International Journal of Chemistry and Pharmaceutical Sciences
ISSN: 2321-3132 | CODEN (USA): IJCPNH |Publisher: Pharma Research Library
Journal Home Page: http://www.pharmaresearchlibrary.com/ijcps
DOI: https://doi.org/10.30904/j.ijcps.2025.4811
Int. J. of Chem. Pharm. Sci., 13(1), 2025: 30-35

Stimulatneous Estimation of Ledipasvir and Sofosbuvir by RP–HPLC Method Devlopment and Validation

D. Anusha*[1], K. Susmitha[1], G. Navya Sri[1], Dr.S.Sujatha[2]

[1]Department of Pharmaceutical Analysis, University College of Pharmaceutical Sciences, Palamuru University, Mahabubnagar, Telangana-509001, India
[2]Assistant Professor, University College of Pharmaceutical Sciences, Palamuru University, Bandameedipally, Mahabubnagar, Telangana-509001, India

Abstract

The optimization of chromatographic conditions for the analysis of Ledipasvir and Sofosbuvir was successfully achieved using a Platisil C18 Column (150×4.6mm, 3µm) at a flow rate of 1.0 ml/min, ensuring excellent peak shape and resolution. System suitability tests confirmed compliance with essential parameters, including resolution, tailing factor, and theoretical plate count, ensuring reliability. The retention times (RT) were recorded as 2.919 min for Ledipasvir and 3.934 min for Sofosbuvir, demonstrating distinct peak separation. Linearity studies indicated strong correlation coefficients (>0.999), enabling accurate quantification. Precision assessments revealed low %RSD values, confirming reproducibility, while accuracy tests showed recovery rates within 98-102%, validating method reliability. Robustness and ruggedness were evaluated by modifying chromatographic conditions such as flow rate and mobile phase composition. Despite slight variations, the method remained stable, maintaining consistent retention times and peak separation. Intermediate precision studies further supported assay consistency across different systems and days. The limits of detection (LOD) and limits of quantification (LOQ) were determined as 0.01 µg/ml for Ledipasvir and 0.02µg/ml for Sofosbuvir, highlighting the method's sensitivity for trace analysis. In conclusion, the developed chromatographic method for Ledipasvir and Sofosbuvir is precise, accurate, linear, sensitive, and robust, making it suitable for routine pharmaceutical analysis and quality control. The validation results confirm compliance with regulatory standards, ensuring reliable identification and quantification of these antiviral drugs. Given its high stability and reproducibility, this method provides an efficient and dependable approach for analyzing Ledipasvir and Sofosbuvir in pharmaceutical formulations.

Keywords: Ledipasvir, Sofosbuvir, Chromatographic Optimization, HPLC, System Suitability, Retention Time, Linearity, Accuracy, Precision, Robustness, Pharmaceutical Analysis.

Citation: *K.Susmitha,et al. (2025) Stimulatneous Estimation of Ledipasvir and Sofosbuvir by RP–HPLC Method Devlopment and Validation. Int. J. of Chem. and Pharm. Sci., 13(1), 30-35*

Contents:

***Corresponding author**
D. Anusha
Department of Pharmaceutical Analysis,
University College of Pharmaceutical Sciences,
Palamuru University, Mahabubnagar, TG-509001, India

Article History:
Received : 07 Mar 2025
Revised : 29 Mar 2025
Accepted : 28 April 2025
Published : 18 May 2025

1. Introduction

High-Performance Liquid Chromatography (HPLC) is a powerful analytical technique used for separating, identifying, and quantifying components in a mixture, particularly valuable in pharmaceutical research and quality control. The technique operates on the principle of differential distribution of compounds between a stationary phase (solid adsorbent) and a mobile phase (liquid), with separation achieved based on compound solubility and molecular size. The system typically uses high pressure to force the mobile phase through a packed column, enabling efficient separation and analysis. HPLC is essential in developing and validating analytical methods for new drugs, especially when standard procedures are not available in pharmacopoeias. Method validation ensures precision, accuracy, specificity, and robustness of analytical

techniques, covering parameters like system suitability, linearity, limit of detection (LOD), and limit of quantification (LOQ). Stability-indicating methods (SIMs) are critical for ensuring drug efficacy, safety, and quality throughout the shelf life, by detecting active ingredients apart from degradation products under stress conditions. In the context of Ledipasvir and Sofosbuvir two potent antiviral drugs used in Hepatitis C treatment there is a continued need for optimizing treatment strategies, understanding resistance mechanisms, improving outcomes in special populations, ensuring safety, evaluating cost-effectiveness, and exploring efficacy in HIV-HCV co-infected patients. Studying these drugs not only enhances treatment protocols and patient care but also contributes to a deeper understanding of HCV biology, resistance, and potential for broader therapeutic applications.

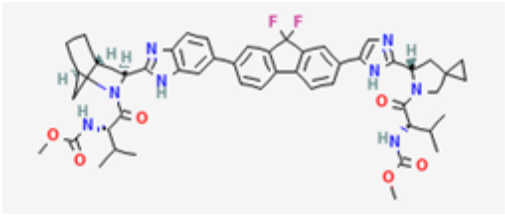

Fig.no.1. Molecular structure of Ledipasvir

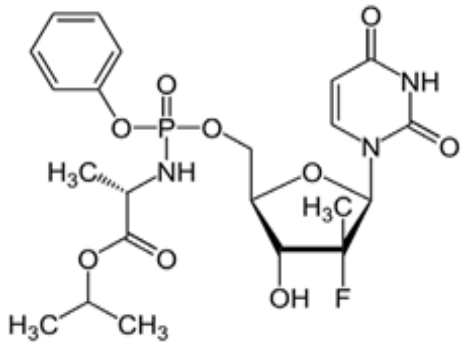

Fig.no.2. Molecular structure of Sofosbuvir

2. Materials and Methods

List of Proposed Materials:

The chemicals, standards, and reagents used for the estimation of Sofosbuvir and Ledipasvir include Potassium dihydrogen orthophosphate (HPLC grade, Qualigens) and Formic acid (HPLC grade, Qualigens), both specifically employed in the analysis of Sofosbuvir and Ledipasvir. Additionally, high-purity Water and Acetonitrile (both HPLC grade, Qualigens) along with Methanol (HPLC grade, Rankem) are used as common solvents and mobile phase components in the estimation of all drugs, ensuring accurate and reproducible chromatographic performance.

Equipments and instruments used in the study:

The equipment used in the estimation of drugs includes an Electronic Balance (Model SAB2032, manufactured by Scaletec) for precise weighing, and an Ultra-Sonicator (Model SE60US, Labman Scientific India) to ensure proper dissolution of samples. A Thermal Oven (Model i-THERM A17782, Dwaraka Scientific) is used for controlled heating purposes, while a pH Meter (Model ORION STAR A111, Thermo Scientific) is employed to measure the pH of solutions. Filtration is carried out using 0.45-micron Filter Paper from Millipore to remove particulates prior to HPLC analysis. The main analytical instrument used is the HPLC System (Model WATERS 2690 Separation Module, manufactured by Waters), which enables accurate and efficient separation and quantification of the drugs.

Method Developmet

Wave length selection:

UV spectrum of 10µg/ml **Sofosbuvir and Ledipasvir** in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm and the isobestic λmax of both the drugs obtained at 225 nm.

Optimization of Column:

Platisil C18 Column, (150×4.6mm, 3µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimization of chromatographic conditions:

Optimized chromatographic conditions: The optimized chromatographic conditions were established using a High Performance Liquid Chromatography (HPLC) system equipped with an Auto Sampler and a PDA or UV detector. The separation was carried out at ambient temperature on a Platisil C18 column (150 × 4.6 mm, 3 µm particle size). The mobile phase consisted of a mixture of 40% potassium dihydrogen orthophosphate buffer (pH 3) 60% acetonitrile (ACN), delivered at a flow rate of 1.0 mL/min. The detection was performed at a wavelength of 225 nm, with an injection volume of 20 µL and a total run time of 8 minutes.

Preparation of buffer and mobile phase:

Preparation of KH2PO4 Buffer pH 3:

To prepare KH2PO4 Buffer solution, by adding 6.8Grams of Potassium dihydrogen orthophosphate in 1000ml water. Adjust this solution to pH 3.0 by using sodium hydroxide.

Preparation of mobile phase: Mix a mixture of above ACN 600ml (60%), 400 ml KH2PO4 (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

ACN: KH2PO4 (600:400ml) ratio.

ASSAY:

Standard Solution Preparation:

Accurately weigh and transfer 20 mg of **Sofosbuvir** and 4.5 mg **Ledipasvir** working standard into a 20 ml clean dry volumetric flasks add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solutions into a 10ml volumetric flasks and dilute up to the mark with Diluents. (60ppm **Sofosbuvir** and 13.5ppm **Ledipasvir**)

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 20 mg of Sofosbuvir and 4.5 mg Ledipasvir equivalent weight of the sample into a 20ml clean dry volumetric flasks add about 7ml of Diluents and sonicate to Dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of each of the above stock solution into a two 10ml volumetric flasks and dilute up to the mark with Diluents. (60ppm Sofosbuvir and 4.5 ppm Ledipasvir).

3. Results and Discussions

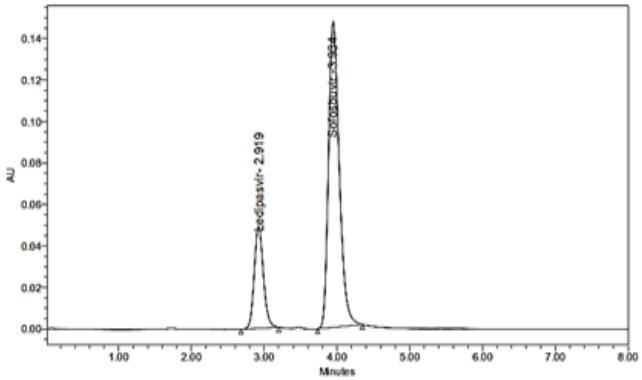

Fig.no.3: Optimized chromatogram

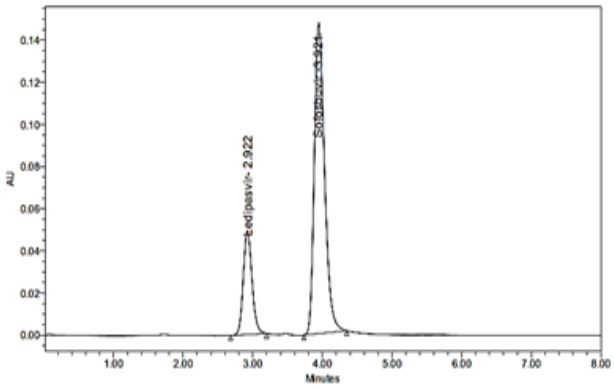

Figure-4: Chromatogram showing system suitability testing of Standard Solution of Ledipasvir and Sofosbuvir.

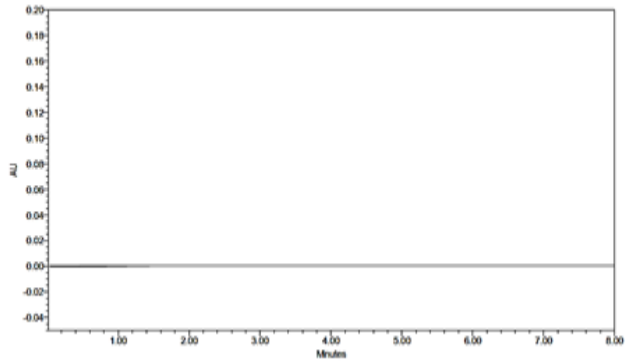

Figure 5: Chromatogram for Blank

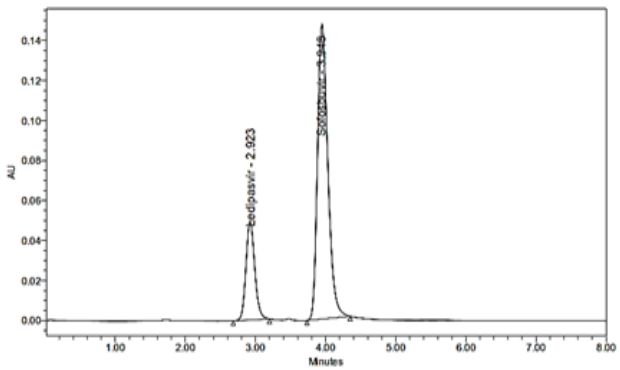

Figure 6: Chromatogram for Standard

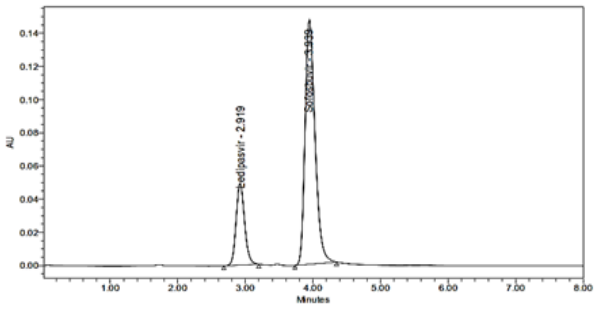

Figure 7: Chromatogram for Sample

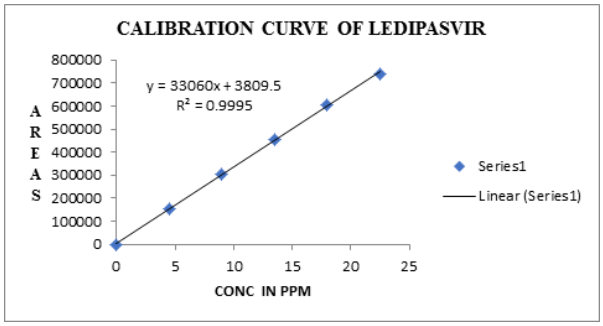

Figure 8: Calibration graph for Ledipasvir

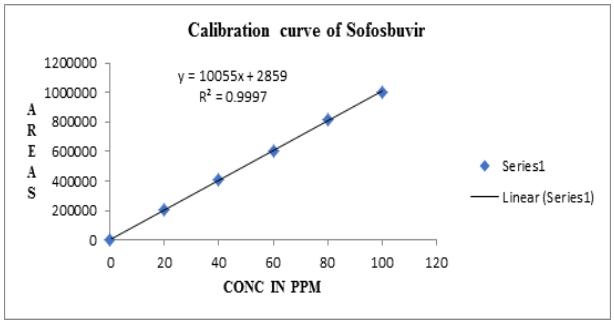

Figure 9: Calibration graph for Sofosbuvir

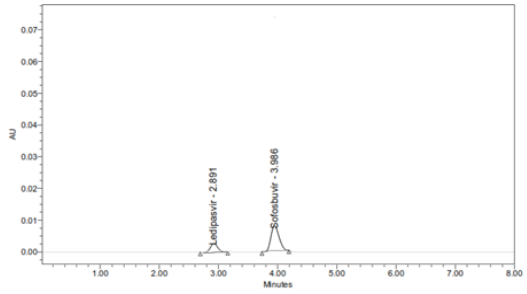

Figure 10: Chromatogram of Ledipasvir and Sofosbuvir showing LOD

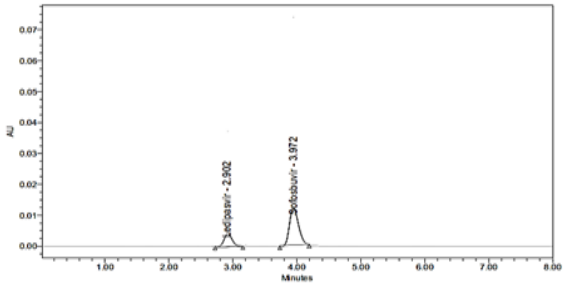

Figure 11: Chromatogram of Ledipasvir and Sofosbuvir showing LOQ

Table.1: Results of optimized chromatogram

Name	Rt	Area	Resolution	Tailing factor	Plate count
Sofosbuvir	2.919	462451	10.7	0.85	9989
Ledipasvir	3.934	6245681		0.91	2987

Table.2: System suitability parameters of Ledipasvir and Sofosbuvir

Name	Rt	Area	Resolution	Tailing factor	Plate count
Ledipasvir	2.919	462451	10.7	0.85	9989
Sofosbuvir	3.934	6245681		0.91	2987

Table .3: Specific responses for Ledipasvir and Sofosbuvir

S.No	Name(STD)	RT(min)	Area(µV sec)	Resolution	USP tailing	USP plate count
1	**Ledipasvir**	2.923	454804	5.2	1.02	3112
2	**Sofosbuvir**	3.948	602458		1.07	3998

NAME (Sample)	RT(min)	AREA(µV sec)	Resolution	USP tailing	USP plate count
Ledipasvir	2.919	454261	5.6	1.25	2987
Sofosbuvir	3.939	601958		1.43	3989

Table 4: Results of Assay for Ledipasvir and Sofosbuvir

DRUG NAMES	Label Claim (mg)	% Assay
Ledipasvir	90mg	101.2
Sofosbuvir	400mg	98.20

Table. 5: Area of different concentration of Ledipasvir and Sofosbuvir

	Concentration (µg/ml od Ledipasvir	Areas of Ledipa	Concentration (µg/ml) Sofosbuvir	Areas of Sofosbuvir
1	4.5	151625	20	204252
2	9	303250	40	408504
3	13.5	454875	60	602746
4	18	606500	80	817028
5	22.5	738125	100	1001240

Table 6: Analytical performance parameters of Ledipasvir and Sofosbuvir

Parameters	Ledipasvir	Sofosbuvir
Slope (m)	33060	10055
Intercept (c)	3809.5	2859
Correlation coefficient (R^2)	0.9995	0.9997

Table 7: Results of Precision for Ledipasvir and Sofosbuvir

Injection	Area of Ledipasvir	Area of Sofosbuvir
Injection-1	462451	6245681
Injection-2	474722	6240567
Injection-3	460932	6149742
Injection-4	469762	6256854
Injection-5	465361	6257342
Injection-6	460631	6240125
Average	465643.2	6231719
Standard Deviation	5603.15	40871.56
%RSD	1.2	0.6

Acceptance criteria: %RSD for sample should be NMT 2

Table 8: Results of Intermediate precision for Ledipasvir and Sofosbuvir

Injection	Area of Ledipasvir	Area OF Sofosbuvir
Injection-1	440310	645602
Injection-2	441024	646602
Injection-3	440023	648028

Injection-4	440010	645821
Injection-5	447302	629046
Injection-6	440103	647124
Average	441462	643703.8
Standard Dev	2886.19	7235.093
%RSD	0.6	1.1

Table 9: Accuracy (recovery) data for Ledipasvir and Sofosbuvir

%Concentration (at specification Level)	Area* of Ledipasvir	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	225625	2.25	2.23	99.00	99.01%
100%	451250	4.5	4.46	99.00	
150%	676892	6.75	6.68	99.01	

Table 10: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Conc. In ppm
Ledipasvir	62	182	2.93	0.01 µg/ml
Sofosbuvir	62	184	2.96	0.02 µg/ml

Table 11: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Conc. In ppm
Ledipasvir	62	618	9.96	0.02 µg/ml
Sofosbuvir	62	608	9.90	0.06 µg/ml

Table 12: Results for variation in flow for Ledipasvir and Sofosbuvir

S. No	Flow Rate (ml/min)	System Suitability Results of Ledipasvir		System Suitability Results of Sofosbuvir	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.8	44959	1.2	647428	1.02
2	1	44963	1.3	647502	1.53
3	1.2	44968	1.7	647214	1.6

4. Conclusion

In conclusion, the validated chromatographic method for Ledipasvir and Sofosbuvir is precise, accurate, linear, sensitive, and robust, with well-defined retention times ensuring reliable identification and quantification. The validation results confirm compliance with regulatory standards, making this method suitable for routine pharmaceutical analysis and quality control applications. Given its stability and reproducibility, it provides an efficient and dependable approach for the analysis of Ledipasvir and Sofosbuvir in pharmaceutical formulations.

Conflict of Interest

We affirm that there are no conflicts of interest.

5. References

[1] Becket and Stenlake, Practical pharmaceutical chemistry, part 24th edition CBS publications and distributors, 2005.

[2] P.D. Sethi, HPLC quantitative analysis of pharmaceutical formulations CBS publications and distributors, 1st edition, 2001.

[3] B.K Sharma, instrumental method of chemical analysis, 23rd edition, goal publishers 2004.

[4] Practical HPLC method development Lloyd R.Snyder, Joseph J. Kirkland, Joseph L. Glajch, second edition.

[5] Validating chromatographic methods, David M.Bliesner. International conference on harmonization**:** ICH Q2 (R1) Validation of Analytical Procedures: Text and Methodology 199.

[6] HassounaME, Abdelrahman MM, Mohamed MA. Assay and dissolution methods development and validation for simultaneous determination of sofosbuvir and ledipasvir by RP-HPLC method in tablet dosage forms. J Forensic Sci & Criminal Inves. 2017;1(3):001-11.

[7] Devilal J, Durgaprasad B, Pal N, Rao AS. New method development and validation for the determination of ledipasvir in bulk drug form by using reverse phase HPLC technique. World J Pharm Pharm Sci. 2016 Jun 8;5(8):1312-21.

[8] Baker MM, Hammad SF, Belal TS. Development and validation of a versatile HPLC-DAD method for simultaneous determination of the antiviral drugs daclatasvir, ledipasvir, sofosbuvir and ribavirin in presence of seven potential impurities.

Application to assay of dosage forms and dissolution studies. Drug Development and Industrial Pharmacy. 2019 Jul 3;45(7):1111-9.

[9] Mastanamma SK, Chandini SK, Reehana SK, Saidulu P. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of Sofosbuvir and Ledipasvir in bulk and their combined dosage form. Future journal of pharmaceutical sciences. 2018 Dec 1;4(2):116-23.

[10] ghante mr, d sawant sa, shinde a, godse s, nikam vs. development and validation of stability-indicating reverse-phase high-performance liquid chromatography method for the estimation of ledipasvir in bulk and tablet dosage form. development. 2019;12(3).

[11] P, Hamrapurkar P, Mukhedkar P. Implementation of Quality by Design approach to develop Yeram and validate stability indicating assay method for simultaneous estimation of sofosbuvir and ledipasvir in bulk drugs and tablet formulation. Int. J. Pharm. Sci. 2019 Jan 1;10:180-8.

[12] Abdallah OM, Abdel-Megied AM, Gouda AS. Development a validated highly sensitive LC–MS/MS method for simultaneous quantification of Ledipasvir, sofosbuvir and its major metabolite GS-331007 in human plasma: Application to a human pharmacokinetic study. Journal of pharmaceutical and biomedical analysis. 2017 Sep 5;143:305-10.

[13] Evangelin MP, Elsebaei F, Hammad SF, El-Brashy AM. UPLC method development and validation for simultaneous estimation of sofosbuvir and ledipasvir in tablet dosage form. World J Pharm Med Res. 2018;4:225-9.

[14] El-Kafrawy Baker MM, DS, Mahrous MS, Belal TS. Validated spectrophotometric and chromatographic methods for analysis of the recently approved hepatitis C antiviral combination ledipasvir and sofosbuvir. InAnnales pharmaceutiques francaises 2018 Jan 1 (Vol. 76, No. 1, pp. 16-31). Elsevier Masson.

[15] Reddy BA, Alam I, Khanam NA, Krishnanand PR. An innovative method development and forced degradation studies for simultaneous estimation of sofosbuvir and ledipasvir by RP HPLC. Int J Pharm Pharm Sci. 2019;11:1-8.

[16] Gandla K, Lalitha R, Kumar DV, Shruthi PV. Analytical Method Development & Validation for the Simultaneous Estimation of Ledipasvir and Sofosbuvir in Bulk and IT's Dosage Form by Rp-hplc. International Journal of Pharmaceutics and Drug Analysis. 2020;8(4):6-15.

[17] Kashif M, Nasiri MI, Khaliq SA, Akhtar MM, Shah SS. RP-HPLC Method Development and Validation for Simultaneous Determination of Sofosbuvir and Ledipasvir: A Pharmaceutical Application Study. Hamdard Journal of Pharmacy. 2023;3(2).

[18] Mehmood Y, Khan IU, Shahzad Y, Khalid SH, Irfan M, Asghar S, Yousaf AM, Hussain T, Khalid I. Development and validation of a stability-Indicating RP-HPLC method for simultaneous estimation of sofosbuvir and velpatasvir in fixed dose combination tablets and plasma. Pak. J. Pharm. Sci. 2019 Jul 1;32(4):1835-42.

[19] Ezzeldin E, Abo-Talib NF, Tammam MH, Asiri YA, Amr AE, Almehizia AA. Validated reversed-phase liquid chromatographic method with gradient elution for simultaneous determination of the antiviral agents: sofosbuvir, ledipasvir, daclatasvir, and simeprevir in their dosage forms. Molecules. 2020 Oct 10; 25(20): 4611.

[20] EL-Shorbagy HI, Elsebaei F, Hammad SF, El-Brashy AM. A green stability-indicating RP-HPLC-UV method using factorial design for determination of ribavirin, sofosbuvir and ledipasvir: Application to average content, acid degradation kinetics and in vitro drug interactions study. Microchemical Journal. 2020 Nov 1;158: 105251.

[21] Zaman B, Siddique F, Hassan W. RP-HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form and its application to in vitro dissolution studies. Chromatographia. 2016 Dec, 79: 1605-13.

[22] Kishore MS, Rambabu C. Development and Validation of UV Spectrophotometric method for the estimation of Sofosbuvir and Ledipasvir in combined Pharmaceutical dosage forms. Int. J. Chem Tech Res. 2019;12:33-40.

[23] Bhavani RP, Maduri MS. Stability indicating method development and validation for the simultaneous estimation of ledipasvir and sofosbuvir in bulk drug by using RP-HPLC. World Journal of Current Medical and Pharmaceutical Research. 2020, 2.

[24] Rai SY, Prajapati Y, Patni P. Development and validation of RPHPLC and UV spectroscopy methods for simultaneous estimation of Sofosbuvir and Ledipasvir in their combined tablet dosage form. An International journal of Pharmaceutical Sciences. 2017 Apr; 8(2).