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ORIGINAL

RP-HPLC Method for Simultaneous Determination of Sofosbuvir and Ledipasvir in Tablet Dosage Form and Its Application to In Vitro Dissolution Studies

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Abstract A reversed-phase high-performance liquid chromatographic method was developed for the simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form. The analysis was performed on Luna analytical column 250 × 4.6 mm, 5 µm, octyl silica packing (Si–$[CH_2]_7$–CH_3) C8, using ammonium acetate buffer solution pH 7.0 and acetonitrile 35:65 % v/v as mobile phase at flow rate of 0.7 mL min^{-1} for isocratic elution. Detection of sofosbuvir and ledipasvir was performed on a UV detector at 245 nm. The retention times of sofosbuvir and ledipasvir were 4.468 ± 0.013 min and 8.242 ± 0.012 min, respectively, and the total run time was 20 min. The method was validated according to the requirements of the United States Pharmacopeia (category I). The overall recovery of both analytes was 100 ± 1 %; the relative standard deviation for precision and intraday precision was less than 2.0 %. The method was linear with correlation coefficient (r) >0.9999, limits of detection 0.485 and 0.175 µg mL^{-1}, and limits of quantification was 1.619 and 0.586 µg mL^{-1} for sofosbuvir and ledipasvir, respectively. The method was successfully applied to the assay and in vitro dissolution studies of sofosbuvir and ledipasvir in tablet dosage form.

Keywords Sofosbuvir · GS-7977 · Ledipasvir · RP-HPLC · In vitro dissolution

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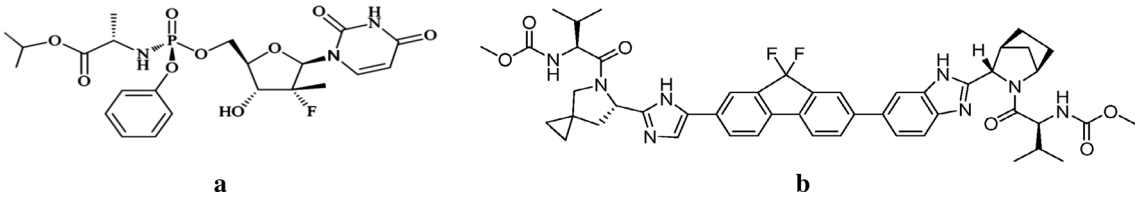
Introduction

Hepatitis C virus (HCV) infection is a significant public health concern, with approximately 170–180 million infected individuals worldwide [1, 2]. Patients infected with HCV are at risk of life-threatening complications and can lead to cirrhosis, decompensated liver disease (liver failure), hepatocellular carcinoma, and need for liver transplantation [3, 4]. Until the most recent developments of treatment options for six major genotypes and subclasses of HCV, different complicated standards of care (SOC) with suboptimal sustained virologic response (SVR) were available as different combinations of interferon, pegylated alpha interferon, ribavirin, telaprevir, and boceprevir; however, these were associated with different toxicities, poor tolerability, and adverse reactions [5–11]. Sofosbuvir (SOF), also known as GS-7977, is a nucleotide analogue polymerase inhibitor of non-structural protein (NS-5B) and is among recent, novel, potent, and safe promising prodrugs, having high SVR rates, used in combination with other medicinal products for the treatment of HCV infections [12–14]. Ledipasvir (LED), also known as GS-5885, is an NS-5A inhibitor and antiviral against HCV (genotypes 1a and 1b) [15, 16] that was approved by the US Food and Drug Administration (FDA) as a fixed-dose combination with SOF in 2014 for the treatment of chronic infection of HCV genotype 1 [17, 18].

SOF is white to off-white non-hygroscopic crystalline solid with the chemical formula $C_{22}H_{29}FN_3O_9P$ and its structure is shown in Fig. 1a. SOF belongs to biopharmaceutics classification system (BCS) class III, having pH-independent high solubility across a pH range from 1.2 to 7.7 and low apparent intestinal permeability. LED is a white to off-white or yellowish, slightly hygroscopic crystalline solid with the chemical formula $C_{49}H_{54}F_2N_8O_6$



a **b**

Fig. 1 **a** Sofosbuvir (GS-7977), **b** ledipasvir (GS-5885)

and its structure is shown in Fig. 1b. LED belongs to BCS class II and exhibits low pH-dependent solubility and high apparent permeability [19, 20].

Considering the BCS classification, solubility, and the influence of gastrointestinal pH on solubility of SOF and LED drug substances, in vitro dissolution studies of formulations containing both drugs are very important to check the release and solubilization of the active drug substance from the drug product and to predict the in vivo performance of different formulations [21–24].

Various methods are described in the literature using different analytical techniques for analysis of SOF and LED for clinical purposes, e.g., UPLC–ESI–MS/MS for SOF and GS-331007 in human plasma [25], SPE-LC for SOF in human plasma [26], UPLC–MS/MS for SOF, GS-331007, and ribavirin in rat plasma [27], and LC–MS/MS for SOF anabolites in cells [28]. To the best of our knowledge no reversed-phase high-performance liquid chromatography (RP HPLC)–UV method is available for the simultaneous determination of SOF and LED in dosage forms for in vitro dissolution studies.

In this study a simple, isocratic RP-HPLC method was developed for the simultaneous determination of SOF and LED in tablet dosage form for assay determination and in vitro dissolution studies. This method would help to quantify the drug content of SOF and LED in tablet dosage form and establish in vitro dissolution profile to predict the in vivo performance of the product. FDA specified conditions [29, 30] are used for in vitro dissolution and the method was validated according to the requirements of United States Pharmacopeia (USP) [31] and International Council for Harmonization (ICH) [32] guidelines.

Experimental

Materials and Chemicals

Generic products of fixed-dose combination SOF 400 mg + LED 90 mg tablets and excipients of tablets for placebo i.e., copovidone (USP), microcrystalline cellulose (USP), lactose monohydrate British Pharmacopeia (BP), magnesium stearate (BP), croscarmellose sodium (BP), polyvinyl alcohol (USP), colloidal anhydrous silica European Pharmacopeia (EP), titanium dioxide (USP), macrogol (EP), and purified talc (BP) were provided by Genome Pharmaceuticals (Pvt) Ltd. SOF and LED tartrate reference standards were obtained from Sigma-Aldrich (Germany); innovator product Harvoni (Gilead Sciences, Inc. USA) was purchased from a local pharmacy; methanol HPLC, acetonitrile HPLC, ammonium acetate, ammonia solution, butylated hydroxytoluene (BHT), polyoxyethylene sorbitan fatty acid esters (Polysorbate 80) were purchased from Sigma-Aldrich (Germany).

Instrumentation

The HPLC system comprised a Cecil Adept CE-4104 low pressure quaternary gradient pump, Adept CE 4200 variable wavelength UV detector (Cecil Instruments Limited UK), controlled by PowerStream chromatography manager software version 4.2. A Luna analytical column (Phenomenex USA) 250 × 4.6 mm, 5 µm, octyl silica packing (Si–$[CH_2]_7$–CH_3) C8, was used for analysis, and USP Dissolution Apparatus (Galvano Scientific Pak) was used for in vitro dissolution studies. A Shimadzu UV-1601 (Shimadzu Japan) double beam spectrophotometer with 1-cm quartz cell was used for optimization of wavelength absorbance. A Shimadzu AW220 electronic balance (Shimadzu Japan), SONOREX ultrasonic bath (Bandelin Germany), Millipore vacuum filtration assembly, and Milli-Q water distillation system (Millipore USA) were also used in this work.

Solution Preparation

Mobile Phase and Dissolution Medium

Ammonium acetate buffer pH 7.0 was prepared by dissolving 0.75 g of ammonium acetate in 400 mL purified water using a 500-mL volumetric flask. The pH was adjusted to pH 7.0 ± 0.5 with 1.0 % ammonia solution. Mobile phase was prepared by mixing ammonium acetate buffer pH 7.0 and acetonitrile in the ratio 35:65 % v/v and filtering through a 0.2-µm nylon membrane filter using a Millipore vacuum filtration assembly.

Potassium dihydrogen orthophosphate 0.01 M solution was prepared by dissolving 8.16 g in about 4.0 L purified water using a 6.0-L volumetric flask; 90 g of Polysorbate 80 was then added to this solution and dissolved by mechanical shaking and heating to 37 °C. The pH of this solution was adjusted to 6.0 ± 0.5 by using 0.0075 mg mL^{-1} solution of BHT, and the volume was adjusted to 6.0 L using purified water. This solution was used as dissolution medium for in vitro dissolution studies.

Reference and Sample Stock Solutions

The potency and purity factor of SOF and LED tartrate reference standards was adjusted; stock solutions were prepared by dissolving equivalent to 20.0 mg of SOF and 4.5 mg LED separately in 30 mL of acetonitrile in 50-mL amber-colored volumetric flasks and diluted to volume with ammonium acetate buffer pH 7.0.

Sample solution of tablet dosage form was prepared by dissolution of 10 tablets in 1 L dissolution medium. Ten milliliters of this solution was further diluted with mobile phase to a composite sample stock solution containing SOF 0.4 mg mL^{-1} and LED 0.09 mg mL^{-1} using 100-mL amber-colored volumetric flasks, and filtered through 0.45-µm nylon filter paper from Millipore.

The reference and sample stock solutions were stored at 2–8 °C, protected from light, and further diluted for composite and individual standards when required in method development, validation, and in vitro dissolution studies.

In Vitro Dissolution Studies

Dissolution parameters, i.e., USP dissolution apparatus type II (paddle type), at 75 rpm ± 4 % and 900 mL ± 1 % dissolution medium containing 1.5 % Polysorbate 80 in 10 mM potassium phosphate buffer with 0.0075 mg mL^{-1} BHT, pH 6.0 ± 0.5, were selected as per FDA guidelines [29] for in vitro dissolution. The medium was equilibrated at 37.0 ± 0.5 °C, and tablet samples were added to each dissolution bowl in series with a time gap of 2 min to manage sample collection as per the prescribed schedule at 5, 10, 15, 20, 30, 45, and 60 min. At specified time intervals 10 mL of sample was collected using a bent SS cannula from half way between the top of the medium and the top of the paddle, not less than 1 cm away from the wall of the bowl. The sampled volume was replaced with an equal volume of the dissolution medium to maintain a constant total volume. At the end of each test time, each sample aliquot was filtered through a 0.45-µm nylon filter (Millipore) and diluted to the required concentration using mobile phase as dilution medium. The content of SOF and LED dissolved was determined using the proposed method.

Method Development and Optimization

SOF and LED are UV-active compounds as a result of the conjugated group and benzene ring in their structures. SOF and LED show absorbance maximum at $\lambda = 261 \pm 1$ and 334 ± 1 nm, respectively, and the specific absorbance ($A = 1$ %) at the λ maximum is 178.5 ± 4 and 564.3 ± 5, respectively. To get the optimum wavelength for the simultaneous detection at a single wavelength of the UV detector, the absorbance of separate and composite reference solution containing SOF 80 µg mL^{-1} and LED 18 µg mL^{-1} was measured from 200 to 400 nm (Fig. 2). Thus, 245 nm was selected as the most suitable absorbance for both molecules at the same wavelength.

A set of conditions suitable for better resolution of both analytes was selected, considering the solubility and nature of each analyte, by systematic elution of different mobile phases at different flow rates over different stationary phases. Reference solutions of both analytes individual and composite were analyzed by isocratic elution of mobile phase with different ratios of acetonitrile and methanol with phosphate and acetate buffers over different columns, i.e., octyl silica (Si–$[CH_2]_7$–CH_3) C8, Athena phenyl (Si–$[CH_2]_n$–C_6H_5), and octadecyl silica (Si–$[CH_2]_{17}$–CH_3) C18 from different manufacturers. Combination of ammonium acetate buffer solution pH 7.0 and acetonitrile 35:65 % v/v at a flow rate of 0.7 mL min^{-1} over 150 mm × 4.6 cm, 5 µm Luna C8 afforded better resolution and symmetrical peaks for both analytes (Fig. 3a). Owing to better resolution, the retention time of each analyte was quite different, and they can easily be identified and quantified by comparing with individual reference solutions. The set of chromatographic conditions was further validated for as per USP and ICH guidelines.

Method Validation

The analytical method was properly validated according to the requirements of USP [31] (category 1) and ICH guidelines [32] for accuracy, precision, intermediate precision, and linearity. Specificity, limit of detection (LOD), and limit of quantification (LOQ) were established and evaluated.

Accuracy and Recovery

The accuracy of the analytical procedure was established by analyzing ($n = 3$) six concentrations covering a range from 120 to 20 % with known concentrations of SOF (96, 80, 64, 48, 32, and 16 µg mL^{-1}) and LED (21.6, 18, 14.4, 10.6, 7.2, and 3.6 µg mL^{-1}). The value of relative error (RE %) was checked against acceptance limits of ±2 % for recovery, and the value of relative standard deviation (RSD %) was evaluated against acceptable limits of ±2 % for accuracy.

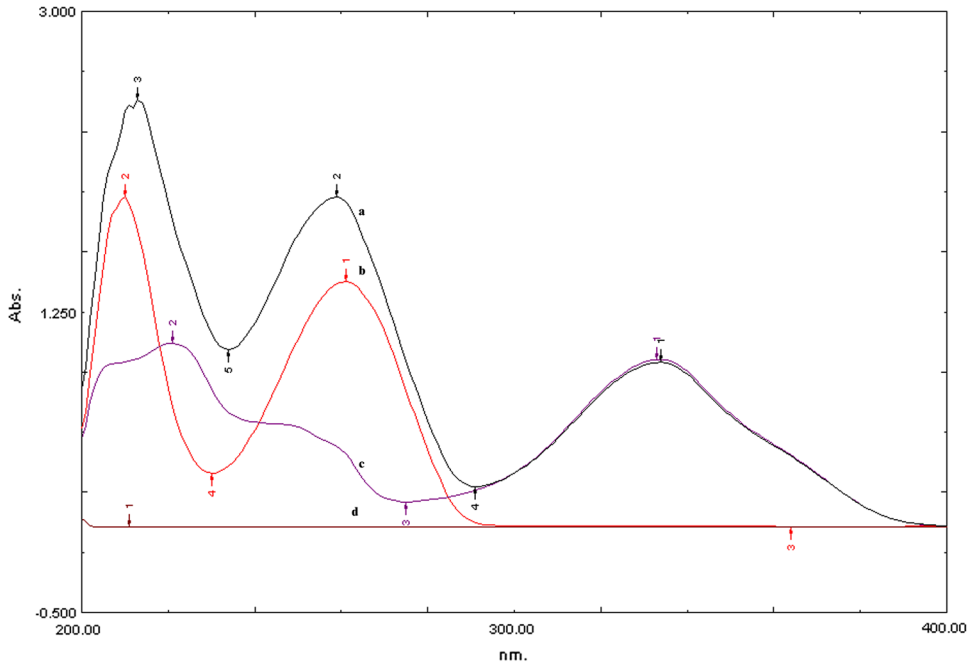


Fig. 2 *a* UV absorbance spectrum a SOF + LED composite sample, *b* SOF, *c* LED, *d* blank/placebo

Precision and Intermediate Precision

Repeatability and precision were assured by analyzing replicates ($n = 6$) of reference solution at high and low concentration (120 and 40 %) with known amounts of SOF (96, 32 µg mL^{-1}) and LED (21.6, 7.2 µg mL^{-1}). The overall RSD % for peak response analyzed on different days was checked against acceptable limits of ±2 % for precision and intermediate precision.

Robustness

Robustness of the method was checked by analyzing ($n = 6$) the solutions used for precision studies with small changes (±2 %) of the given values in dilution medium and mobile phase ratio. The quantitative influence of the variables was determined by evaluating the value of RSD % against acceptable limits of ±2 % for peak response and retention times of each analyte.

Linearity and Range

For linearity assessment seven concentrations ($n = 3$) covering the analysis range 120 to 10 % of assay with known concentrations of SOF (96, 80, 64, 48, 32, 16, 8 µg mL^{-1}) and LED (21.6, 18, 14.4, 10.6, 7.2, 3.6, 1.8 µg mL^{-1}) were analyzed. The peak response (A) was plotted on the Y-axis against concentration and plotted on the X-axis; the relation of concentration and response was evaluated on the basis of the least-square linear regression equation $A =$ slope $C + Y$ intercept, where A is peak area and C is the concentration. LOD and LOQ were calculated using the expression 3.3δ/slope and 10δ/slope, respectively.

Specificity and Placebo Interference

Specificity of the method is important to check interference of excipients and dissolution medium on the response of the drug substance. A composite solution of placebo was prepared from all excipients of tablets, i.e., except the active ingredient, in the same medium. This solution was analyzed using the same chromatographic conditions, and baseline was evaluated for peak response. Placebo interference was also ensured by spiking the reference solution with appropriate levels of excipients and evaluating for any interference or additional peak other than known peaks of SOF and LED.

Stability of Solution

LED is sensitive to light and degrades in solution upon exposure to light; therefore, all solutions were stored protected from light in amber-colored flasks. Stability of sample solutions was assessed by analyzing three concentrations, i.e., 100, 60, and 20 %, containing known concentrations of SOF (80, 48, 16 µg mL^{-1}) and LED (18, 10.6, 3.6 µg mL^{-1}), respectively. Replicates ($n = 3$) were exposed to different conditions, i.e., room temperature (15–25 °C) for 7 days, and cool temperature (2–8 °C) 14 days. Results were evaluated by comparing with assays of freshly prepared solutions of reference standards.

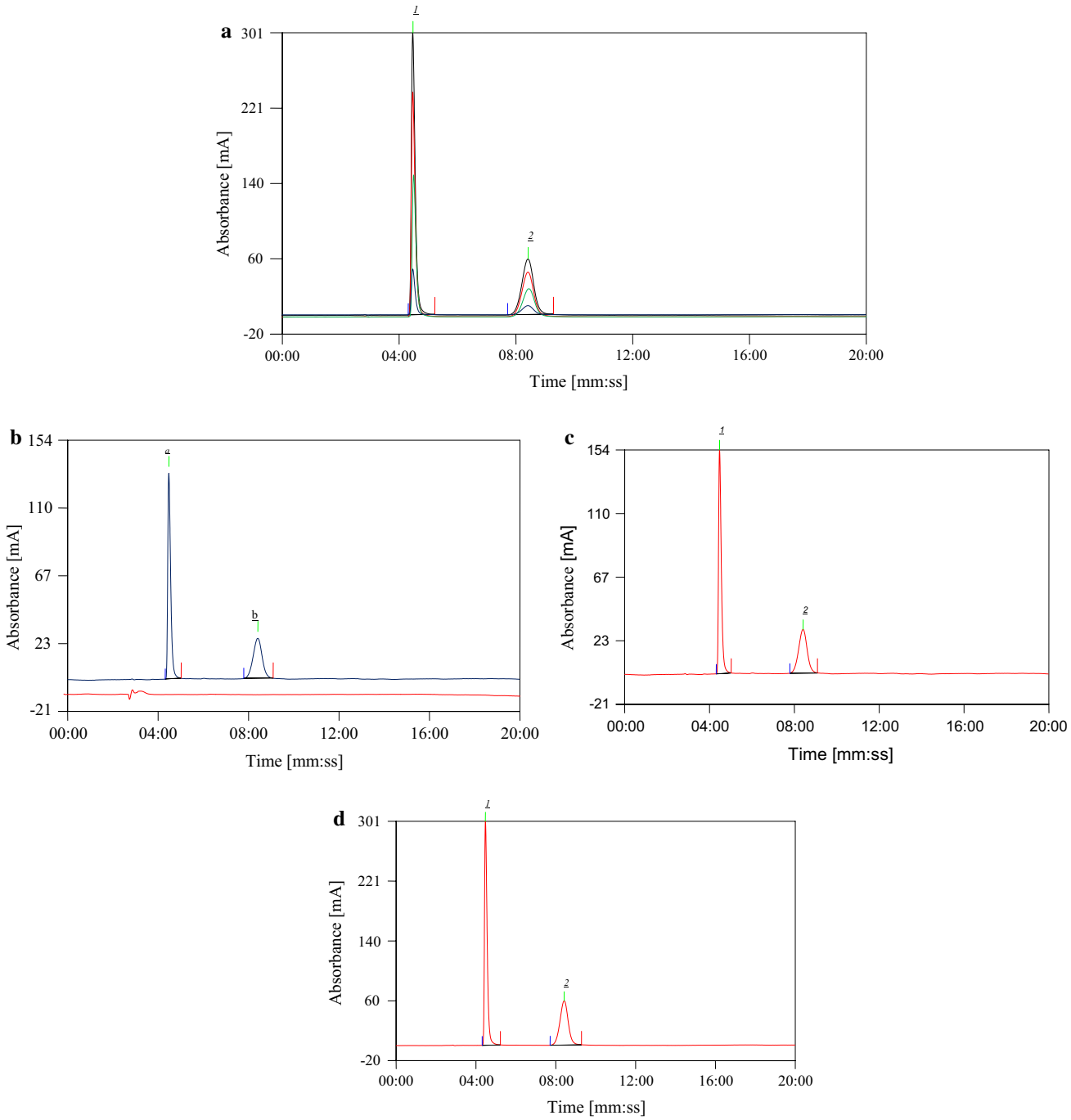


Fig. 3 **a** Chromatogram of SOF + LED recovery studies at four concentrations: 20, 60, 100, and 120 %. **b** Chromatogram of SOF + LED composite reference solution *a* SOF, *b* LED, and *c* blank/placebo. **c** Chromatogram of SOF + LED tablet (assay sample). **d** Chromatogram of SOF + LED tablet (dissolution sample)

Results and Discussion

System Suitability

A set of optimized conditions, i.e., combination of ammonium acetate buffer solution pH 7.0 and acetonitrile 35:65 % v/v as mobile phase at a flow rate of 0.7 mL min^{-1} over a Luna C8 150 mm × 4.6 cm, 5 µm, was selected and system suitability was assessed according to USP guidelines. Statistical data of different parameters like peak area (*A*), retention time (tR), RSD, theoretical plates (*N*), symmetry factor (As), mass distribution ratio (*K*), and resolution of SOF and

Table 1 Results of system suitability parameters

Parameters	SOF	LED
Peak area (*A*)	2330.20 ± 11.2	1459.93 ± 11.04
Relative standard deviation (RSD)	0.481 %	0.756 %
Retention time (tR)	4.468 ± 0.013	8.242 ± 0.012
Relative retention time (tRR)	–	1.845 ± 0.005
Theoretical plates (*N*)	6135.18 ± 211.037	2376.81 ± 124.15
Symmetry factor (As)	1.143 ± 0.015	1.025 ± 0.008
Capacity factor (*K*)	2.723 ± 0.008	7.242 ± 0.012
Resolution (Rs)	–	7.413 ± 0.282

LED were calculated for peak response by PowerStream chromatography manager software version 4.2. The results (Table 1) showed that all the performance parameters of the analytical method comply with USP requirements for system suitability. The RSD for *A* and tR of both analytes was less than 2.0 %, resolution of LED as compared to SOF was more than 2.0, tailing factor (As) was less than 2.0, capacity factor (*K*) was 2–10, and the number of theoretical plates was more than 2000. The method was suitable for simultaneous analysis of LED and SOF and successfully applied for determination of both analytes in tablet dosage form and comparative dissolution studies.

Method Validation

Accuracy and Recovery

Results of the six concentrations ranging from 120 to 20 % analyzed ($n = 3$) for recovery studies are shown in Table 2. The overall recovery of SOF and LED was 100 ± 1 % at each concentration, and the RSD % and RE % of recovery studies were less than 2.0 %. The results show that the method is accurate and suitable for assay of SOF and LED in tablets and in vitro dissolution studies.

Precision and Intermediate Precision

The results (Table 3) for precision and intermediate precision studies show that the RSD for peak response of both analytes at 20 and 120 % concentrations, analyzed replicates ($n = 6$), on different days is at most 2.0 %. The results indicate that the given method is precise and repeatable within the acceptable limits and criteria.

Robustness

The results (Table 4) show that minor changes ±2 % of the given values in chromatographic conditions do not influence the results for SOF and LED. The value of RSD for replicates ($n = 6$) at each concentration (i.e., 20 and 120 %)

Table 2 Results of recovery studies

Concentration (%)	SOF			LED		
	Recovery %	RSD %	RE %	Recovery %	RSD %	RE %
120	99.67 ± 0.40	0.4	0.33	99.88 ± 0.69	0.69	0.12
100	100.05 ± 0.65	0.65	−0.05	99.76 ± 0.59	0.59	0.24
80	99.39 ± 0.55	0.55	0.61	99.04 ± 0.78	0.78	0.96
60	99.71 ± 0.58	0.58	0.29	99.19 ± 0.54	0.54	0.81
40	99.31 ± 0.41	0.41	0.69	100.08 ± 0.4	0.4	−0.08
20	98.95 ± 0.61	0.62	1.05	99.33 ± 0.51	0.51	0.67

Table 3 Results of precision and intermediate precision

Sample/days	SOF				LED			
	20 % (16 µg/mL)		120 % (96 µg/mL)		120 % (21.6 µg/mL)		20 % (3.2 µg/mL)	
	A	tR	*A*	tR	*A*	tR	*A*	tR
Day 1	464.8 ± 4.4	4.45 ± 0.02	2799.6 ± 18.7	290.3 ± 1.9	8.25 ± 0.02	4.44 ± 0.03	1741.4 ± 16.6	8.24 ± 0.02
RSD	0.943 %	0.517 %	0.669 %	0.678 %	0.206 %	0.563 %	0.955 %	0.23 %
Day 2	454.2 ± 2.9	4.43 ± 0.01	2810.5 ± 20.4	282.0 ± 3.4	8.26 ± 0.01	4.44 ± 0.02	1748.9 ± 17.01	8.26 ± 0.03
RSD	0.648 %	0.271 %	0.727 %	1.21 %	0.169 %	0.541 %	0.975 %	0.279 %
Day 3	471.9 ± 5.2	4.47 ± 0.02	2792.6 ± 24.4	288.1 ± 5	8.25 ± 0.021	4.452 ± 0.025	1727.2 ± 8.5	8.248 ± 0.03
RSD	1.092 %	0.358 %	0.872	1.729 %	0.255 %	0.562	0.492	0.339

Table 4 Results of robustness studies

Analyte	Conc. (µg/mL)	*A*	RSD %	tR	RSD %
SOF	6.0	491.6 ± 7.4	1.497	4.51 ± 0.02	0.421
	96.0	2819.2 ± 25.2	0.896	4.51 ± 0.03	0.665
LED	3.2	300.4 ± 3.1	1.01	8.37 ± 0.03	0.37
	21.6	1756.4 ± 20.5	1.167	8.31 ± 0.05	0.566

Table 5 Statistical data derived from calibration curve

	SOF	LED
Correlation *r*	0.9999	0.9999
Slope	29.145	80.524
Intercept	−5.495	2.672
SD	4.728	5.915
LOD (µg/mL)	0.485	0.175
LOQ (µg/mL)	1.619	0.586

of at most 2.0 % indicates that the method is robust and suitable for routine analysis assay and dissolution of SOF and LED in tablet dosage form.

Linearity

Results (Table 5) for the statistical data derived from linearity studies showed a good correlation between concentration and peak response. The linear regression equations for linearity of SOF and LED were $A = 29.145C - 5.495$ and $A = 80.524C + 2.672$, respectively, and the correlation coefficient for both SOF and LED was $r = 0.9999$. The LODs were 0.485 and 0.175 µg mL^{-1} and the LOQs were 1.619 and 0.586 µg mL^{-1} for SOF and LED, respectively.

Specificity and Placebo Interference

There is no significant peak of placebo (Fig. 3b) at the given retention time, and the influence of tablet excipients on the peaks of SOF and LED at under the given chromatographic conditions is negligible. The results show that the proposed method is specific for quantification of SOF and LED in tablet dissolution medium.

Stability of Solution

Stability of SOF and LED in solution was investigated and summarized in Table 6; the results show that the solutions are stable for 7 days at room temperature (15–25 °C) and for 14 days at cool temperature (2–8 °C) stored in amber-colored flasks protected from light.

Table 6 Results of stability studies

Analytes	Concentration analyzed (µg/mL)	Concentration recovered %	
		15–25 °C, 7 days	2–8 °C, 14 days
SOF	16	100.18 ± 0.69	99.68 ± 0.41
	48	99.53 ± 0.2	99.48 ± 0.76
	80	98.79 ± 0.73	99.59 ± 0.31
LED	3.6	99.26 ± 0.54	99.5 ± 0.4
	10.6	99.44 ± 0.47	99.55 ± 0.09
	18	99.5 ± 0.23	99.83 ± 0.48

Application of Method and In Vitro Dissolution of Tablets

The method was successfully applied to the quantitative determination of SOF and LED in tablet dosage form (Fig. 3c) and dissolution samples (Fig. 3d). The recovery of SOF and LED was ensured by recovery studies on six concentrations covering a range from 120 to 20 % (Table 2). For recovery of SOF and LED in dissolution samples, comparative dissolution studies were performed on two different generic brands A and B compared with innovator brand Harvoni tablets using the same conditions for dissolution as described by the FDA [29]; the results are summarized in Table 7. The amount of SOF and LED dissolved in medium at physiological conditions was determined using the same validated method. To obtain the in vitro dissolution profile of the product, the cumulative percentage of SOF and LED released was plotted against time (Fig. 4).

According to the graphical presentation and statistical evaluation of results, the dissolution of both generic brands showed similar behavior to the innovator brand. All the brands met the USP criteria ($Q = 80$ %) in 30 min for dissolution.

Conclusions

In vitro dissolution studies of pharmaceutical dosage form are very important and a vital criterion for the product quality control. In vitro dissolution is used to evaluate the release and solubilization of the active drug substance from the drug product and to predict correctly the delivery of the required drug substance to the patients. In this context, it is important to have an accurate and precise RP-HPLC analytical method to quantify the amount of drug substance in dissolution medium simultaneously. The proposed RP-HPLC analytical method was successfully validated according to the requirements of USP and ICH guidelines for validation of analytical procedure (category I). All the results of the

Table 7 Results of comparative dissolution studies

Time	Generic brand A		Generic brand B		Innovator brand	
	SOF	LED	SOF	LED	SOF	LED
5	66.95 ± 1.86	58.94 ± 1.8	77.82 ± 1.82	62.17 ± 1.6	82.72 ± 1.24	69.44 ± 2.2
10	80.75 ± 1.87	77.5 ± 1.25	86.54 ± 1.54	83.57 ± 1.63	93.93 ± 1.53	87.98 ± 1.07
20	93.13 ± 1.66	95.91 ± 0.75	96.28 ± 1.45	98.38 ± 0.65	99.39 ± 1.21	99.82 ± 0.91
30	98.63 ± 1.93	99.63 ± 0.85	98.27 ± 0.83	99.72 ± 0.58	100.14 ± 1.71	99.61 ± 0.62
45	99.58 ± 1.05	99.34 ± 0.53	98.49 ± 2.64	100.03 ± 0.59	99.86 ± 0.61	99.58 ± 0.58
60	99.81 ± 1.49	99.16 ± 0.44	98.78 ± 1.1	99.76 ± 0.88	99.25 ± 1.3	98.91 ± 1.33

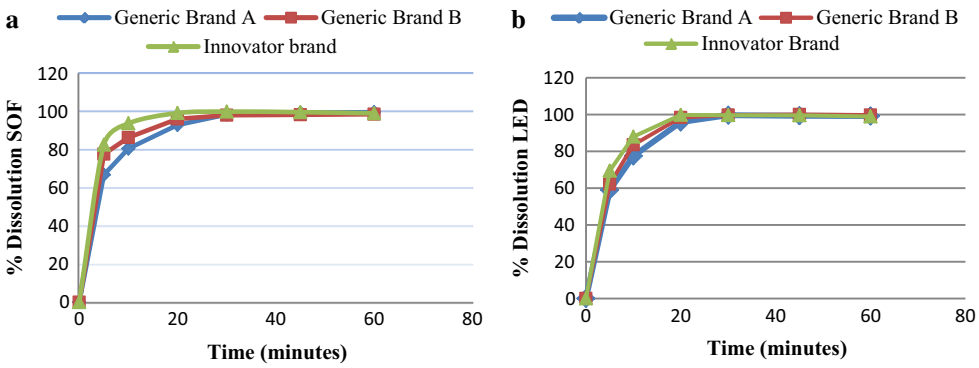


Fig. 4 Dissolution profile of **a** SOF and **b** LED

tests carried out for the validation of the method are in complete agreement with the required limits and criteria. The validated method was successfully applied to the simultaneous determination of SOF and LED in their dosage form and the simultaneous determination of dissolution profiles of the tablet dosage form, using USP apparatus II. It is concluded that the method is accurate, precise, linear, and specific for the simultaneous determination of SOF and LED and can be applied to routine quality control analysis of SOF and LED dosage form in vitro dissolution and comparative dissolution studies for development of new formulations.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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