How to Cite:
Mujawar, T., Imran, M., Bairagi, V., Mahajan, K., Pulate, C., & Ahmad, S. (2022). Development and validation for the simultaneous estimation of Sofosbuvir and Ledipasvir by UV spectrophotometer method in bulk and tablet dosage forms. *International Journal of Health Sciences*, *6*(S6), 6343–6357. https://doi.org/10.53730/ijhs.v6nS6.11840

Development and validation for the simultaneous estimation of Sofosbuvir and Ledipasvir by UV spectrophotometer method in bulk and tablet dosage forms

Dr. Tabrej Mujawar
Department of Pharmacology, Gangamai College of Pharmacy, Nagaon, Dist. Dhule, Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India
Email: tabrej_27@yahoo.com

Dr. Md. Imran
Department of Quality Assurance, K. B. H. S. S. Trust's Institute of Pharmacy, Malegaon Camp, Nashik - 423105, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India
Email: imransa99@gmail.com

Dr. Vinod Bairagi
Department of Pharmacology, K. B. H. S. S. Trust's Institute of Pharmacy, Malegaon Camp, Nashik - 423105, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India
Email: kovin12@gmail.com

Dr. Kiran Mahajan
Department of Pharmaceutics, Sharadchandra Pawar College of Pharmacy, Otur, Tal. Junnar, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India
Email: kirancmahajan@gmail.com

Chetan Pulate
Department of Pharmaceutical Chemistry, Sharadchandra Pawar College of Pharmacy, Otur, Tal. Junnar, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India
Email: pulatechetan2222@gmail.com

Dr. Sufiyan Ahmad
Department of Quality Assurance, Gangamai College of Pharmacy, Nagaon, Dist. Dhule, Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India
*Corresponding author email: sufipharm1712@gmail.com

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
Manuscript submitted: 9 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 27 July 2022

***Abstract*---**The day-by-day new combinations drugs are being introduced in market. Then the multiple therapeutic agents which acts at different sites are used in the management of various diseases and disorders are done. Thus it is necessary to develop methods for analysis with the help of number of analytical techniques which are available for the estimation of the drugs in combination. An accurate, precise and reproducible UV spectrophotometer method was developed for the simultaneous quantitative determination of Sofosbuvir and Ledipasvir in tablet dosage forms. Methods: Younglin (S. K.) gradient system UV detector and C_{18} column with 250 mm x 4.6 mm i. d. and 5µm particle size Acetonitrile: OPA water (80: 20v/v) pH 2.5 was used as the mobile phase for the method. The detection wavelength was 283 nm and flow rate was 0.9 ml/min. Results: In the developed method, the retention time of Sofosbuvir and Ledipasvir were found to be 6.366 min and 8.616 min. The developed method was validated according to the ICH guidelines. Conclusion: In this methods linearity, precision, range, robustness were observed. The method was found to be simple, accurate, precise, economic and reproducible. So the proposed methods can be used for the routine quality control analysis of Sofosbuvir and Ledipasvir in bulk drug as well as in formulations.

***Keywords*---**sofosbuvir, ledipasvir, method- development, validation, HPLC.

Introduction

Pharmaceutical Analysis plays a vital role in quality assurance and quality control of bulk drugs and their formulations. Pharmaceutical analysis is a particular branch of analytical chemistry, which includes isolating, identifying and determining the relative amounts of compounds in a sample matter. It is concerned with chemical characterization of matter both quantitative and qualitative. In recent years many analytical techniques have been developed. Analytical method is a particular utilization of a procedure to solve a problem. Analytical instrumentation assumes an imperative part in the production and evaluation of new products and protection of Consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, medications, water and air.

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation. Globally, 130-150 millions of people have chronic hepatitis C infection. A significant number of those who are chronically infected will develop liver cirrhosis or liver cancer. Gilead Sciences overcome most common related liver diseases by its

Great invention (Harvoni). Harvoni (90 mg ledipasvir/400 mg sofosbuvir) approved by United States FDA. (Harvoni, 2016; Gilead Files, 2014) [1-2]. Chronic hepatitis C virus (HCV) infection is one of the most common etiologies of liver-related mortality throughout the world. Among the six HCV genotypes, genotype 1 was significantly more aggressive Among the available treatments for HCV genotype 1, the combination therapy of ledipasvir/sofosbuvir provides several advantages compared to other regimens, including use of a single-pill regimen, possibility to shorten the duration of treatment to 8 weeks, efficacy in patients exposed to protease inhibitors, safety in decompensated cirrhosis, and potential to avoid ribavirin [3-5]. Sofosbuvir and ledipasvir are inhobits HCV NS5B and HCV NS5A polymerase respectively. A fixed-dose combination of sofosbuvir–ledipasvir was approved in 2014 for treatment of patients chronically infected with genotype 1HCV[6].

Sofosbuvir (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-3-hydroxy-4-methyltetrahydrofuran-2-yl) methoxy)-(phenoxy) phosphorylamino) propanoate . It has a molecular formula of $C_{22}H_{29}FN_3O_9P$ and a molecular weight of 529.45 (Figure 1). Sofosbuvir is a 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 [7]. Sofosbuvir is a pangenotypic inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is essential for viral replication [8]. Sofosbuvir is a nucleotide prodrug that undergoes intracellular activation to form GS-461203 (active triphosphate, not detected in plasma), and ultimately the inactive, renally eliminated metabolite GS-331007 [9]. The pharmacologically active uridine analog triphosphate (GS-461203) can be incorporated by HCV NS5B and acts as a chain terminator. In a biochemical assay, GS-461203 inhibits the polymerase activity of the recombinant NS5B from HCV genotype 1b, 2a, 3a and 4a with an IC50 value ranging from 0.7 to 2.6 μM. GS-461203 is neither an inhibitor of human DNA and RNA polymerases nor an inhibitor of mitochondrial RNA polymerase [10]. Sofosbuvir/Ledipasvir is a fixed-dose combination (FDC) tablet containing Sofosbuvir (a previously approved NS5B polymerase inhibitor) and ledipasvir, a new NS5A-inhibitor [7].

Ledipasvir (LDV); is chemically known as Methyl [(2S)-1-{(6S)-6-[5-(9,9-difluoro-7-{2-[(1R,3S,4S)-2-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}-2-azabicyclo[2.2.1] hept-3-yl]-1H-benzimidazol-6-yl}-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-azaspiro[2.4] hept-5-yl}-3-methyl-1-oxobutan-2-yl] carbamate. It has a molecular formula of $C_{49}H_{54}F_2N_8O_6$ and a molecular weight of 889.00 (Figure 2). 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 ledipasvir is 3.8 and the pKa1 is 4.0 and pKa2 is 5.0 [7]. Ledipasvir is an HCV inhibitor targeting the HCV NS5A protein, which is essential for both RNA replication and the assembly of HCV virions. Biochemical confirmation of NS5A inhibition of ledipasvir is not currently possible as NS5A has no enzymatic function. In vitro resistance selection and cross-resistance studies indicate ledipasvir targets NS5A as its mode of action [10]. The combination of these two drugs is not official in any pharmacopoeia [11-12]. Very 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 have been determined by HPLC [13-14]. SOF's disposition was characterized into various in vivo cell types [15].

Sofosbuvir in human plasma was determined by UPLC-MS/MS method [16]. Quantification of Sofosbuvir and its metabolite, GS-331007, in human plasma has been determined by UPLC-ESI-MS/MS method [17]. Simultaneous quantification of ribavirin, sofosbuvir and its metabolite in rat plasma by UPLC-MS/MS has been reported [18]. SOF in pure form [19], in bulk and tablet dosage form was determined by RP-HPLC [20]. Finally, sofosbuvir (SOF) was used as an internal standard (IS) in an UPLC-MS/MS method for the determination of daclatasvir (DAC) in human plasma [21]. While for LDV, only two methods have been published for its individual determination in bulk drug form by simple UV spectrophotometry [22] and by RP-HPLC [23]. Both sofosbuvir and ledipasvir in human plasma were determined by UPLC-MS/MS method [24] and besides some antiviral agents [25]. Ledipasvir, sofosbuvir and its metabolite in rat plasma were also, determined by UPLC-MS/MS [26].

According to the best of our knowledge, only three HPLC methods [27-28] have been published, during the preparation of the present work for publishing. The present study aimed to develop a simple, sensitive, short retention time and accurate RP-HPLC method for the simultaneous determination of both sofosbuvir and ledipasvir together in pure and tablet dosage forms with high sensitivity, selectivity that can be used for the routine analysis of production samples.

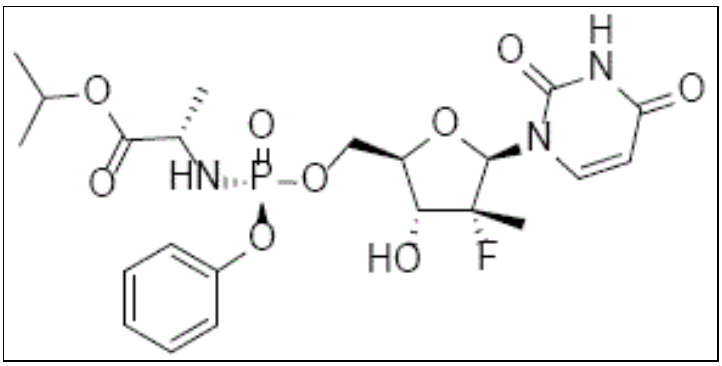


Fig. 1. Structure of Sofosbuvir

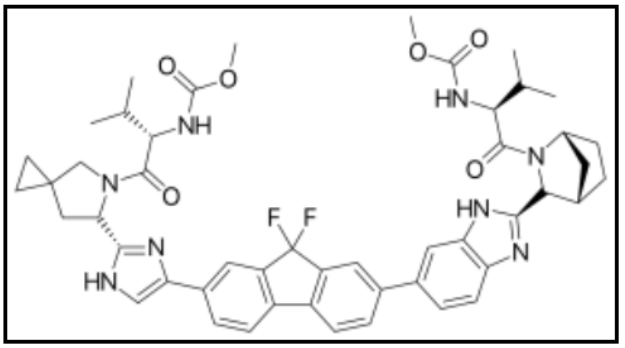


Fig. 2. Structure of Ledipasvir

Materials and Methods

Materials and Reagents

The analysis of the drug was carried out on Youngline (S. K.) Gradient System UV Detector. Equipped with reverse phase (Grace) C_{18} column (4.6mm x 250mm; 5µm), a SP930D pump, a 20µl injection loop and UV730D Absorbance detector and running autochro-3000 software. Sofosbuvir and Ledipasvir were procured from R.S.I.T.C Jalgaon. Orthophopsphoric acid (OPA) (Avantor Performance material India Ltd. Thane, Maharashtra) and methanol, acetonitrile, (HPLC grade Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.), water, 0.45 µm filter (Millipore, Bangalore). A combination of Sofosbuvir (400 mg) and Ledipasvir (90 mg) in tablet formulation was procured from Hetero drugs Ltd. Mumbai (Ledifos brand).

Chromatographic Conditions

Column C_{18} (250 mm× 4.6 mm); particle size packing 5µm ; detection wavelength of 283 nm; flow rate 0.9 ml/min; temperature ambient; sample size 20 µl; mobile phase Acetonitrile: water (OPA 0.1% PH 2.5 with TEA) (80:20); run time of 12 mins.

Preparation of standard stock solution

40 mg of Sofosbuvir and 10 mg of Ledipasvir were weighed accurately and transferred to a 10 ml volumetric flask dissolved in methanol and diluted to 10 ml with the mobile phase Acetonitrile + 0.1% OPA water with TEA(80 + 20% v/v) to give a stock solution of 4000 µg/ml Sofosbuvir and 1000 µg/ml Ledipasvir (Table 1 and Fig. 3).

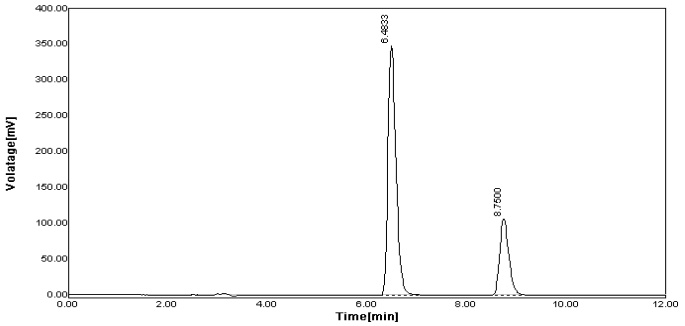


Fig. 3. Chromatogram of standard combination of Sofosbuvir and Ledipasvir

Table 1
Details of chromatogram of standard combination containing Sofosbuvir and Ledipasvir

Sr. No.	Name of drug	RT[min]	Area [mV*s]	Area%	TP	TF	Resolution
1	Sofosbuvir	6.483	3883.2092	73.98	7934.8	1.333	0.0000
2	Ledipasvir	8.7500	1365.7129	26.02	10613.9	1.2273	9.7647
Sum			5248.9219				

Method development and validation

Serial dilutions were done to prepared various concentration stock (Standard solution and diluted to get required concentration for calibration plot and which was injected [29-30].

Assay preparation for commercial formulation

For analysis of the tablet dosage form, weigh 20 Sofosbuvir and Ledipasvir combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 12.2 mg Sofosbuvir and Ledipasvir into 10 ml volumetric flask. Add about 10ml ACN of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm nylon membrane filter. Then volume was made up to the mark with Acetonitrile + 0.1% OPA water with TEA(80 + 20% v/v). The simple chromatogram of test Sofosbuvir and Ledipasvir shown in (Fig. 4). The amounts of Sofosbuvir and Ledipasvir per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for % Label claim for % RSD Calculated, Result was shown in (Table 2).

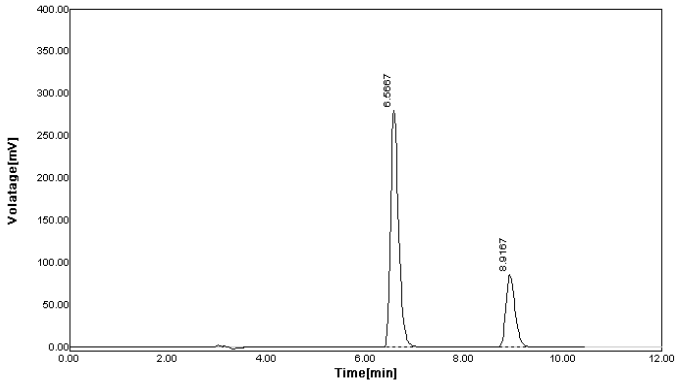


Fig. 4. Chromatogram for Marketed Formulation

Table 2
Analysis of marketed formulation

Assay	Drug	Label claimed	Amt. Found	% Label claim	SD	%RSD
RP-HPLC Method	SOFO	80	80.31	100.39	0.02	0.01
	LEDI	20	20.00	100.00	0.01	0.01
	SOFO	80	80.28	100.35	0.28	0.01
	LEDI	20	19.99	99.95	0.00	0.01

Results

Linearity and Range

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 20-100µg/mL for Sofosbuvir and 5-25µg/mL for Ledipasvir (Table 3 and 4) depict the calibration data of Sofosbuvir and Ledipasvir. The respective linear equation for Sofosbuvir was y = 38.01x + 80.60andLedipasvir equation y = 54.47x +7.385where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Sofosbuvir and Ledipasviris depicted in (Fig. 5 and 6).

Table 3
Linearity data for Sofosbuvir

Method	Conc. µg/ml	Peak area (µV.sec)		Average peak area (µV.sec)	S. D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	20	849.7955	850.6942	850.24	0.64	0.07
	40	1598.8525	1599.3652	1599.109	0.36	0.02
	60	2340.5071	2380.40	2339.454	1.49	0.06
	80	3132.3569	3135.1005	3133.729	1.94	006
	100	3883.2092	3885.1035	3885.156	1.34	0.03

	Equation	y = 38.011x + 80.60
	R^2	0.999

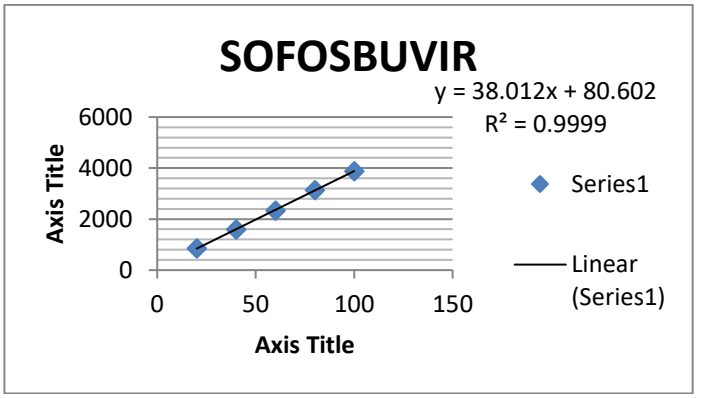
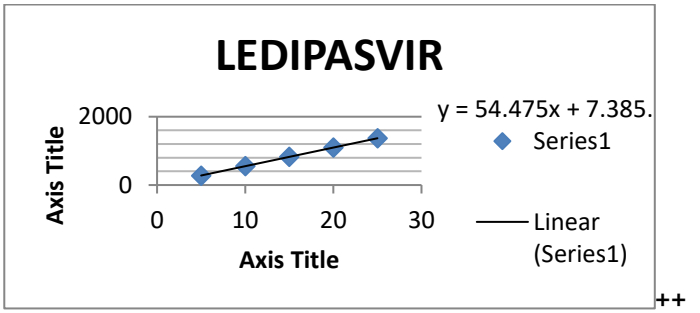


Fig. 5. Calibration curve of Sofosbuvir

Table 4
Linearity data for Ledipasvir

Method	Conc. µg/ml	Peak area (µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	5	276.2312	277.2356	276.7334	0.71	0.26
	10	552.436	552.213	552.3245	0.16	0.03
	15	829.4583	830.2341	829.8462	0.55	0.07
	20	1097.2723	1098.1311	1097.702	0.61	0.06
	25	1365.7129	1365.1014	1365.907	0.27	0.02
	Equation		y = 54.47x + 7.385			
	R^2		0.999			



++

Fig. 6. Calibration curve of Ledipasvir

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To a pre-analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed**.** The % recovery

was found to be within 98-101% (Table 5 and 6). Statistical validation of recovery studies are shown in (Table 5, 6 and Fig. 7, 8 and 9).

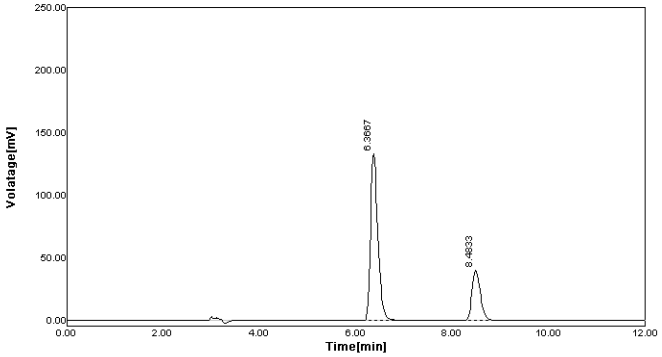


Fig. 7. Chromatogram of Accuracy 80%

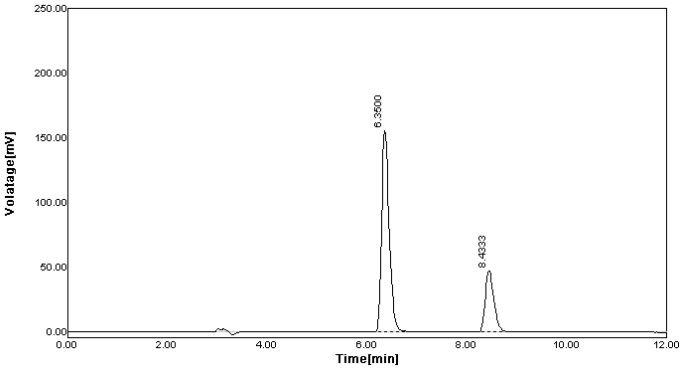


Fig. 8. Chromatogram of Accuracy 100%

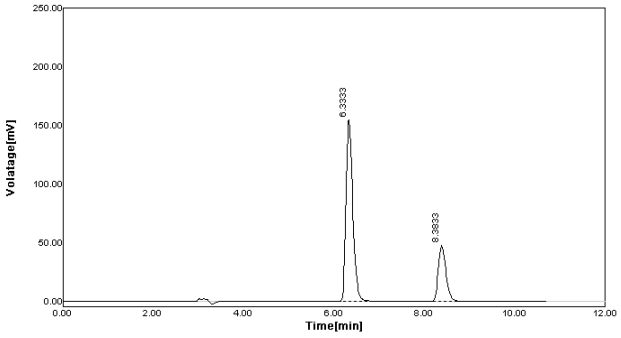


Fig. 9. Chromatogram of Accuracy 120%

Table 5
Result of Recovery data for Sofosbuvir and Ledipasvir

Method	Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RP-HPLC Method	SOFO	80%	20	16	1453.68± 0.06	16.12±0.06	100.77±0.34
		100%	20	20	1604.77± 0.02	20.58±0.02	100.50±0.09
		120%	20	24	1755.25±0.03	24.05±0.03	101.58±0.11
	LEDI	80%	5	4	496.06±0.01	3.97±0.01	99.46±0.12
		100%	5	5	547.8± 0.06	4.91±0.06	98.42±1.33
		120%	5	6	604.93±0.01	5.97±0.01	101.58±0.18

*mean of each 3 reading for RP-HPLC method

Table 6
Statistical validation of recovery studies Sofosbuvir and Ledipasvir

Method	Level of Recovery (%)	Drug	Mean % Recovery	S. D.*	% RSD
RP-HPLC Method	80%	SOFO	100.77	0.34	0.34
		LEDI	99.46	0.12	0.12
	100%	SOFO	100.50	0.09	0.09
		LEDI	98.42	1.33	1.35
	120%	SOFO	101.58	0.11	0.11
		LEDI	101.58	0.18	0.18

*Denotes average of three determinations for RP-HPLC

System suitability parameters

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Sofosbuvir and Ledipasvir system suitability parameters were studied. The result shown in below (Fig. 10 and Table 7).

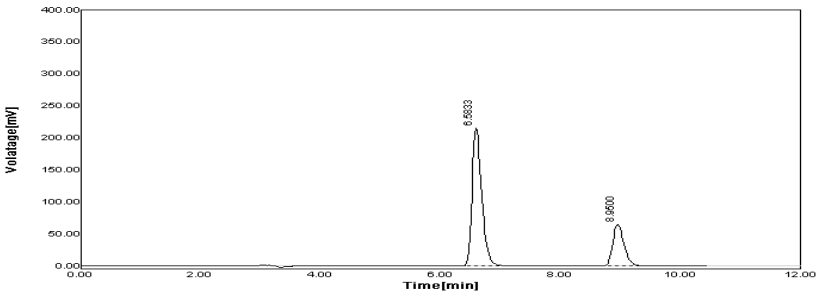


Fig. 10. Chromatogram of System suitability -1

Table 7
Repeatability studies on RP-HPLC for Sofosbuvir and Ledipasvir

Method	Conc. of SOFO and LEDI (mg/ml)	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method for SOFO	60	1419.4238	61.52	102.53
	60	2418.9814		
		Mean	61.52	
		SD	0.31	
		%RSD	0.01	
RP-HPLC Method for LEDI	15	828.8961	15.09	100.60
	15	830.4302		
		Mean	1.08	
		SD	0.13	
		%RSD		

Precision

The method was established by analyzing various standards of Sofosbuvir and Ledipasvir. All the solution were analyzed thrice in order to record any intra-day & inter-day variation in the result. The result obtained for interday and intraday variation are shown in the (Table 8 and Fig. 11).

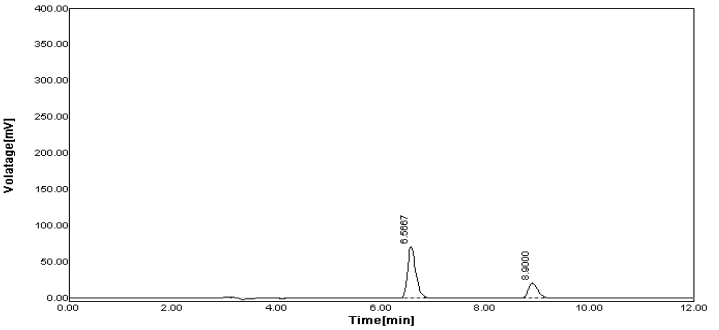


Fig. 11. Chromatogram of Precision

Table 8
Result of Intra day and Inter day Precision studies on RP-HPLC method for Sofosbuvir and Ledipasvir

Method	Drug	Conc. (µg/ml)	Intraday Precision		Intraday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
Rp-HPLC Method	SOFO	20	849.39±1.52	101.40	846.66±1.93	100.75
		60	2340.47±1.11	99.53	24.08.27±9.80	102.05
		100	3794.04±67.83	97.69	3882.20±1.48	100.01
	LEDI	5	278.54±0.81	99.56	279.08±0.96	98.00
		15	828.83±1.01	100.53	830.67±0.50	100.73

		25	1356.12±0.15	99.04	1356.46±0.91	99.04

*Mean of each 3 reading for RP-HPLC method

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The results indicate that less variability in retention time and tailing factor were observed (Table 9 and 10).

Table 9
Result of Robustness study of Sofosbuvir

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.8ml	60	2232.99±32.73	1.47
Chromatogram of flow change 1.0 ml	60	2425.59±0.36.82	1.52
Chromatogram of comp change 79ml ACN+21ml water	60	2288.50±10.69	0.47
Chromatogram of comp change 81mlACN +19ml water	60	2401.26±15.20	0.63
Chromatogram of comp change wavelength change 282nm	60	2248.80±7.70	0.34
Chromatogram of comp change wavelength change 284nm	60	2478.70±4.81	0.19

Table 10
Result of Robustness Study of Ledipasvir

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.8ml	15	766.25±4.08	0.53
Chromatogram of flow change 1.0 ml	15	829.12±1.83	0.22
Chromatogram of comp change 79ml ACN +21ml water	15	790.40±1.19	0.15
Chromatogram of comp change 81mlACN +19ml water	15	828.50±1.17	0.14
Chromatogram of comp change wavelength change 282nm	15	759.10±0.40	0.05
Chromatogram of comp change wavelength change 284nm	15	860.32±2.68	0.31

Discussion

The proposed methods for simultaneous estimation of SOFO and LEDI in tablet dosage forms were found to be simple, accurate, economical and rapid [31]. The method was validated as per the ICH Q2 (R1) guidelines.

Standard calibration yielded correlation coefficient (r^2) 0.999 for both SOFO and LEDI at all the selected wavelengths [32]. The values of % RSD are within the prescribed limit of 2 %, showing high precision of methods and recovery was close to 100% for both drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination with virtually no interference of any additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of SOFO and LEDI in formulations.

Conclusion

The developed HPLC methods in that linearity, precision, range, robustness were found to be more accurate, precise and reproducible. The methods were found to be simple & time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

Acknowledgements

The authors are thankful to the Principal, Gangamai College of Pharmacy, Nagaon, Dist. Dhule for providing necessary facilities for research work. Authors are grateful to two anonymous reviewers for their valuable comments on the earlier version of this paper. They are also grateful to Hetero drugs Ltd. Mumbai for giving gift samples of pure drugs.

References

1. (2014) European Pharmacopeia (8th edn). The United States Pharmacopoeia 38th Revision (2015) NF 33.
2. (2014) Harvoni tablets for oral use. US Prescribing Information Gilead Sciences, Inc. Foster City, USA.
3. Afdhal N, Reddy KK, Nelson DR, Lawitz E, Gordon SC, Schiff E, et al. Investigators, Ledipasvir and sofosbuvir for previously treated HCV genotype1 infection, N. Engl. J. Med. 2014;370:1483.
4. Ariaudo A, Favata F, De Nicolò A, Simiele M, Paglietti L, et al. (2016) A UHPLC-MS/MS method for the quantification of direct antiviral agents simeprevir, daclatasvir, ledipasvir, sofosbuvir/GS-331007, dasabuvir, ombitasvir and paritaprevir, together with ritonavir, in human plasma. J Pharm Biomed Anal 125: 369-375.
5. Bakht Zaman, Faisal Siddique.,Waseem Hassan, 2016. RP-HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form and its application to in vitro dissolution studies.Chromatographia.79(23),pp.1605–1613.
6. Bruno S, Crosignani A, Facciotto C, Rossi S, Roffi L, Redaelli A, et al. Sustained virologic response prevents thedevelopment of esophageal varices in compensated, Child-Pugh class Ahepatitis C virus-induced cirrhosis. A 12-year prospective follow-up study, Hepatology 2010;51:2069.
7. European Medicines Agency (2014) Committee for Medicinal Products for Human Use (CHMP) EMA/702742/2014.

8. Gandamayu, I. B. M., Antari, N. W. S., & Strisanti, I. A. S. (2022). The level of community compliance in implementing health protocols to prevent the spread of COVID-19. International Journal of Health & Medical Sciences, 5(2), 177-182. https://doi.org/10.21744/ijhms.v5n2.1897
9. Gilead Files for U.S. Approval of Ledipasvir/Sofosbuvir Fixed-Dose Combination Tablet for Genotype 1 Hepatitis C. Gilead Sciences. 2014.
10. Harona, M., Avulaa, B., Shia, Q., Lia, X., Ashfaqa, M., Baea, J., Guanc, S., Hincheec, M ., Khan, I., Khan, S., 2019. Quantitative determination and pharmacokinetic study of fusaricidin A in mice plasma and tissues using ultra-high performance liquid chromatography-tandem mass spectrometry. J. Pharm. Biomed. Anal. 170, 187–192.
11. Harvoni (Ledipasvir/Sofosbuvir) Tablets Product Monograph. Gilead Sciences Inc. Foster City, CA 94404 USA, Date of Preparation, 2016.
12. Harvoni, 2014. Tablets for oral use. US Prescribing Information Gilead Sciences, Inc. Foster City, USA.
13. J Devilal, B Durgaprasad, Narottam Pal, A Srinivasa Rao (2016) New method development and validation for the determination of ledipasvir in bulk drug form by using reverse phase HPLC technique. World Journal of pharmacy and pharmaceutical Sciences 5(8): 1312-1321.
14. Joseph E Rower, Leah C Jimmerson, Xinhui Chen, Jia-Hua Zheng, Ariel Hodara, et al. (2015) Validation and application of an LC-MS/MS method to determine the concentrations of sofosbuvir. Antimicrobial Agents and Chemotherapy 59(12): 7671-7679.
15. Kale, S. A., & Shaikh, K. S. (2022). Validated UV-Vis spectrophotometric method for the estimation of Sorafenib Tosylate in bulk and nanoparticles. *International Journal of Health Sciences*, *6*(S3), 4011–4019. *https://doi.org/10.53730/ijhs.v6nS3.6719*
16. Keating GM (2014) Sofosbuvir: a review of its use in patients with chronic hepatitis C. Drugs 74(10): 1127-1146.
17. Kirby BJ, Symonds WT, Kearney BP, Mathias AA (2015) Pharmacokinetic, Pharmacodynamic and Drug-Interaction Profile of the Hepatitis C Virus NS5B Polymerase Inhibitor Sofosbuvir. Clin Pharmacokinet 54(7): 677-690.
18. Nawfal, I. N. K., & Hanon, I. T. (2022). The use of high-performance liquid chromatography technique for the simultaneous determination of amlodipine and valsartan. *International Journal of Health Sciences*, *6*(S4), 6076–6086. *https://doi.org/10.53730/ijhs.v6nS4.9530*
19. Nebsen M, Elzanfaly ES (2016) Stability-Indicating Method and LC-MS-MS Characterization of Forced Degradation Products of Sofosbuvir. J Chromatogr Sci.
20. Ng V, Saab V. Effects of a sustained virologic response on outcomes of patients with chronic hepatitis C. Clin. Gastroenterol. Hepatol. 2011;9:923.
21. Pan C, Chen Y, Chen W, Zhou G, Jin L, et al. (2016) Simultaneous determination of ledipasvir, sofosbuvir and its metabolite in rat plasma by UPLC-MS/MS and its application to a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci 1008: 255-259.
22. Ranjana S, Nitin S, Ganesh T, Gholve SB (2016) Development and Validation of Simple UV Spectrophotometric Method for the Determination of Ledipasvir in Bulk Form and Stress Degradation Studies. Inventi Rapid: Pharm Analysis & Quality Assurance 3: 1-5.

23. Ravikumar Vejendla, CVS Subramanyam, G Veerabhadram (2016) Estimation and validation of sofosbuvir in bulk and tablet dosage form by RP-HPLC. International Journal of Pharmacy 6(2): 121-127.
24. Rezk M.R., Bendas E.R, Basalious E.B., Karim I.A.,2016.Quantification of sofosbuvir and ledipasvir in human plasma by UPLC-MS/MS method: Application to fasting and fed bioequivalence studies. J Chromatogr B Analyt Technol Biomedical Life Sciences. 1028, pp.63-70.
25. Rezk MR, Basalious EB, Amin ME (2016) Novel and sensitive UPLC-MS/MS method for quantification of Sofosbuvir in human plasma: application to a bioequivalence study. Biomed Chromatogr 30: 1354-1362.
26. Rezk MR, Basalious EB, Karim IA (2015) 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: 97-104.
27. Rezk MR, Bendas ER, Basalious EB, Karim IA (2016) Development and validation of sensitive and rapid UPLC-MS/MS method for quantitative determination of daclatasvir in human plasma: Application to a bioequivalence study. J Pharm Biomed Anal 128: 61-6.
28. Rezk MR, Bendas ER, Basalious EB, Karim IA (2016) Quantification of Sofosbuvir and ledipasvir in human plasma by UPLC-MS/MS method: Application to fasting and fed bioequivalence study. J Chromatogr B Analyt Technol Biomed Life Sci 1028: 63-70.
29. Shi X, Zhu D, Lou J, Zhu B, Hu AR, et al. (2015) 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 Analyt Technol Biomed Life Sci 1002: 353-357.
30. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
31. Swain D, Samanthula G, Bhagat S, Bharatam PV, Akula V, et al. (2016) Characterization of forced degradation products and in silico toxicity prediction of Sofosbuvir: a novel HCV NS5B polymerase inhibitor. J Pharm Biomed Anal 120: 352-363.
32. The United States Pharmacopoeia Convention Inc.
33. Veldt BJ, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, et al. M.P. Sustained virologicresponse and clinical outcomes in patients with chronic hepatitis C andadvanced fibrosis. Ann. Intern. Med. 2007; 147:677.
34. Vikas PM, Satyanarayana T, Kumar DV, Mounika E, Sri LM, Sathish Y (2016) Development and validation of new RP-HPLC method for the determination of sofosbuvirin pure form. World Journal of pharmacy and pharmaceutical Sciences 5(5): 775-781.