Vol 5, Issue 1, 2018 ISSN 2349-7041

Research Article

DIFFERENT SPECTRAL DATA PROCESSING TECHNIQUES FOR DETERMINATION OF LEDIPASVIR AND SOFOSBUVIR IN THEIR PURE AND DOSAGE FORMS; A COMPARATIVE STUDY

FATHY M SALAMA, KHALID A ATTIA, AHMAD A ABOUSERIE, AHMED EL-OLEMY, EBRAHIM ABOLMAGD *

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, 11751, Nasr City, Cairo, Egypt.

Email: Eb.Abolmagd@yahoo.com

ABSTRACT

Four techniques were described for simultaneous determination of sofosbuvir and ledipasvir in their pure and pharmaceutical dosage forms.The first and second methods describe the development of classical least squares (CLS) and principle component regression (PCR) chemometric models, the third and fourth methods describe the development and validation of spectrophotometric methods namely; simultaneous equation and amplitude modulation. The four methods were successfully applied to quantify sofosbuvir and ledipasvir in laboratory prepared mixtures and real market sample. The investigated methods were found to be accurate, precise and can resolve the overlapped spectra of the mixture without any preliminary separation steps.

Key words: Sofosbuvir; Ledipasvir; CLS; PCR; Amplitude modulation; Simultaneous equation.

INTRODUCTION

The new anti-viral combination *Sofolanork plus*® containing Ledipasvir and sofosbuvir is one of the most effective protocol that can manage and completely cure the Hepatitis C virus patients.

Ledipasvir (LED); (Fig. 1.) is methyl *N*-[(2*S*)-1-[(6*S*)-6-[5-[9,9-difluoro-7-[2 [(1*S*,2*S*,4*R*) -3[(2*S*)-2(methoxy carbonyl amino)-3-methyl butanoyl] -3-azabicyclo [2.2.1]heptan-2-yl]-3*H*-benzimidazol-5-yl] fluoren-2-yl]- 1H- imidazol-2-yl] - 5 - azaspiro [2.4] heptan - 5 - yl] - 3 - methyl - 1 - oxobutan -2-yl] carbamate. It is an inhibitor of an important viral phosphor-protein, NS5A, which is involved in viral replication, assembly and secretion [1]. Physically it is a white to tinted (off-white, tan, yellow, orange, or pink), slightly hygroscopic crystalline solid. It is freely soluble in methanol, ethanol and DMSO and slightly soluble in acetone. Its molecular weight is 889. [2]

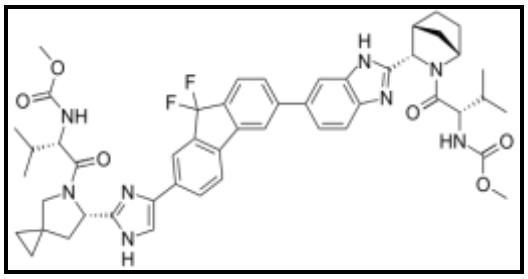

Fig.1: Structural formula of Ledipasvir. (Mol. Formula: $C_{49}H_{54}F_2N_8O_6$)

Sofosbuvir (SOF) ;(Fig. 2.) is (*S*) - isopropyl 2- ((*S*)-(((2*R*,3*R*,4*R*,5*R*) - 5-(2,4 dioxo-3,4 dihydropyrimidin-1(2H)-yl)-4-fluoro-3 hydroxy-4 methyl tetra hydro furan-2-yl) methoxy) - (phenoxy) phosphorylamino) propanoate. Itis potent in inhibiting the HCV NS5B RNA-dependent RNA polymerase, it undergoes intracellular metabolism to produce GS-461203, active uridine analog triphosphate which inhibits the polymerase activity of the NS5B from HCV genotype 1b, 2a, 3a and 4a with IC50 values ranging from 0.7 to 2.6 μM estimated in a biochemical assay.Physically it is a white crystalline solid soluble in the pH range of 2-7.7 at temperature 37ºC and has slight aqueous solubility. Its molecular weight is 529.45. [3]

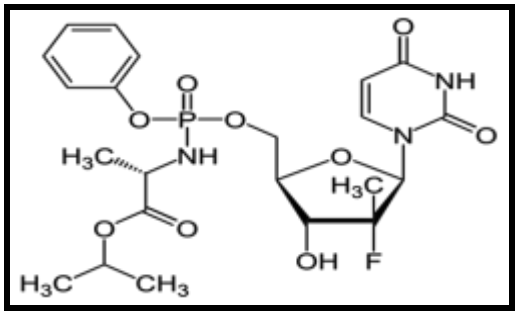

Fig.2: Structural formula of Sofosbuvir. (Mol. Formula $C_{22}H_{29}FN_3O_9P$)

Since this anti-viral combination is newly formulated, few analytical techniques have been reported in the literature for the quantitative determination of SOF alone or in combinations e.g., UPLC–ESI–MS/MS for Sofosbuvir and GS-331007 in human plasma [4], SPE-LC for Sofosbuvir in human plasma [5], UPLC–MS/MS for SOF, GS-331007 and ribavirin in rat plasma [6] and LC–MS/MS for SOF anabolites in cells [7]. While there is only one (RP-HPLC)-UV method for determination of SOF and LED in dosage form simultaneously [8], a TLC densitometric method was also developed by the same authors of this article for simultaneous determination of SOF and LED [9], in addition; only few spectrophotometric methods were available for the simultaneous determination of LED and SOF and only one method for determination of LED in presence of SOF [10] in their dosage form. To the best of our knowledge there is few spectrophotometric methods and *no* chemometric methods available for simultaneous determination of LED and SOF.

To the best of our knowledge there is few spectrophotometric methods and no chemometric methods available for simultaneous determination of LED and SOF.

Hence, the aim of this work was to develop accurate and precise spectrophotometric and chemometric methods for simultaneous determination of LED and SOF in their dosage form. The four methods have the advantage of being able to quantitatively determine the components without interference from each other or from tablet excipients.

Theory of amplitude modulation method (AM)

If we have a mixture of X and Y where Y is extended over X. The absorbance of the zero order absorption spectrum at of mixture of X and Y at certain point as follows:

$$[A_m] = [a_X C_X] + [a_Y C_Y] \quad (1)$$

Dividing eq (1) with normalized spectrum of Y as a divisor (1µg/mL), to get ratio spectrum with isosbestic point (at the same wavelength of the zero order) so the following equation was obtained:

$$[A_m] / [a_Y C_{Y'}] = [a_X C_X] / [a_Y C_{Y'}] + [a_Y C_Y] / [a_Y C_{Y'}] \quad (2)$$

$$[A_m] / [a_Y C_{Y'}] = [a_X C_X] / [a_Y C_{Y'}] + \text{Constant} \quad (3)$$

$$Pm = PX + PY$$

Where, (P_m) is the amplitude of ratio spectrum of the mixture, (P_X) is the amplitude of component X and (P_Y) is the amplitude of component Yi.e the recorded amplitude at the chosen point is equal to the sum of amplitude corresponding to X and that corresponding to Y.

The amplitude representing the component Y(P_Y) was the constant [$a_Y C_Y$] / [$a_Y C_{Y'}$] and it can be measured directly from the spectrum at the straight line that is parallel to the wavelength axis in the region where Y spectrum is extended. [11]

Since, we use normalized divisor of Y so, $C_{Y'}$ = 1µg mL^{-1}

$$PY = [a_Y C_Y] / [a_Y C_{Y'}]$$

$$PY = [C_Y] \quad (4)$$

So the recorded amplitude of the constant was modulated to concentration and it was representing the concentration of Y [C_Y],($C_{Recorded}$ of Y). For determination of amplitude of X in the mixture, If we subtract the measured value of the constant from that of the mixture at

The chosen point of the ratio spectrum Eq. (2);

$$P_X = P_m - P_Y$$

$$P_X = \{[a_X C_X] / [a_Y C_{Y'}] + \text{Constant}\} - \text{Constant} \quad (5)$$

$$P_X = [a_X C_X] / [a_Y C_{Y'}] \quad (6)$$

So at that chosen point $a_X = a_Y$ and normalized divisor of Y $C_{Y'}$ = 1µg mL^{-1}

$$P_X = [a_X C_X] / [a_Y C_{Y'}] \quad (7)$$

$$P_X = [C_X] \quad (8)$$

Theory of simultaneous equation method (SE)

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} different from the other, it may be possible to determine both drugs by the technique of simultaneous equations. The information required is (a) The aborptivities of X at and λ_1 and λ_2 areax_1 and ax_2 respectively (b) The aborptivities of Y at and λ_1 and λ_2 areay_1 and ay_2 respectively. (c) The absorbances of the diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively. Let C_x and C_y be the concentrations of X and Y respectively in the diluted sample. Two 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 of X and Y [12]

$$\text{At } \lambda_1 A_1 = aX_1\, bC_x + aY_1 bC_y \quad (1)$$

$$\text{At } \lambda_2 A_2 = aX_2\, bC_x + aY_2\, bC_y \quad (2)$$

For measurements in 1 cm cells b=1

$$\text{Rearrange eq. (2) } C_y = (A_2 - aX_2\, bC_x) / aY_2$$

Substituting for C_y in eq. (1) and rearranging

$$C_x = (A_2\, aY_1 - A_1\, aY_2) / aX_2\, aY_1 - aX_1 aY_2 \quad (3)$$

$$C_y = (A_1\, aX_2 - A_2\, aX_1) / aX_2\, aY_1 - aX_1 aY_2 \quad (4)$$

As an exercise one needs to drive modified equation containing a symbol b for path length for application in situations where A_1 and A_2 are measured in cells other than 1 cm path length.

EXPERIMENTAL

Instruments

Shimadzu UV-Vis. 1800*Spectrophotometer*, (Tokyo, Japan), equipped with 10 mm matched quartz cells was used. The scanning speed is 2800 nm/min with 1 nm interval.

Software

UV-Probe personal spectroscopy software version 2.1. (SHIMADZU).

All chemometric methods were implemented in Matlab R2013b (8.2.0.701).

CLS and PCR were carried out by using PLS toolbox software version 2.1.

The student *t*-test and F value were performed using Microsoft-Excel.

All calculations were performed using a Quad core CPU, 1.47 GHz, 4.00 GB of RAM under Microsoft Windows 7 ™.

Materials and Reagents

Pure LED and SOF were kindly supplied by Mash Premiere for Pharmaceutical and Cosmetics Industries, Third Industrial Zone, Badr City, Egypt. Their purity were (99.25 %) and (99.7 %) respectively according to the company certificates.

Pharmaceutical preparation: Sofolanork Plus® tablets (Batch no. M 169916) manufactured by Mash Premiere for Pharmaceutical and Cosmetics Industries. It is labelled to contain (400 mg of SOF and 90 mg of LED) per tablet and purchased from local pharmacies.

Methanol was purchased from (El-Nasr Chemicals Co. Abu- Zabaal, Cairo, Egypt).

Standard solutions

Standard solutions for chemometric methods

- A *Standard solution* of LED (450 µg mL^{-1}) was prepared by dissolving 45 mg of LED in 50 mL of methanol and the volume was completed to 100 mL with methanol. *Working solution* of LED (45 µg mL^{-1}) was prepared by further dilution of the stock solution with methanol.
- A *Standard solution* of SOF (1 mg mL^{-1}) was prepared by dissolving 100 mg of SOF in 50 mL of methanol and the volume was completed to 100 mL with methanol. *Working solution* of SOF (200 µg mL^{-1}) was prepared by was prepared by further dilution of the stock solution with methanol.

Standard solutions for spectrophotometric methods

- A *stock standard solution* of LED (90 µg mL^{-1}) was prepared by dissolving 9 mg of LED in 50 mL of methanol and the volume was completed to 100 mL with methanol. *Working standard* solution (9 µg mL^{-1}) was prepared by further dilution of the stock solution with methanol.
- A stock *standard* solution of SOF (400 µg mL^{-1}) was prepared by dissolving 40 mg of SOF in 50 mL of methanol and the volume was completed to 100 mL with methanol. *W*orking *standard* solution (40 µg mL^{-1}) was prepared by further dilution of the stock solution with methanol.

PROCEDURES

For chemometric techniques (CLS and PCR)

Experimental design

A 5 levels, 2 factors experimental design was used in which 0.8, 0.9, 1, 1.1 or 1.2 mL aliquots of both LED and SOF working solutions equivalent to (36, 40.5, 45, 49.5 and 54 µg mL^{-1}) for LED and (160, 180, 200, 220 and 240 µg mL^{-1}) of SOF were combined and diluted to 10 mL with methanol resulting in 25 mixtures. [13]

The central level of the design is 4.5 µg mL^{-1} and 20 µg mL^{-1} for LED and SOF respectively. The chosen concentrations for each compound are based on their linearity and the ratio between both compounds involved in their pharmaceutical preparation. The concentrations details are given in table 1.

The absorption spectra of the prepared mixtures were recorded over the wavelength range 200-400 nm with 1 nm interval thus the produced spectral data matrix has 25 rows representing different samples and 201 columns representing wavelengths (25 x 201).13 mixtures of this design (odd numbers) were used as a calibration set and the other 12 mixtures (even numbers) were used as a validation set to test the predictability of the proposed multivariate models.

For spectrophotometric methods

Amplitude modulation method

Different aliquots from working solutions of LED (9 µg mL^{-1}) and SOF (40 µg mL^{1})equivalent to (9-90 µg mL^{-1}) and (40 - 400 µg mL^{-1}) for LED and SOF respectively, were accurately transferred into two separate sets of 10-mL volumetric flasks and completed to the mark with methanol. The absorption spectra (from 210 to 380 nm) of these solutions were recorded using methanol as a blank, and then divided by the normalized absorption spectrum of LED (1µg mL^{-1}). The amplitude of ratio spectra at 334 nm resembling the constant values were plotted versus the concentrations of LED and regression equation was derived. While the amplitude of ratio spectra at 270 nm after subtraction of the constant were plotted versus the concentrations of SOF and regression equation was derived.

Simultaneous equation method

Different aliquots from working solutions of LED (9 µg mL^{-1}) and SOF (40 µg mL^{-1}) equivalent to (9-90 µg mL^{-1}) and (40 - 400 µg mL^{-1}) for LED and SOF respectively, were accurately transferred into two separate sets of 10-mL volumetric flasks and completed to the mark with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank. The absorbance of each component at 220 nm and 260 nm were recorded and the absorptivity values were calculated. The absorbance and absorptivity values were used for calculating the concentration of LED and SOF by using the equations (3) and (4) mentioned under theory of the method.

Application to laboratory prepared mixtures

Amplitude modulation method

Into a series of 10 mL volumetric flasks, aliquots equivalent to (18-72 µg) and (80-320 µg) of LED and SOF respectively, were accurately transferred from their working solutions (9 µg mL^{-1}) and (40 µg mL^{-1}) respectively, and the volume was completed to mark with methanol. The concentrations of LED and SOF were calculated from the corresponding regression equations.

Simultaneous equation method

Into a series of 10 mLvolumetric flasks, aliquots equivalent to (18-72 µg) and (80-320 µg) of LED and SOF respectively, were accurately transferred from their working solutions (9 µg mL^{-1}) and (40 µg mL^{-1}) respectively, and the volume was completed to mark with methanol. The concentrations of LED and SOF were calculated from equation (3) and (4) mentioned under the theory of the method.

Application of the method to pharmaceutical formulation

Ten tablets of Sofolanork Plus® (400/90 mg) were finely powdered and an amount equivalent to one tablet (400 mg of SOF and 90 mg of LED) was extracted three times with 25 mL of ethanol, filtered into 100 mL volumetric flask then the volume was adjusted with methanol to obtain a solution labelled to contain (4000 µg mL^{-1} of SOF and 900 µg mL^{-1} of LED). This solution was diluted to obtain solution labelled to contain (400 µg mL^{-1} of SOF and 90 µg mL^{-1} of LED).The spectra of these solutions were scanned from 200 to 400 nm, stored in the computer and analysed by the proposed methods.

RESULTS AND DISCUSSION

The UV spectra of SOF and LED show certain degree of overlap Fig. 3, which creates difficulty in the simultaneous analysis of this mixture. Therefore, different methods based on spectral data processing were applied to resolve the overlapped spectra of SOF and LED in both pure form as well as in their pharmaceutical formulation.

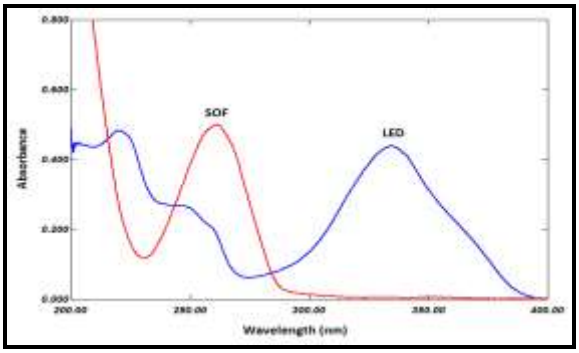

Fig. 3: Zero order absorption spectra of (20 µg mL^{-1}) SOF and (4.5 µg mL^{-1}) LED.

For Chemometric techniques (CLS and PCR)

For the CLS method, the training set was used for constructing CLS model or (K) matrix (i.e. absorptivity at different wavelengths) but poor predictions were obtained. The results were greatly improved by using the CLS model with nonzero intercept.

The predicted concentrations were compared with the known concentrations of the compounds in each calibration sample. The root mean squares error of cross-validation (RMSECV) was calculated for each method for examining the errors in the predicted concentrations. The optimum number of factors was selected by following the criterion of [14].

The selected model was that with the smallest number of factors such that RMSECV for that model was not significantly greater than RMSECV from the model with additional factor. A number of factors were found to be optimum for the mixture of LED and SOF using PCR Fig. 4.

The percentage recoveries of the validation samples are shown in table 2 indicated the high predictive abilities of CLS and PCR models.

When results obtained by applying the proposed methods for analysis of LED and SOF compared to those obtained by applying the reported method [10] they showed no significant difference regarding accuracy and precision; and results were given in table 7.

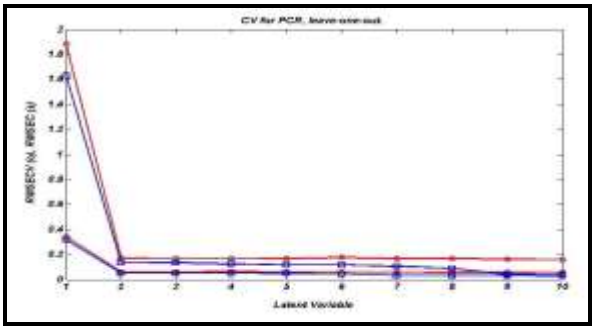

Fig. 4: RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PCR model.

For spectrophotometric methods

Spectral characteristics

Amplitude modulation method

By dividing the spectra of the both LED and SOF by the normalized LED (1µg mL^{-1}) spectrum, we obtain the ratio spectra of their

mixture Fig.5. The amplitude value of the constant can be determined at the plateau region at 334 nm, which is equal to the amplitude constant value of LED along the whole spectrum. At 270 nm, the amplitude of the ratio spectra at this point will be equal to the sum of the amplitudes of SOF and LED. After the subtraction of the recorded amplitude at 334 nm from the previously obtained values at 270 nm, we get the corresponding recorded amplitude of SOF Fig.6.While the constant values is equal to LED concentrations.

Simultaneous equation method

The absorbance of LED and SOF was recorded at two wavelengths 220 nm and 260 nm for LED and SOF simultaneously. The absorptivity coefficients of each component at both wavelengths were determined from the calibration graph where absorptivity is equal to the slope. Their concentrations in laboratory mixture and pharmaceutical formulation were determined by substituting the absorbance and absorptivity coefficient in the equation (3) and (4) in the method theory.

Methods validation

The proposed spectrophotometric methods were validated in compliance with the ICH guidelines [15]. Linearity, range LOD and LOQ, were shown in **Table 3.** Accuracy and precision of the proposed methods were shown in **Table 4** while **Table 5** shows the specificity; recovery of the laboratory prepared mixture of LED and SOF.

The validity of the proposed procedures is further assessed by applying the standard addition technique showing no excipients interference. The results obtained were shown in **Table 6.**

Analysis of real market sample

The proposed procedure was applied for determination of LED in presence of SOF in Sofolanork plus® tablets. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method [10]. No significant differences were found by applying *t*-test and F-test at 95% confidence level [16] indicating good accuracy and precision of the proposed methods for the analysis of the studied drug in its pharmaceutical dosage form, as shown in Table 7.

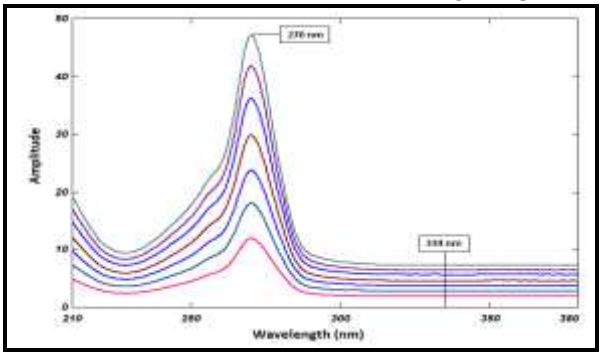

Fig. 5: Ratio spectra of laboratory prepared mixtures (1.8-7.2 µg mL^{-1}) LED with (8-32 µg mL^{-1}) SOF using normalized spectrum of LED (1 µg mL^{-1}) as a divisor.

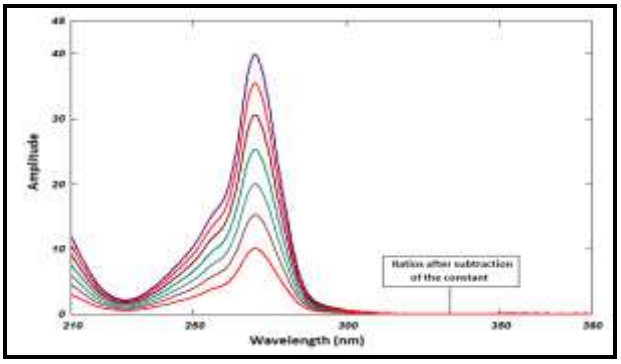

Fig. 6: Ratio spectra of laboratory prepared mixtures (1.8-7.2 µg mL^{-1}) LED with (8-32 µg mL^{-1}) SOF using normalized spectrum of LED (1 µg mL^{-1}) as a divisor after subtraction of the constant at 334 nm.

Table 1: The 5-level, 2-factor experimental design shown as concentrations of the mixture components in µg mL^{-1}.

Mixture number	LED	SOF
1	4.5	20
2	4.5	16
3	3.6	16
4	3.6	24
5	5.4	18
6	4.05	24
7	5.4	20
8	4.5	18
9	4.05	18
10	4.05	22
11	4.95	24
12	5.4	22
13	4.95	20
14	4.5	24
15	5.4	24

16	5.4	16
17	3.6	22
18	4.95	16
19	3.6	20
20	4.5	22
21	4.95	22
22	4.95	18
23	4.05	16
24	3.6	18
25	4.05	20

Table 02: The shaded rows represent the validation set.

Mixture number	CLS		PCR	
	LED	SOF	LED	SOF
1	101.54	101.57	101.33	101.61
2	101.31	97.73	101.56	97.76
3	100.10	100.20	100.27	100.16
4	100.69	103.08	100.62	103.01
5	98.88	98.80	98.88	98.77
6	101.16	98.37	101.10	98.39
7	99.98	99.38	100.06	99.35
8	101.02	100.09	100.84	100.18
9	101.46	98.91	101.31	98.97
10	100.89	99.00	100.90	98.99
11	99.57	98.58	99.47	98.62
12	101.08	97.06	100.96	97.07
Mean (%R)	100.64	99.40	100.61	99.41
%RSD	0.820	1.705	0.797	1.691
RMSEP	0.0420	0.2015	0.0399	0.2012

Table 3: Calibration data for simultaneous determination of LED and SOF by the proposed amplitude modulation and simultaneous equation methods:

Calibration parameters	Amplitude modulation		Simultaneous equation	
	LED	SOF	LED	SOF
Wavelength (nm)	334	270	220	260
Linearity range (µg mL^{-1})	0.9-9	4-40	0.9-9	4-40
LOD (µg mL^{-1})*	0.168	0.479	0.184	0.530
LOQ (µg mL^{-1})*	0.509	1.454	0.557	1.606
Slope ± SD	1.001 ± 0.246	1.2445 ± 0.315	0.1077 ± 0.205	0.0249 ± 0.112
Intercept ± SD	0.0087 ± 0.051	0.3733 ± 0.181	0.0015 ± 0.006	0.007 ± 0.004
determination coefficient (r^2)	0.9997	0.9998	0.9997	0.9997

*** The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines from the following equations: LOD = 3.3 σ/S and LOQ = 10 σ/S. Where σ: the standard deviation of y-intercepts of regression lines, S: is the slope of the calibration curve**

Table 4: Accuracy and precision of the proposed amplitude modulation and simultaneous equation methods.

parameters		Amplitude modulation		Simultaneous equation	
		LED	SOF	LED	SOF
Accuracy (%R)*		98.18	100.74	99.03	99.15
precision, (% RSD)	**Repeatability***	0.547	0.361	0.875	0.766
	Intermediate precision*	0.881	0.682	1.073	0.904

*** Average of three replicates determinations of three concentrations (3.6, 4.5, and 5.4) and (16, 20 and 24) µg ml-1 of LED and SOF respectively.**

Table 5: Determination of LED and SOF in their synthetic mixtures by the proposed amplitude modulation and simultaneous equation methods.

LED (µg mL^{-1})	SOF (µg mL^{-1})	Amplitude modulation		Simultaneous equation		% Recovery			
						Amplitude modulation		Simultaneous equation	
		LED found (µg mL^{-1})	SOF found (µg mL^{-1})	LED found (µg mL^{-1})	SOF found (µg mL^{-1})	LED	SOF	LED	SOF
1.8	8	1.77	7.94	1.78	7.86	98.57	99.28	98.96	98.25
2.7	12	2.66	12.06	2.68	12.07	98.67	100.54	99.38	100.59
3.6	16	3.61	15.89	3.60	15.94	100.14	99.33	100.11	99.64
4.5	20	4.52	20.08	4.51	20.13	100.43	100.42	100.20	100.66
Mean						99.45	99.89	99.78	99.66
%RSD						0.965	0.679	1.121	0.595

Table 6: Recovery study by applying standard addition technique for simultaneous determination of LED and SOF by the proposed spectrophotometric methods.

Sofolanork plus® tablets added µg ml^{-1}		Pure added µg ml^{-1}		% Recovery of Sofolanork plus® added				% Recovery of pure found			
				Amplitude modulation		Simultaneous equation		Amplitude modulation		Simultaneous equation	
LED	**SOF**	**LED**	**SOF**	**LED**	**SOF**	**LED**	**SOF**	**LED**	**SOF**	**LED**	**SOF**
2.7	12	0.9	4	101.83	101.27	101.78	101.46	101.57	101.08	101.67	101.60
		1.8	8					101.73	101.55	101.33	101.43
		2.7	12					100.56	101.37	100.94	101.62
Mean								101.29	101.33	101.31	101.55
%RSD								0.639	0.245	0.370	0.108

Table 7: Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of LED and SOF in Sofolanork plus® tablets.

Method	Drug	Mean	N*	S.D	% RSD	*t***	F**
CLS	**LED**	100.95	**5**	0.891	0.835	1.501 (2.306)	4.808 (6.388)
	SOF	99.37		0.927	0.874	0.837 (2.306)	4.519 (6.388)
PCR	**LED**	100.94		0.853	0.845	0.499 (2.306)	4.924 (6.388)
	SOF	99.38		0.871	0.876	0.754 (2.306)	4.541 (6.388)
Amplitude	**LED**	101.83		0.247	0.243	1.092 (2.306)	2.457 (6.388)
modulation	**SOF**	101.27		0.311	0.307	0.773 (2.306)	1.798 (6.388)
Simultaneous equation	**LED**	101.78		0.495	0.494	0.912 (2.306)	1.683 (6.388)
	SOF	101.46		0.463	0.464	1.225 (2.306)	1.274 (6.388)
Reported method [10]	**LED**	101.61		0.379	0.381	-------	-------
	SOF	101.94		0.419	0.411	-------	-------

*** No. of experimental. ** The values in the parenthesis are tabulated values of t and F at (p= 0.05). *** Absorbance subtraction method at which a mathematically estimated factor representing the absorbance ratio (A262.4/A325) for pure LED was calculated, then this factor was used for simultaneous quantitation of LED and SOF using an equation computed at λ iso (262.4 nm) [10].**

CONCLUSION

In this study, simple multivariate chemometric models and spectrophotometric methods were developed. It was found that LED and SOF can be determined simultaneously in their tablets in presence of excipients and additives by using the developed methods.

The developed methods has the advantages of being simple and inexpensive unlike HPLC procedure which is time consuming and expensive.

The developed methods can be applied for routine and analysis of both LED and SOF in their pure form and pharmaceutical dosage form.

REFERENCES

1. Wikipedia [internet], [reviewed 2016 Sep 2; cited 2016 Sep 30]. Available from: https://en.wikipedia.org/wiki/Ledipasvir, September 30th, 2016.
2. European Medicines Agency [Internet], Committee for Medicinal Products for Human Use (CHMP) [reviewed 2014 Sep 25; cited 2015 Sep 30]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Public_assessment_report/human/003850/WC500177996 pdf.
3. V.R. Ghayathri, C. Vinodhini, S. Gayatri, K. Chitra, Drug Profile of Sofosbuvir - A Nucleotide Analog Inhibitor of the Hepatitis C Virus Polymerase, World J. Pharm. Pharmaceutical Sci., 3(5), (2014) 1411-1416.
4. M.R. Rezk, E.B Basalious, I.A. Karim, Development of a sensitive UPLC–ESI–MS/MS method for quantification of Sofosbuvir and its metabolite GS-331007 in human plasma: application to a bioequivalence study. J Pharm Biomed Anal. 114 (2015) 97-104.
5. L. Qu, W. Wang, D. Zeng, Y. Lu, Z. Yin, Quantitative performance of online SPE-LC coupled to Q-Exactive for the analysis of sofosbuvir in human plasma, RSC Adv. 5 (2015) 98269-98277.
6. X. Shi, D. Zhu, J. Lou, B. Zhu, A.R. Hu, D. Gan, Evaluation of a rapid method for the simultaneous quantification of ribavirin Sofosbuvir and its metabolite in rat plasma by UPLC–MS/MS, J Chromatogr B. 1002 (2015) 353-357.
7. J.E. Rower, L.C. Jimmerson, X. Chen, J.H. Zheng, A. Hodara, L.R. Bushman, P.L. Anderson, J.J. Kiser, Validation and application of a liquid chromatography-tandem mass spectrometry method to determine the concentrations of sofosbuvir anabolites in cells, Antimicrob Agents Chemother. 59 (2015) 7671-7679.
8. B. Zaman, F. Siddique, W. Hassan, RP‑HPLC Method for Simultaneous Determination of Sofosbuvir and Ledipasvir in Tablet Dosage Form and Its Application to In Vitro Dissolution Studies, Chromatographia. (2016) 1-9.
9. Fathy M. Salama, Khalid A. Attia, Ahmed A. Abouserie, Ahmed Elolemy and Ebrahim Abolmagd, Application of TLC densitometric method for simultaneous estimation of the newly co-formulated antiviral agents ledipasvir and sofosbuvir in their tablet dosage form, anal. chem. let, 7(2), (2017), 241-247.
10. N. S. Abdelwahab and N. F. Farid, Innovative spectrophotometric methods for determination of newly discovered combination for hepatitis C treatment, anal chem let. 6(6), (2016), 783-794.
11. H.M. Lotfy, absorbance subtraction and amplitude modulation as novel spectrophotometric methods for the analysis of binary mixtures, Int. J Pharm and Pharmaceutical Sci, 6(1) (2014) 735-741.
12. M. Rohitas, A. Agrawal, A. K. Jain, N. K. Lariya, A. K. Kharya, G. P. Agrawal, development of simultaneous spectrophotometric method of mesalazine and prednisolone in same dosage form, Int. J app pharm, 2(4) (2010) 8-11.
13. R.G. Brereton, Multilevel multifactor designs for multivariate calibration, Analyst. 122 (1997) 1521-1529.
14. D.M. Haaland, E.V. Thomas, Partial least-squares methods for spectral analyses. Relation to other quantitative calibration methods and the extraction of qualitative information, Anal. Chem. 60 (1988) 1193-1202.
15. ICH Q2 (R1): Validation of analytical procedure. Text and methodology. Geneva: International conference on Harmonization, 2005.
16. P. Armitage, G. Berry., Statistical methods in medical research. 3rd ed. Oxford, UK: Blackwell; 1994.