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VALIDATED CAPILLARY ZONE ELECTROPHORESIS APPROACH FOR SIMULTANEOUS SEPARATION AND DETERMINATION OF HEPATITIS C SOFOSBUVIR AND LEDIPASVIR IN TABLET DOSAGE FORM

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ABSTRACT

Our strategy in this study concerned with the development of a simple, highly sensitive and precise capillary zone electrophoresis method for the simultaneous separation and quantification of hepatitis C drugs sofosbuvir and ledipasvir in pure forms and their combined tablets. Under optimum electrophoretic conditions fused silica capillary of 57 and 50 µm i.d. total length and effective length, respectively was applied for the separation of the selected drugs using 20 mmol L^{-1} acetate buffer pH = 4 as ground electrolyte and the running potential 25 kV with hydrodynamic injection 5 s and 70 mbar pressure. The temperature was adjusted at 25˚C and the diode array detection was carried out at 260 nm. Daclatasvir was used as internal standard. The electrophoretic separation was performed over the linear relationship of 5-600 and 20-400 µg mL^{-1} with correlation coefficients of 0.9995 and 0.9997 for sofosbuvir and ledipasvir, respectively. Excellent quantification/detection ratios of 5/1.5 and 20/6.0 µg mL^{-1} were obtained for sofosbuvir and ledipasvir, respectively. The method was validated to ensure its suitability using ICH guidelines.

KEYWORDS: Sofosbuvir, Ledipasvir, Hepatitis C, Pharmaceutical formulations, Capillary zone electrophoresis.

INTRODUCTION

Hepatitis C is a liver infectious disease caused due to the infection by hepatitis C virus (HCV).[1] The patient who suffers from hepatitis C has no symptoms in the initial state of infection. Occasionally a mild fever with abdominal pain as well as dark urine and yellow skin are observed.[2] Through many years complicated symptoms will be developed such as liver cirrhosis and liver cancer. The human body immune system is unable to eliminate the virus from the body. Nowadays, advanced number of medications is recommended to treat hepatitis C. The diagnosis of hepatitis C can be performed by subjecting the patient to different numbers of diagnostic tests, including HCV antibody enzyme immunoassay, RNA polymerase chain reaction (PCR) and recombinant immunoblot assay.[3] The chronic infection with hepatitis C is known by the persisting HCV infection for more than six months and the presence of its RNA.[4] Due to the chronic infection with hepatitis C is typically asymptomatic, it is mostly discovered by the investigation of elevated liver enzyme levels.[5] Sofosbuvir (SOF) is recommended for the treatment of hepatitis C under the brand name of solvadi. It is used with the combination of other antiviral medications such as ribavirin, ledipasvir and daclatasvir. It is chemically known as isopropyl (2S)-2-{[(s)-{(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydro-1(2H)-pyrimidinyl)-4-fluro-3-hydroxy-4-methyltetrahydro furanyl] methoxy}(phenoxy) phosphoryl] amino}propanoate[6] "Figure 1a". The mechanism of its action based on the inhibition of viral RNA synthesis.[7]

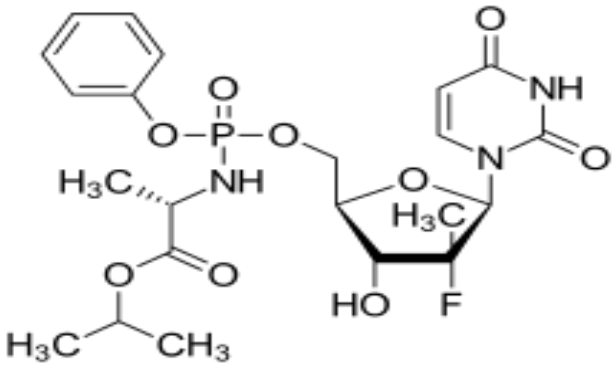

Figure 1a: Chemical structure of sofosbuvir

Several articles concerned with the detection of SOF in single form or combined with other medication by different analytical methods have been published. Among these methods reversed phase high performance liquid chromatography,[8-12], Liquid chromatography-Mass spectrometry,[13,14] Ultra performance liquid chromatography coupled with mass spectrometry,[15-17] and spectrophotometry.[18]

Ledipasvir (LDV) is a medical treatment of hepatitis C. Its mechanism of action is based on the inhibition of RNA replication via NS5A. LDV chemical name is methyl [2S)-1-{(6S)-6-[5-(9,9-difluro-7-{2-[(1R,3S,4S)-2-{(2S)-2-[(methoxycarbonyl) amino]-3-methylutanoyl}-2-azaicyclo [2.2.1] hept-3-yl]-1Hbenzimidazol-6-yl}-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-azaspiro[2.4]hept-5-yl}-3-methyl-1-oxobutan-2yl]carbamate " Figure 1b".

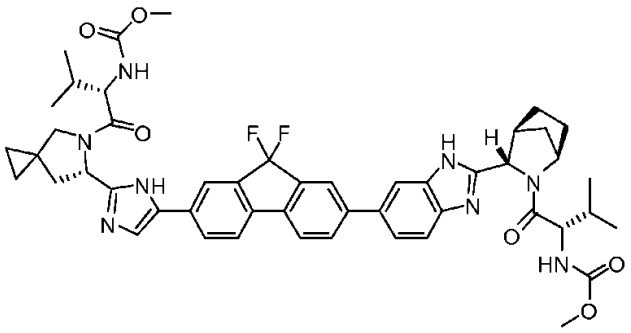

Figure 1b: Chemical structure of ledipasvir

Nowadays, LDV in combination with SOF are recommended in the treatment of hepatitis C. Different published articles were concerned with the detection of LDV including spectrophotometry,[19] reversed phase high performance liquid chromatography,[12, 20] Ultra performance liquid chromatography coupled with mass spectrometry.[21] Although, the selected drugs were detected using different chromatographic techniques, the introduction of new CZE method faced many advantages, such as minimizing solvent consuming, cost benefit technique and high separation speed. The point of view in this article is the suggestion of a new CZE approach to separate and detect SOF and LDV in their pure forms and combined tablets. The described method was evaluated with respect to its validity and suitability using ICH guidelines.[22]

EXPERIMENTAL

Instrumentation and software: The PrinCE 770-Technology instrument was used to separate and estimate two important drugs which are usually recommended in hepatitis C treatment. The device is connected with diode-array (DAD) detector and has a fused silica capillary 57 and 50 µm i.d. total length and effective length. The samples were automatically injected under high voltage using an autosampler. Furthermore, the temperature was controlled by adjusting the thermostat of column cartridge. Data acquisition was carried out using WinPrinCE-770, DA×3D software. HANNA microprocessor pH-meter model 211 (Cluj, Romania) is the device which used to adjust the pH through the conducted study.

Materials and reagents: To detect the selected hepatitis C drugs SOF and LDV, all chemicals are pure analytical grade and the spectroscopic HPLC solvents were used. 0.1mol L^{-1} of glacial acetic acid as well as sodium acetate was used to prepare acetate buffer of pH = 4 as a ground electrolyte. BDH, Philadelphia, USA supplied different kinds of solvents such as ethanol (EtOH), methanol (MeOH), isopropyl alcohol (IPA) and acetonitrile (ACN). WinLab, East Midlands, UK provided sodium acetate, sodium hydrogen phosphate, sodium dihydrogen phosphate, boric acid and sodium hydroxide ≥ 99.0%. Pure grade of SOF, LDV and DAC were purchased from Shandong Mingyuan. Co., (Shandong, China). Harvoni® 90 mg ledipasvir/ 400 mg sofosbuvir/tablet was purchased from online suppliers.

Preparation of analytical samples

Standard solution: Stock solutions of each SOF and LDV were daily prepared at a concentration of 1000 µg mL^{-1} by dissolving approximately 100 mg of each pure drugs in 100 mL methanol. Daclatasvir dihydrocloride (DAC) was used as internal standard (IS). Approximately, 100 µg mL^{-1} was prepared by dissolving 10 mg of DAC in 100 mL deionized water. The working solutions were freshly prepared by serial dilution of the selected drugs in the ranges of 5 - 600 and 20 - 400 µg mL^{-1} for SOF and LDV, respectively using the same solvent.

Preparation of authentic binary mixtures: The introduced CZE method was applied to detect the selected drugs in their binary authentic mixture. The evaluation was carried out using 200 µg mL^{-1} standard solutions of each drug in the presence of 2 mL of 100 µg mL^{-1} IS. The evaluation was performed by using the CZE method to analyze the final concentrations 1:1, 1:2, 2:3, 2:5, 3:4, 5:7 (w/w) of SOF and LDV, respectively.

Preparation of Harvoni® tablet solutions: Not less than 10 tablets were weighed and the average weight of one tablet was determined. The tablets were finely powdered and accurate amount equivalent to the average weight of one tablet was transferred into a 100-mL volumetric flask and dissolve using methanol. Approximately, 10 mL of the solution was transferred into a 100-mL volumetric flask and diluted using methanol, then filtrated using 0.45 Millipore membrane paper. After filtration the volume was completed to mark using the same solvent.

Electrophoretic conditions: The developed CZE method was used to detect the selected hepatitis C drugs SOF and LDV in their binary mixture in the presence of DAC as IS. Before

beginning the separation process the capillary should be conditioned using 0.1 mol L^{-1} sodium hydroxide for 2 min, followed by deionized water for 2 min and then equilibrated with running electrolyte for 5 min. The investigated drugs were separated under optimum condition by using fused silica capillary of 57 and 50 µm i.d. total length and effective length, respectively, 20 mmol L^{-1} acetate buffer pH = 4 as ground electrolyte, running potential 25 kV with hydrodynamic injection 5 s and 70 mbar pressure. The temperature was adjusted at 25˚C and the diode array detection was carried out at 260 nm. The reproducibility of the separation was evaluated and the capillary was replenished using 0.1 mol L^{-1} sodium hydroxide for 5 min, deionized water for 5 min and running buffer electrolyte for 10 min.

Calibration graph: The calibration graph of each drug of interest was constructed over the concentration range of 5-600 and 20-400 µg mL^{-1} for SOF and LDV, respectively in the presence of 2 mL of DAC 100 µg mL^{-1} as IS. Triplicate analysis for each concentration was carried out and the calibration graph was plotted using the peak area ratio of each concentration to IS *vs.* the relating standard concentration. The regression equation of each drug was derived.

RESULTS AND DISCUSSION

The developed CZE method was employed to detect mixture of SOF: LDV (50: 100 µg mL^{-1}) in the presence of DAC as IS. The typical electropherogram of the laboratory mixture of both drugs was shown in Figure 2. Excellent separation was recorded at 2.45 and 4.20 min for SOF and LDV, respectively. The introduced method was also employed to detect the investigated drugs in their dosage formula.

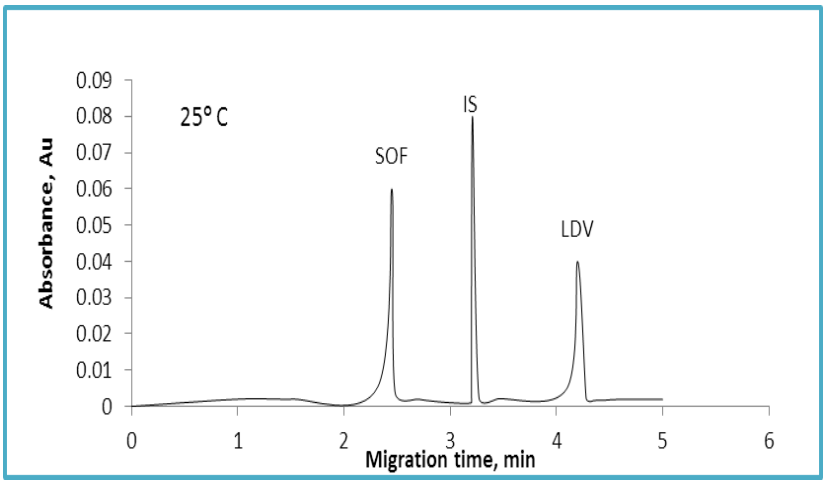

Figure 2: Typical electropherogram of a mixture of SOF (50 µg mL^{-1}) and LDV (100 µg mL^{-1}) in the presence of 2 mL of 100 µg mL^{-1} IS

Optimization of CZE conditions: Several parameters including the degree of ionization of the drug of interest, electrophoretic mobility, migration time and the pH of running buffer solution should be optimized to improve the electrophoretic separation.

Selection of running buffer solution: The selection of the suitable running electrolyte is considered as one of the most essential parameters which should be optimized before carrying out the separation process. Therefore, at constant instrumental condition such as applied pressure, applied voltage, injection time, wavelength and temperature, 10-50 mmol L^{-1} of acetate, phosphate and borate buffer solutions were investigated. The acceptable results were recorded displaying excellent separation with a sharp signal intensity and suitable migration time was achieved by using 20 mmol L^{-1} acetate buffer of pH = 4.

Effect of pH: The electrophoretic separation using CZE technique is very sensitive to pH changes rather than in other chromatographic separation such as high performance liquid chromatography. Therefore, a small change in pH will greatly influences the separation process. As shown in Figure 3, Excellent separation for SOF, LDV and IS by using an acetate buffer of pH = 4 was obtained.

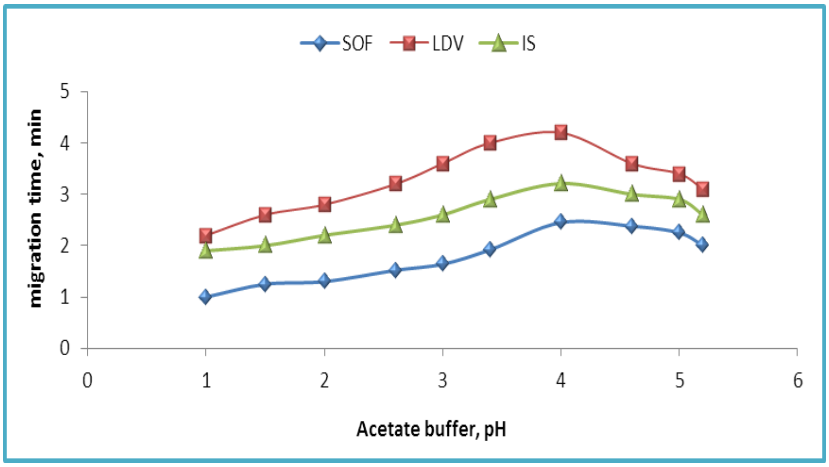

Figure 3: Effect of buffer pH on the migration time: optimum conditions 20 mmol L^{-1} acetate buffer pH = 1-5, injection time 5 s, 25°C, 70 mbar applied pressure, 260 nm, 25 kV and 50 µg mL^{-1} of each of the tested drugs and 100 µg mL^{-1} of IS

Effect of running buffer concentration

The separation process in CZE is greatly affected by the concentration of the running buffer. The mechanism of its action is controlled by a stacking phenomenon which is explained by keeping the conductivity of the buffer more than the conductivity of the sample. In this

section we investigated different acetate buffer concentrations ranging from 5-50 mmol L^{-1}. It was found that high separation with reasonable migration time was achieved by using 20 mmol L^{-1} under constant electrophoretic conditions: acetate buffer of pH = 4, applied pressure 70 mbar, applied voltage 25 kV, 25°C and 260 nm "Figure 4".

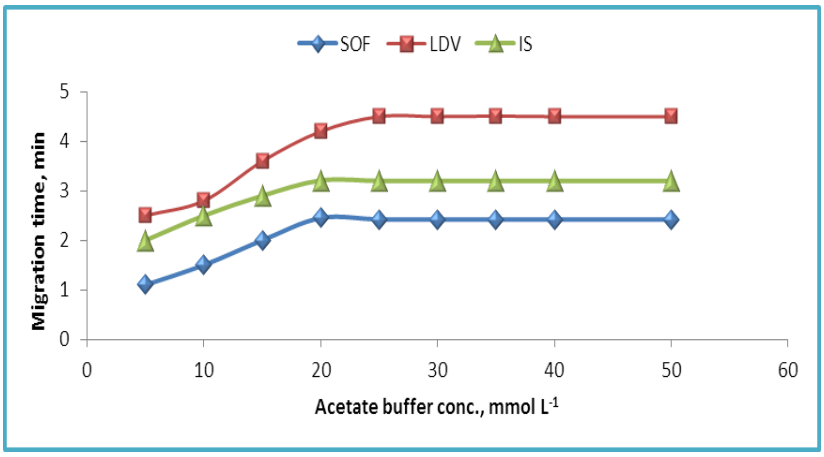

Figure 4: Effect of buffer concentration on the migration time: optimum conditions 5-50 mmol L^{-1} acetate buffer pH = 4, injection time 5 s, 25ºC, 260 nm, 25 kV and 50 µg mL^{-1} of each of the tested drugs and 100 µg mL^{-1} of IS

Effect of additives and organic modifiers

Some additives including 10-30 mmol L^{-1} sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and beta- cyclodextrin (β-CD) were investigated to study their effect on the electrophoretic system. No significant improvement in the system separation was observed with the addition of (β-CD). Meanwhile, the addition of SDS and CTAB in the level above the critical micelle concentration increases the surface aggregation and interaction of the hydrophobic molecules leading to the change in the sample mobility.[23] Therefore, no additive compounds were added during this study. Furthermore, the effect of some organic modifiers such as ethanol (EtOH), methanol (MeOH), isopropanol (IPA) and acetonitrile (ACN) was tested. Approximately 10 - 50% of each modifier is added to the running buffer. It was observed that a considerable increase in migration time and viscosity of the running buffer was obtained, but no significant improvement in the separation of both drugs was recorded by using the organic modifiers "Figure 5".

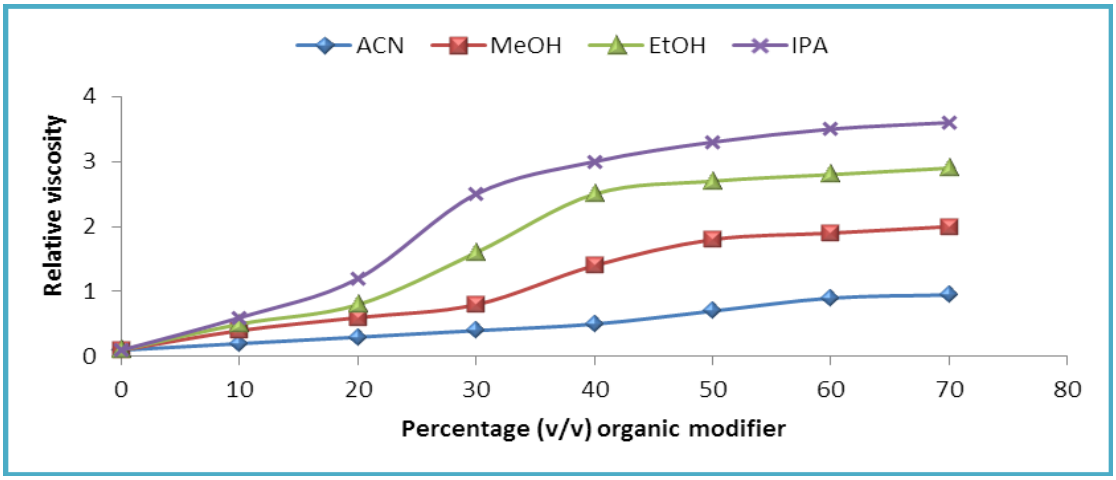

Figure 5: Effect of percentage (v/v) organic modifiers on the viscosity of the running buffer solution

Effect of applied voltage: To investigate the effect of the applied voltage on the electrophoretic separation, several runs were performed using an applied voltage ranging from 10 - 30 kV. A direct relationship between the efficiency of resolution (Rs) and the applied voltage was observed. Therefore, increasing applied voltage from 10 - 25 kV causes an increase in Rs. While, excessive increasing of applied voltage than 25 kV may cause the generation of excessive Joule heat, which will cause a significant inhibition in Rs of the capillary. Excellent resolution was obtained at 25 kV " Figure 6 ".

Selection of injection time: The peak width and peak height in the electrophoretic separation are greatly influenced by the injection time. Therefore, the investigated samples were tested using hydrodynamic injection in the time range of 2 - 10 s under pressure 70 mbar. It was noticed that after 5 s, peaks deformation was observed. The hydrodynamic injection time was considered as 5 s.

Selection of internal standard: The role of IS in the CZE technique is the improvement of the separation performance, decreases the injection error and lowers the migration time of the separation process. The molar masses of SOF, LDV and IS are 529.458, 947.098 and 738.89 g mol L^{-1}, respectively. By using acetate buffer pH = 4, the expected separation is SOF, IS and LDV respectively.

Selection of the detection wavelength

The suitable wavelength for the electrophoretic separation was selected by carrying out the analysis at 200-400 nm. Excellent separation with high resolution and the best signal to noise ratio was achieved at 260 nm.

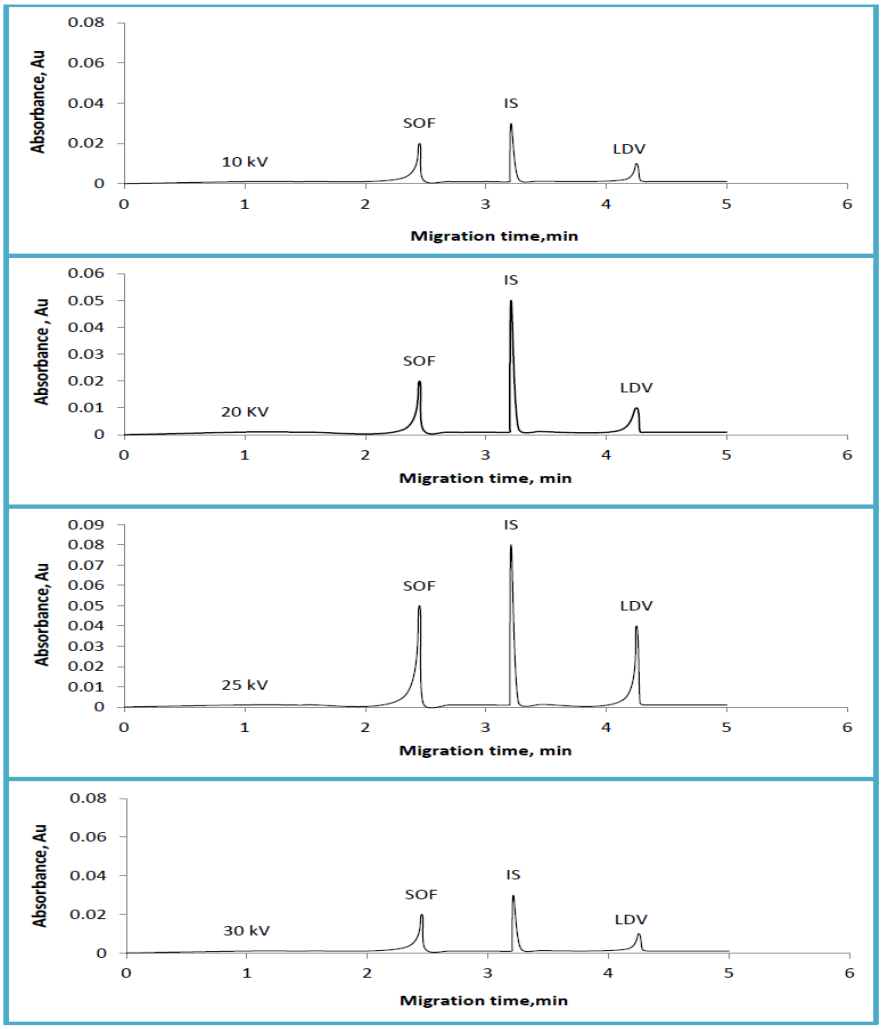

Figure 6: Effect of different voltages on separation of SOF (50 µg mL^{-1}) and LDV (100 µg mL^{-1}) and 100 µg mL^{-1} IS; running buffer; 20 mmol L^{-1} acetate buffer; injection 70 mbar for 5 s; separation voltage (10-30 kV); capillary temperature 25°C and DAD detection at 260 nm

Method validation: The suitability of the electrophoretic separation method was validated by obeying ICH guidelines.[22] The validation parameters were tested such as linearity, system stability, specificity, accuracy, and precision etc.

Linearity: The peak area ratio (drug/IS) was plotted vs. drug concentration to evaluate the linear relationship of the developed CZE method for the detection of SOF and LDV. It was found that the proposed method displayed linearity over the concentration ranges of 5-600 and 20-400 µg mL^{-1} for SOF and LDV, respectively. As illustrated in Figure 7, an excellent separation with good resolution was conducted with regression equations Y_{SOF} = 0.0012 C + 0.1741 and Y_{LDV} = 0.0017 C + 0.1793 and correlation coefficients (r) of 0.9995 and 0.9997 for SOF and LDV, respectively.

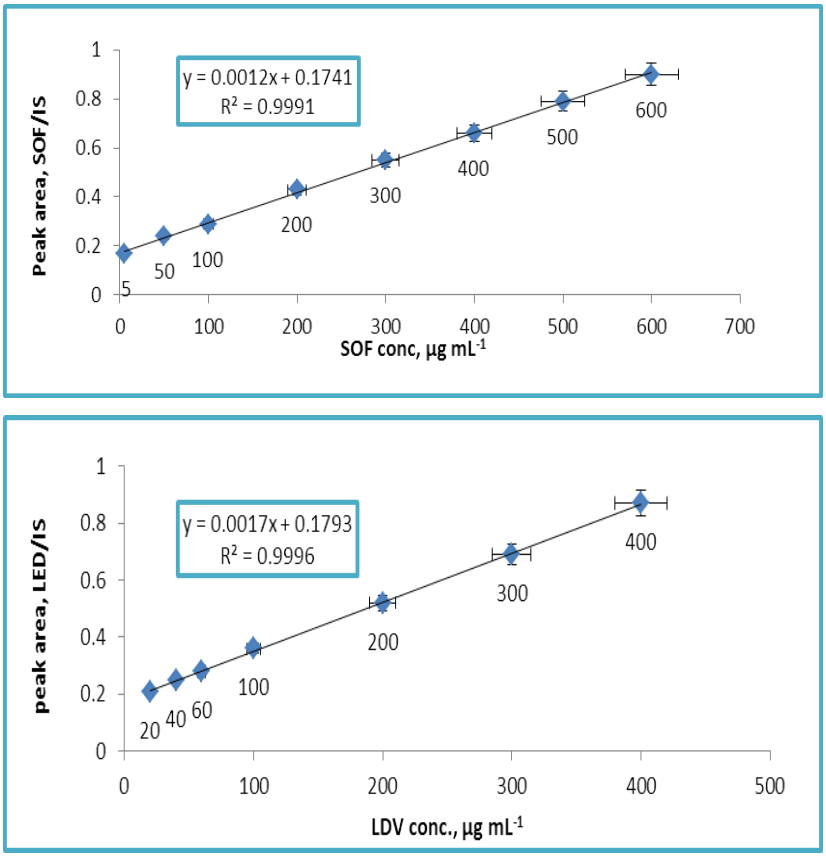

Figure 7: Typical calibration graphs of the investigated drugs SOF and LDV using the CZE method under the optimum conditions: running buffer; 20 mmol L^{-1} acetate buffer pH = 4, under pressure 70 mbar for 5 s; separation voltage 25 kV; capillary temperature 25°C and DAD detection at 260 nm.

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

The guidelines ICH Q2 (R1) was used to evaluate the limits of detection LOD and quantification LOQ by applying the following equations: LOD = 3.3 S_a/b and LOQ = 10 S_a/b. Where, S_a is the standard deviation of the intercept and b is the slope.

Table 1, summarized the critical performance results of the investigated drugs using the developed CZE method. The investigated drugs were detected with LOQ/LOD ratios of 5/1.5 and 20/6.0 µg mL^{-1} for SOF and LDV, respectively.

Table 1: Critical performance data of the determination of the investigated drugs SOF and LDV using CZE method

Parameter	SOF	LDV
Linearity range (µg mL^{-1})	5-600	20-400
Regression equation	Y_{SOF} = 0.0012 C + 0.1741	Y_{LDV} = 0.0017 C + 0.1793
Correlation coefficient (r)	0.9995	0.9997
Standard deviation of residuals, $S_{y/x}$	0.2871	0.0029
Standard deviation of intercept, S_a	0.0311	0.0004
Standard deviation of slope, S_b	0.0001	0.0002
Limit of detection (LOD)	1.5	6.0
Limit of quantification (LOQ)	5	20
%RSD	0.5	0.8
%Error*	0.002	0.001

**% Error was calculated by SD/$\sqrt{n}$.*

Accuracy and precision: The accuracy of the developed CZE method was evaluated using standard solutions of the tested drugs SOF and LDV. After calculating the mean percentage recoveries considering (n = 6) the obtained data were assessed statistically and evaluated with respect to the results of a published method, reversed phase high-performance liquid chromatographic separation and determination of SOF and LDV in tablets. The chromatographic separation was carried out under optimum conditions Eclipse XDB C18 column, mobile phase 0.02 mol L^{-1} potassium dihydrogen phosphate of pH = 3 and 5.7 mmol L^{-1} hexane sulfonate: acetonitrile (50:50 v/v), flow rate 1.5 mL min^{-1} with injection volume 10 µL and UV detection at 254 nm.[12]

Table 2, summarized the obtained results which indicated good agreement with those of a reported method. Moreover, inter-day and intra-day was applied to evaluate the precision of the suggested CZE method. The % RSD was calculated and the obtained results were listed in Table 3.

Table 2: Analytical results of the determination of SOF and LDV in bulk powder using CZE method and reference method

Drug	Proposed CZE method			Reference method [12]		
	Taken (µg mL^{-1})	Found (µg mL^{-1})	% Recovery	Taken (µg mL^{-1})	Found (µg mL^{-1})	% Recovery
SOF	5	4.99	99.8	40	39.98	99.9
	10	9.98	99.8	60	59.68	99.5
	50	49.95	99.9	100	99.75	99.8
	100	99.96	99.9	200	199.99	99.9
	200	199.87	99.9	300	295.85	98.6

	400	389.99	97.5	500	498.71	99.7
	600	595.72	99.3			
Mean± SD	**99.4±0.8**			**99.6±0.5**		
n	**7**			**6**		
Variance	**0.64**			**0.25**		
***%SE**	**0.30**			**0.20**		
t-test	**0.361 (2.201)****					
F-test	**2.56 (4.39)****					
LDV	20	19.98	99.9	30	29.99	99.9
	40	39.87	99.7	50	49.84	99.7
	80	79.99	99.9	80	79.87	99.8
	100	99.95	99.9	100	99.99	99.9
	300	292.84	97.6	200	199.63	99.8
	400	399.96	99.9	300	292.47	97.5
Mean± SD	**99.5±0.9**					
n	**6**			**99.4±1.0**		
Variance	**0.81**			**6**		
***%SE**	**0.37**			**1.00**		
t-test	**0.911 (2.228)****			**0.58**		
F-test	**1.23 (5.05)****					

** %SE= SD/$\sqrt{n}$ **Figures in parentheses are the tabulated values of t- and F- testes at 95% confidence limit*[24]

Table 3: Analytical data of inter-day and intra-day precisions for the determination of SOF and LDV using CZE method

Parameter	**SOF Taken (µg mL^{-1})**			**LDV Taken (µg mL^{-1})**		
Inter-day	**5**	**100**	**600**	**20**	**100**	**400**
% Found	99.25 99.14 99.82	99.28 99.73 99.87	99.84 99.36 99.42	98.99 100.00 99.85	99.92 99.36 99.82	100.00 99.93 99.47
Mean ± SD **% RSD** **% SE**	**99.40±0.37** **0.37** **0.21**	**99.63±0.31** **0.31** **0.18**	**99.54±0.26** **0.26** **0.15**	**99.61±0.55** **0.55** **0.31**	**99.70±0.29** **0.29** **0.17**	**99.80±0.29** **0.29** **0.17**
Intra-day	**5**	**100**	**600**	**20**	**100**	**400**
% Found	99.16 99.87 99.93	99.75 99.63 99.89	99.52 99.36 99.97	99.96 99.38 99.74	99.18 99.53 99.21	99.47 98.88 99.69
Mean ± SD **% RSD** **% SE**	**99.65±0.13** **0.13** **0.07**	**99.86±0.09** **0.09** **0.05**	**99.62±0.32** **0.32** **0.18**	**99.69±0.29** **0.29** **0.17**	**99.31±0.19** **0.19** **0.11**	**99.35±0.42** **0.42** **0.24**

Robustness of the developed method

To evaluate the robustness of the suggested CZE method, minor deliberated changes in method parameters were carried out. These parameters include the change in the

concentration of the running buffer (20±5 mmol L^{-1}), running buffer solution pH (4±0.5), the temperature of capillary cartridge 25±2 ºC, injection time 5±1 s and applied voltage 20±2 kV with only one parameter at a time was changed. As presented in Table 4, no significant changes in the peak area ratio and the migration time were observed.

Table 4: Robustness data using 50 µg mL^{-1} SOF and 100 µg mL^{-1} of LDV in the presence of 100 µg mL^{-1} IS

Parameter	Migration time, min		Peak area ratios	
	SOF	LDV	SOF	LDV
Standard	**2.45**	**4.20**	**0.43**	**0.72**
Acetate buffer pH				
3.5	**2.48**	**4.16**	**0.43**	**0.76**
4.5	**2.40**	**4.28**	**0.42**	**0.78**
Acetate buffer concentration, mmol L^{-1}				
15	**2.39**	**4.18**	**0.40**	**0.77**
25	**2.52**	**4.25**	**0.45**	**0.79**
Injection time, s				
4	**2.02**	**4.23**	**0.49**	**0.75**
6	**2.54**	**4.12**	**0.44**	**0.73**
Applied voltage, kV				
23	**2.30**	**4.49**	**0.41**	**0.71**
27	**2.15**	**4.28**	**0.48**	**0.74**
Capillary cartridge temperature,ºC				
23	**2.48**	**4.38**	**0.41**	**0.79**
37	**2.56**	**4.17**	**0.43**	**0.76**

Specificity: The specificity of the investigated drugs were tested to study discriminate them from other interfering species. The standards of SOF and LDV solutions and some possible spiking interfering compounds such as magnesium stearate, microcrystalline cellulose and colloidal silicon dioxide were studied. A diode array was used to record the peak purity of the samples against the standard drugs by prinCE-770 DA × 3D software. At the lower limit of quantification, no separation peaks were recorded revealed the selectivity and specificity of the proposed CZE method for separation of SOF and LDV drugs.

Analytical applications

Quantification of SOF and LED in authentic mixture

The electrophoretic conditions were optimized and the selected drugs were analyzed in their authentic binary mixture. Sample injection was triplicated and the percentage recoveries of each drug concentration were calculated from the calibration graphs. In Table 5, The recorded data were found to be 99.6±0.5 and 99.3±0.2 for SOF and LDV, respectively and

assessed statistically using Student's t-test and variance F-test [24] and obtained results were compared with the results obtained from a reference method.[12]

Table 5: Analytical results of the determination of SOF and LDV in an authentic mixture using CZE method and reference methods

Ratio SOF:LDV % w/w	Taken (µg mL^{-1})	SOF		Reference method [12] Taken (40-500 µg mL^{-1})		LDV		Reference method [12] Taken (30-300 µg mL^{-1})	
		Found (µg mL^{-1})	% Recovery	Found (µg mL^{-1})	% Recovery	Found (µg mL^{-1})	% Recovery	Found (µg mL^{-1})	% Recovery
1:1	20:20	19.85	99.3	39.98	99.9	19.85	99.3	29.58	98.6
1:2	20:40	19.96	99.8	79.84	99.8	39.63	99.1	49.32	98.6
2:3	40:60	39.51	98.8	99.96	99.9	59.74	99.6	80.01	100.0
2:5	40:100	39.87	99.7	199.63	99.9	98.89	98.9	100.00	100.0
3:4	60:80	59.99	99.9	298.87	99.6	79.54	99.4	199.65	99.8
5:7	100:140	100.00	100.0	489.87	97.9	139.12	99.4	299.84	99.9
Mean ± SD		99.6±0.5		99.5±0.8		99.3±0.2		99.5±0.7	
n		6		6		6		6	
Variance		0.25		0.64		0.04		0.49	
*%SE		0.20		0.32		0.08		0.29	
t-test		0.264 (2.228)**							
F-test		2.56 (5.05)**							

** % SE= SD/$\sqrt{n}$ * Figures in parentheses are the tabulated values of t-and F- testes at 95% confidence limit*[24]

The excellent results encouraged the detection of the selected drugs in dosage forms. **Tables 6** listed the percentage recoveries in pharmaceutical formulations as 99.4±0.9 and 99.6±0.4 for SOF and LDV, respectively.

Table 6: Analytical results of the determination of SOF and LDV in dosage forms using CZE method and reference method [12]

Sample	Taken (µg mL^{-1})	SOF		Reference method [12] Taken (40-500 µg mL^{-1})		LDV		Reference method [12] Taken (30-300 µg mL^{-1})	
		Found (µg mL^{-1})	% Recovery	Found (µg mL^{-1})	% Recovery	Found (µg mL^{-1})	% Recovery	Found (µg mL^{-1})	% Recovery
Harvoni® 90:400mg LDV/SOF /Tablet	400:90	400.00	100.00	39.19	97.9	90.00	100.00	29.58	98.6
		398.84	99.7	79.84	99.8	89.85	99.8	49.87	99.7
		389.98	97.5	99.49	99.5	89.74	99.7	79.56	99.5
		400.00	100.0	199.23	99.6	89.14	99.0	100.00	100.00
		399.21	99.8	298.67	99.6	89.92	99.5	199.63	99.8
		399.46	99.9	500.00	100.0	89.51	99.4	299.47	99.8
Mean ± SD		99.4±0.9		99.7±0.7		99.6±0.4		99.5±0.5	
n		6		6		6		6	
Variance		0.81		0.49		0.16		0.25	
*%SE		0.37		0.29		0.16		0.20	
t-test		0.638 (2.228)**				0.390 (2.228)**			
F-test		2.79 (5.05)**				1.56 (5.05)**			

**%SE= SD/$\sqrt{n}$ ** Figures in parentheses are the tabulated values of t-and F- testes at 95% confidence limit*[24]

CONCLUSION

In our study, we focused on the development of a new, simple and highly sensitive CZE method for the simultaneous separation and detection of two impact drugs SOF and LDV on hepatitis C infection. The separation conditions were optimized and excellent separation peak areas with high resolution and acceptable migration time was recorded. The developed method was applied to detect both drugs in their combined pharmaceutical form mixture. The method was validated to ensure its suitability, precision and accuracy by obeying ICH guidelines. The obtained results were of good agreements with those obtained from other previously reported method.

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