the basis of the standard deviation of the *y*-intercept and slope: DL = 3.3 σ/S and QL = 10 σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve of analyte.

Table 8. LOD and LOQ of LEDI and SOF for the proposed methods

Parameters	**UV**		**RP-HPLC**	
	LEDI	**SOF**	**LEDI**	**SOF**
L O D **(µg/ml)**	0.0710	0.209	0.328	0.0707
L O Q **(µg/ml)**	0.2153	0.633	0.995	0.2144

g) Robustness

Robustness of the method was determined by Preparing the Combined standard solutions of LEDI (9 μg/ml) and SOF (40 μg/ml) and analyzed by changing mobile phase ratio, flow rate, and wavelength and measuring the corresponding responses 3 times. Flow rate was changed to 1.0 ± 0.02

ml/min. The mobile phase ratio was changed to ± 2 % for both the components. Wavelength of detection was changed to 281 ± 2 nm. The method was found to be robust with respect to variability in applied conditions. Result of robustness was shown in Table 9.

Table 9. Result of Robustness study of LEDI and SOF (n=3)

Chromatographic parameters	Actual condition	Change condition	%RSD	
			LEDI	SOF
Flow rate ± 0.02 ml/min	1.0	0.98	0.349	0.029
		1.02	0.406	0.035
Wavelength ± 2nm	281	279	0.330	0.027
		283	0.374	0.031
Change in the mobile phase ratio ± 2%	50:20:30	52:19:29	0.325	0.028
		48:21:31	0.393	0.032
[a] mean of 3 determination				

h) Analysis of Marketed formulation

The validated UV spectrophotometric and RP-HPLC methods applied in the analysis of the marketed formulation HEPCINET-LP with a label claim of 90mg mg of LEDI and 400 mg of SOF per Tablet. The results for the assay show good agreement with the label claims. Result of the assay was shown in Table 10.

Table 10. Results of assay in commercial sample

Parameters	UV		RP-HPLC	
	LEDI	SOF	LEDI	SOF
Labeled claim (mg)	90	400	90	400
Amount found[a] (mg)	89.10	397.2	88.95	397.6
% Assay ± SD[a]	99.19 ± 0.50	99.30 ± 0.87	98.83 ± 1.41	99.40 ± 1.68
[a] mean of 6 determination				

Comparison of the UV Spectrophotometric and RP-HPLC Methods

The comparison of the developed UV spectrophotometric and RP-HPLC methods was carried out by applying t- test to the assay results of both the drugs obtained by developed methods. It was found that t_{stat} value was less than $t_{critical}$ value for both the drugs. Hence there was no significant difference between the developed methods. So both the developed methods can be successfully applied for quality control analysis of this drugs in the combined pharmaceutical formulation. Result of statistical analytical comparison was shown in Table 11.

Table 11. Result of t-test for LEDI and SOF

Parameter	LEDI		SOF	
	UV Method	RP-HPLC Method	UV Method	RP-HPLC Method

Mean[a]	99.56333	99.75	99.7633	99.86667
Variance[a]	0.768133	1.0317	1.382033	1.265833
Observations	6	6	6	6
Hypothesized Mean Difference	0		0	
d_f	4		4	
t_{stat}	-0.241		-1.0999	
P(T<=t) two-tail	0.821407		0.917715	
$t_{critical}$ two-tail	2.776445		2.776445	
[a]mean of 6 assay determinations				

CONCLUSION

UV Spectrophotometric (First-order derivative method) and RP-HPLC methods were successfully developed and validated for the simultaneous determination of LEDI and SOF. The developed methods were found to be sensitive, accurate, precise, and robust. The results of the assay of the commercial formulation obtained from the UV and HPLC methods were not significantly different as per statistical analysis. This implies that the proposed UV and HPLC methods can be used for quality control analysis of LEDI and SOF in the combined pharmaceutical formulation.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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