Available online on 15.06.2022 at ijmspr.com

International Journal of Medical Sciences and Pharma Research

Open Access to Medical and Research

Copyright © 2022 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited

Open Access **Research Article**

Development and Validation of Method for Simultaneous Estimation of Sofosbuvir and Ledipasvir in Combined Pharmaceutical Dosage Form

Ganesh Nanaware [1]*, Neetesh Kumar Jain[2], Apoorva Tiwari[1], Kratika Daniel[3]

[1] *Department of Quality Assurance, Faculty of Pharmacy, Oriental University Indore-India*

[2] *Department of Pharmacology, Faculty of Pharmacy, Oriental University Indore-India*

[3] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Oriental University Indore-India*

Article Info:

Article History:

Received 27 April 2022
Reviewed 25 May 2022
Accepted 08 June 2022
Published 15 June 2022

Cite this article as:

Nanaware G, Jain NK, Tiwari A, Daniel K, Development and Validation of Method for Simultaneous Estimation of Sofosbuvir and Ledipasvir in Combined Pharmaceutical Dosage Form, International Journal of Medical Sciences & Pharma Research, 2022; 8(2):91-99

DOI: http://dx.doi.org/10.22270/ijmspr.v8i2.44

*Address for Correspondence:

Ganesh Nanaware, Department of Quality Assurance, Faculty of Pharmacy, Oriental University Indore-India

Email: ganesh.cdsco@gmail.com

Abstract

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. HPLC originally referred to the fact that high pressure was needed to generate the flow required for liquid chromatography in packed columns. In the beginning, instrument components only had the capability of generating pressures of 500psi (35 bar). Sofosbuvir (tradename Sovaldi) is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV) pka value 9.3, Ledipasvir is a direct acting antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus having pka1 = 4.0, pka2 = 5.0. LediHep brand name of combined dosage form as tablet having strength is 90 mg Ledipasvir and 400 mg Sofosbuvir was used for study. Separately weighed 20 tablets and average weight of individual tablets were found out and weight equivalent to LED (90 mg) and SOF (400 mg) was taken into 100 mL volumetric flask and dissolved into 60 mL of HPLC grade Methanol with sonication for 20 minutes. The responses of standard solution measured with UV detector showed a good result at 247 nm for the RP-HPLC method. A validated stability-indicating RP-HPLC analytical method has been successfully applied for quantitative determination of LED and SOF in tablet dosage form. The stability-indicating RP-HPLC method developed meets the system suitability criteria and resolution of the parent drugs from its degraded products.

Keywords: HPLC, Ledipasvir, Sofosbuvir and Validation.

1. INTRODUCTION:

The ability to provide timely, accurate, and reliable data is central to the discovery, development, and manufacture of pharmaceuticals.[1,2] Analytical data are used to screen potential drug candidates, aid in the development of drug synthesis, support formulation studies, monitor the stability of bulk pharmaceuticals and formulated products, and test final products for release. The quality of analytical data is a key factor in the success of a drug development program.

HPLC originally referred to the fact that high pressure was needed to generate the flow required for liquid chromatography in packed columns. In the beginning, instrument components only had the capability of generating pressures of 500psi (35 bar). This was called High Pressure Liquid Chromatography (HPLC).[3]

1.1 SOME OF THE ADVANTAGES ARE:

- Speed (analysis can be accomplished in 20 minutes or less)
- Greater sensitivity (various detectors can be employed)
- Precise and reproducible
- Calculations are done by integrator itself
- Improved resolution (wide variety of stationary phases)
- Reusable columns (expensive columns but can be used for many analysis)
- Ideal for the substances of low volatility
- Easy sample recovery, handling and maintenance
- Instrumentation tends itself to automation and quantitation (less time and less labor)
- Suitable for preparative liquid chromatography on a much larger scale.[4,5]

1.2 INSTRUMENTATION

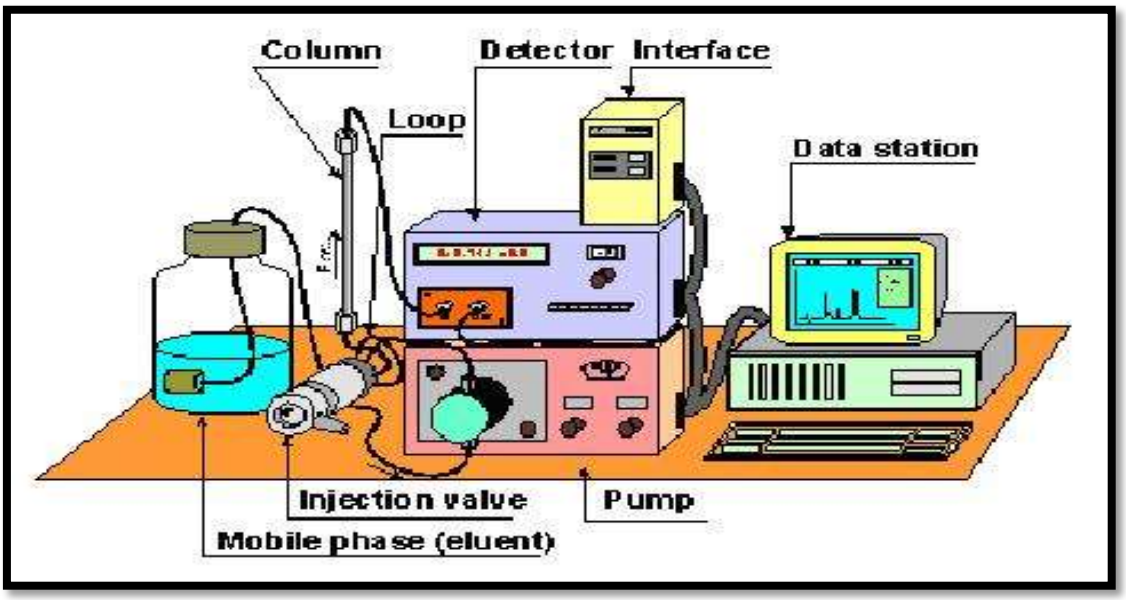

Figure 1: Schematic Diagram of HPLC Instrument[6]

1.3 METHODOLOGY OF ANALYTICAL METHOD VALIDATION[7,8]

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

It is an act of proving that any procedure, process, equipment, material, activity and system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness, etc.When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc.

1.3.1 Analytical Method Validation Parameters:

Before performing validation of analytical method it is necessary to understand the validation parameters. The various Performance parameters, which are addressed in a validation exercise, are grouped as follows.

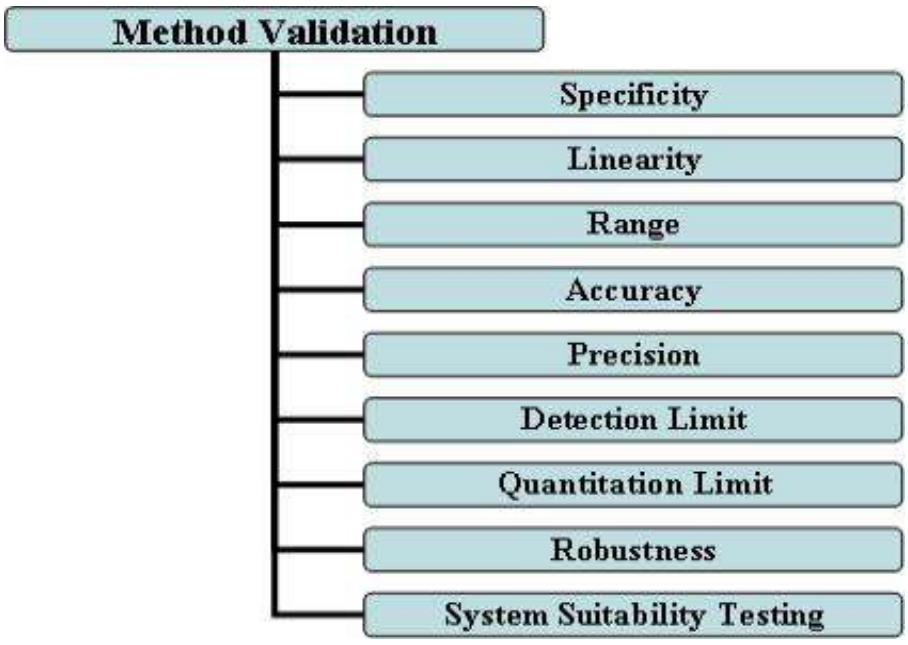

Figure 2: Method Validation Parameters as per USP and ICH

2. DRUG PROFILE:

2.1 DRUG: SOFOSBUVIR[9]

Structure:

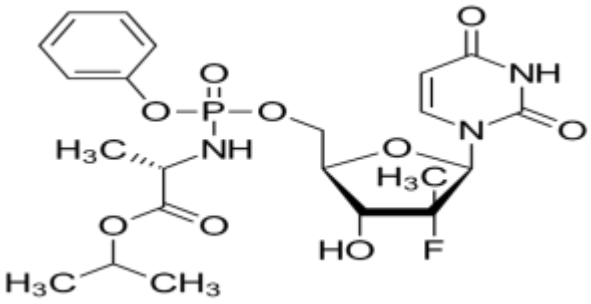

Figure 3: Structure of Sofosbuvir[7]

Description:

Sofosbuvir (tradename Sovaldi) is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients.[9]

Weight: Average: 529.458, Monoisotopic: 529.162544687

Chemical Formula: $C_{22}H_{29}FN_3O_9P$

Property	Value	Source
logP	1.62	FDA Label
pKa	9.3	FDA Label

2.2 LEDIPASVIR[10]

Drug structure:

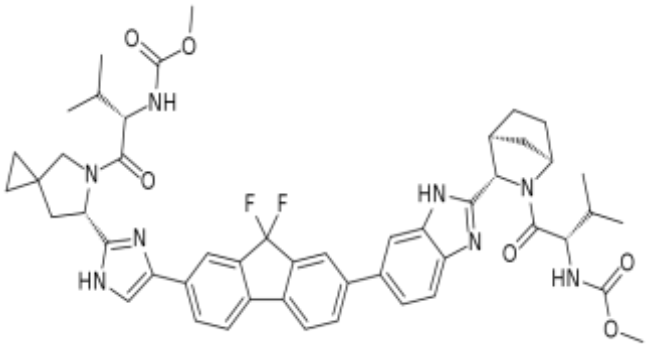

Figure 4: Structure of Sofosbuvir[10]

Description:

Ledipasvir is a direct acting antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV).[10]

Weight: Average: 888.9999, Monoisotopic: 888.41343791

Chemical Formula: $C_{49}H_{54}F_2N_8O_6$

Property	Value	Source
logP	3.8	FDA Label
pKa	pka1 = 4.0, pka2 = 5.0	FDA Label

3. MATERIALS AND METHODS:

3.1 Market Formulation

Brand Nmae	Contents	Manufacturer	Formulation
LediHep	90 mg Ledipasvir and 400 mg Sofosbuvir	Zydus Heptiza	Tablet

3.2 Preparation of Standard Stock Solution of LED and SOF

Accurately weighed 10 mg of LED standard drug powder. It was transferred into 100 mL volumetric flask and diluted up to the mark with HPLC grade Methanol to give a solution having strength of 0.1 mg/mL or 100 ppm of LED, from this solution 1 mL was diluted up to 10 mL with HPLC grade Methanol to give a stock solution having strength of 0.01 mg/mL or 10 ppm of LED. Accurately weighed 100 mg of SOF standard drug powder. It was transferred into 100 mL volumetric flask and diluted up to the mark with HPLC grade Methanol to give a stock solution having strength of 1 mg/mL or 1000 ppm of SOF.[11]

3.3 Preparation of Combined Standard Stock Solution of LED and SOF

1 mL of LED standard stock solution and 1mL of SOF standard stock solution were pipetted out into 10 mL volumetric flask and diluted up to 10 mL with mobile phase to produce final concentration of 1 µg/mL of LED and 100 µg/mL of SOF.[12]

3.4 Preparation of Mobile Phase

Mixed 70 mL of HPLC grade Methanol and 30 mL of Phosphate Buffer. Filtered through 0.45 µ Millipore nylon filter and degassed.[13]

3.5 Preparation of Test Solution

Separately weighed 20 tablets and average weight of individual tablets were found out and weight equivalent to LED (90 mg) and SOF (400 mg) was taken into 100 mL volumetric flask and dissolved into 60 mL of HPLC grade Methanol with sonication for 20 minutes. The solution was filtered through 0.45 µ Millipore nylon filter and the residues were washed thoroughly with HPLC grade Methanol. The filtrate and washings were combined into 100 mL volumetric flask and diluted up to the mark with HPLC grade Methanol to get final concentration of 50 µg/mL of LED and 5000 µg/mL of SOF. From this solution 1 mL was pipetted out into 10 mL volumetric flask and diluted up to 10 mL with HPLC grade Methanol to produce final concentration of 5 µg/mL of LED and 500 µg/mL of SOF. Then 1 mL of this solution was pipetted out into 5 mL volumetric flask and diluted up to 5 mL with mobile phase to produce final concentration of 1 µg/mL of LED and 100 µg/mL of SOF.[14]

3.6 Melting Point Determination:

Melting point of the APIs was determined by using melting point apparatus. The observed melting points of APIs were compared with the reported melting point.

3.7 Infrared Spectroscopy:

IR spectrum of Ledipasvir and Sofosbuvir were taken by KBr pellet method on FTIR and characteristic peaks were compared with IR spectrum of Reference standard given in Indian Pharmacopoeia.

3.8 Solubility Determination

The solubility of Ledipasvir and Sofosbuvir were checked in various solvents like distilled water, methanol, and Dimethyl Sulphoxide etc.

4. METHOD DEVELOPMENT AND VALIDATION:

4.1 Selection of wavelength:

Scan the standard solution and test solution on UV/Visible spectrophotometer, over the spectral range 200 to 400 nm. Use diluent as blank. The UV spectrum of the test solution should exhibit maxima at the same wavelength (±2 nm) as that of a standard solution. Ledipasvir and Sofosbuvir show reasonably good response at 237 nm and 247 nm respectively.[15]

4.2 Method Validation Parameter

As per the ICH guidelines Q2R1, the method validation parameters studied were solution stability, linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and system suitability test.

4.3 Estimation of Sofosbuvir and Ledipasvir in Formulation[16]:

Sample preparations were injected in triplicate and chromatograms were recorded. Responses for the analyte peaks were measured. The concentration of the drug in sample solution was determined using regression equation of calibration curve.

5. RESULT AND DISCUSSION:

5.1 Melting point determination:

Table 1: Melting point determination

Name of Drug	Reported Melting Point	Observed Melting Point
LED	170-178^{0}C	169-178^{0}C
SOF	120-125^{0}C	121-127 ^{0}C

5.2 Infrared Spectroscopy:

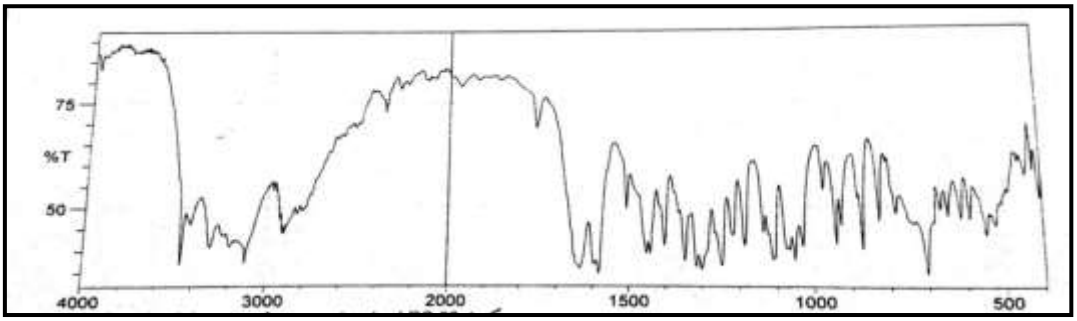

Fig 5: IR Spectra of LED

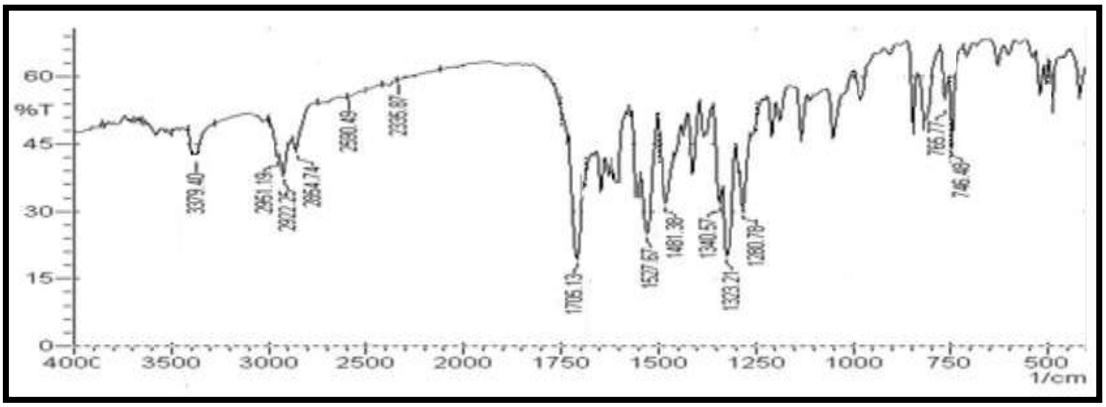

Fig 6: IR Spectra of SOF

5.3 Solubility Studies:

Table 2: Solubility determination of APIs

Sr No.	Drug	Reported	Observed
1	LED	**Water :** Slightly soluble in water **Methanol :** Sparingly soluble in Methanol **Dimethyl Sulphoxide :** Soluble in Dimethyl Sulphoxide	Complies with Reported solubility
2	SOF	**Water :** soluble in water **Methanol :** soluble in Methanol **Dimethyl Sulphoxide :** Soluble in Dimethyl Sulphoxide	Complies with Reported solubility

5.4 Method Development and Optimization:

5.4.1 Selection of Wavelength for Determination:

The standard solutions of LED (1 µg/mL) and SOF (100 µg/mL) were scanned in the range of 200 – 400 nm. The responses of standard solution measured with UV detector showed a good result at 247 nm for the RP-HPLC method.

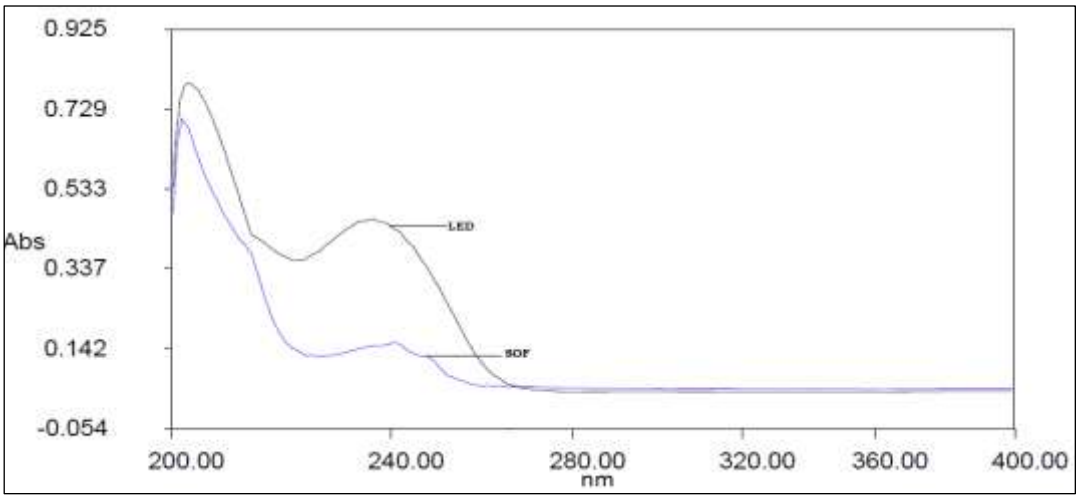

Fig 7: Uv Spectral studies of LED and SOF

5.4.2 Optimized Chromatographic Condition:

Column: Hypersil BDS C18 (250 mm × 4.6 mm) 5µ, Thermoscientic

Mobile Phase: Phosphate Buffer : Methanol (30 : 70 v/v) pH 4.5

Detection wavelength: 226 nm

Flow rate: 1 mL/min

Injection volume: 20 µl

Column oven temperature: 25ºC

5.4.3 Accuracy:

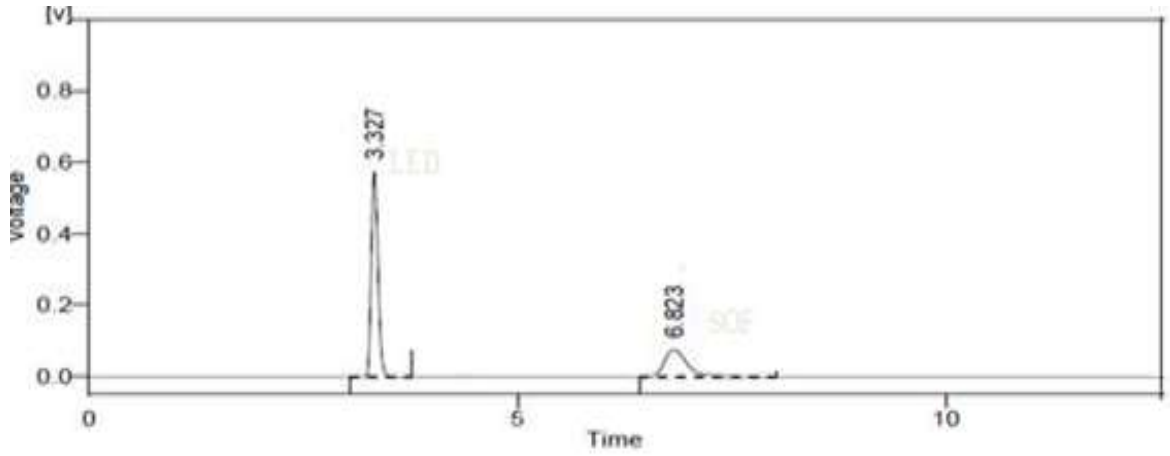

Figure 8: Accuracy for Ledipasvir and Sofosbuvir

Table 3: Accuracy data for Ledipasvir And Sofosbuvir

Peak No.	Ret No.	Name	Area	Area%	Tailing factor	Resolution	Plates
1	3.327	Ledipasvir	3144.798	68.843	0.26	--	8163
2	6.823	Sofosbuvir	1423.301	31.157	1.73	11.326	3370
Total			4568.099	100.000			

5.4.4 Linearity and Range:

Acceptance Criteria: The correlation coefficient value should not be less than 0.995 over the working range.

Linearity of the method was evaluated at five concentration levels by diluting the standard stock solution to give solutions of Sofosbuvir and Metformin in the concentration range from 0.5 - 1.5 µg/mL and 50 - 150 µg/mL. Results show good correlation between peak area and concentration of analytes. The calibration curves were prepared by plotting the area under the response from the detector versus the concentration of standard drugs.

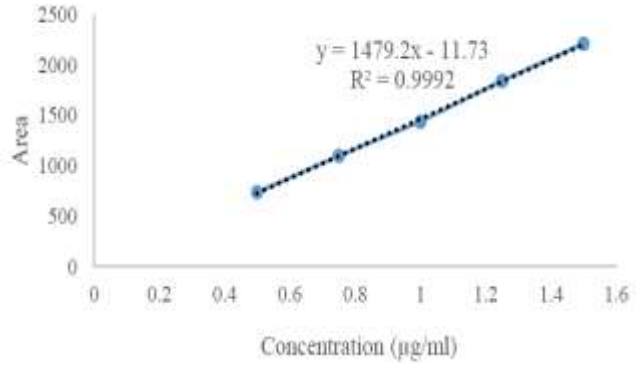

Figure 9: Calibration curve of LED at 247 nm by HPLC method

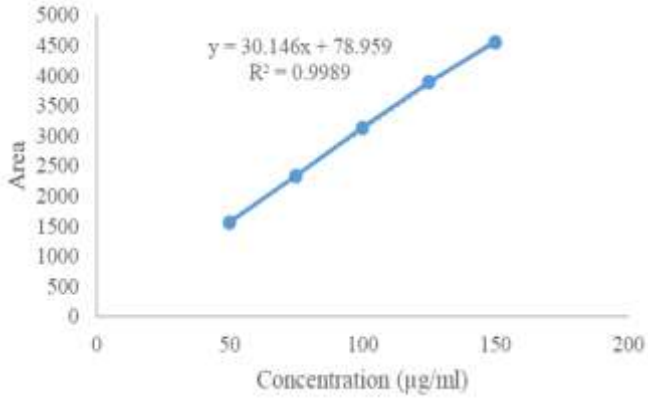

Figure 10: Calibration curve of SOF at 237 nm by RP-HPLC method

5.4.5 % Recovery:

Acceptance Criteria: Recovery for individual and mean value at each level should be 95.0 % to 105.0 % and with % RSD not more than 2.0 %. Recovery and % RSD were calculated at each level and recorded.

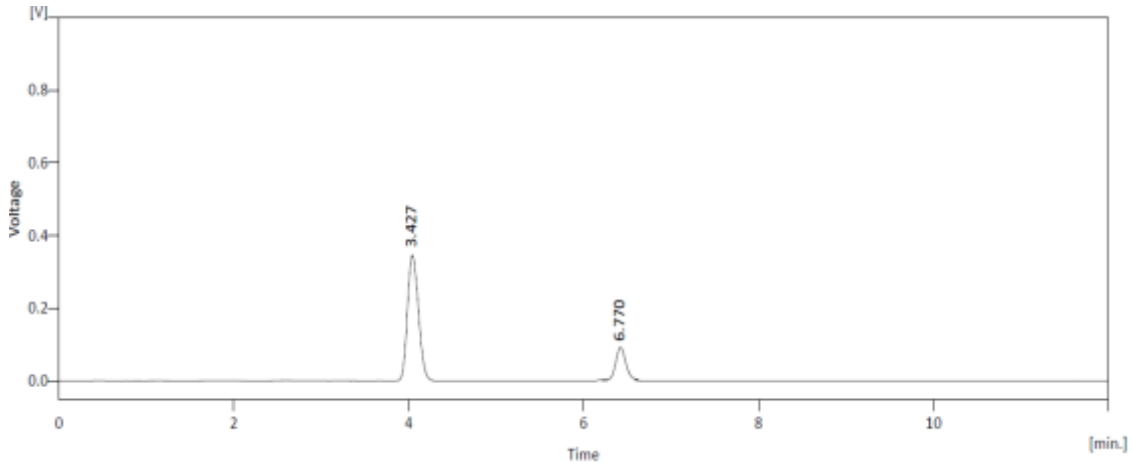

Figure 11: % recovery of LED and SOF

5.4.6 Method Precision (Repeatability)

Acceptance Criteria: The % RSD of assay of six sample preparations should not be more than 2.0 %. Mean, % assay, SD and % RSD of result obtained were recorded.

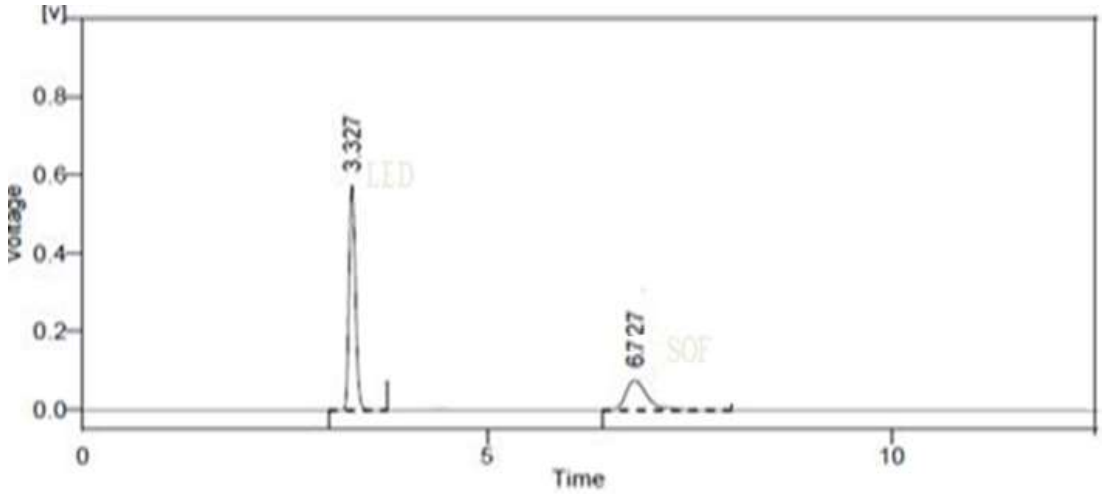

Figure 12: Precision (Repeatability) of LED and SOF

5.4.7 Intermediate Precision (Reproducibility)

Acceptance Criteria: % RSD of intermediate precision should not more than 2.0 % Results of intermediate precision for both intra-day and inter-day are shown.

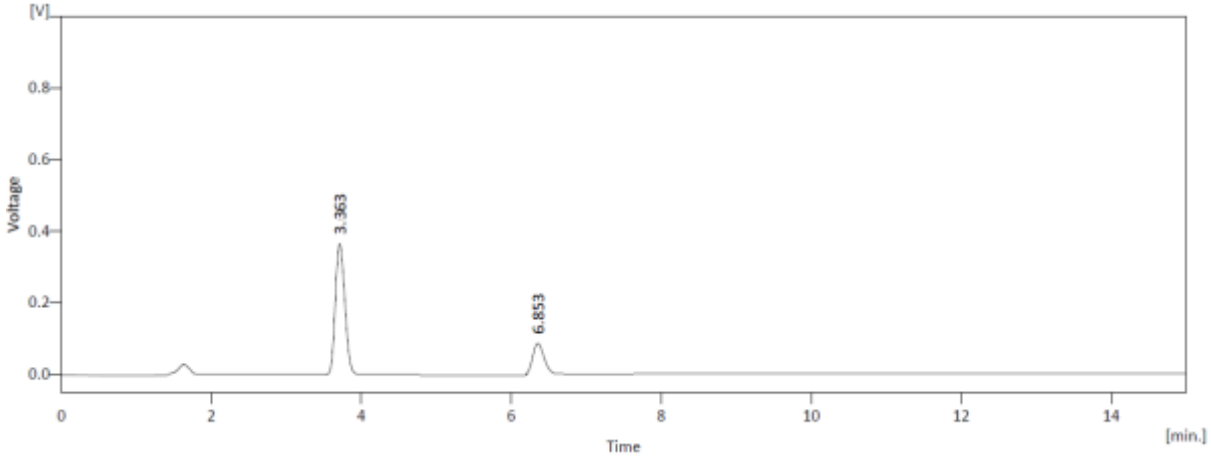

Figure 13: Intermediate Precision (Reproducibility) of LED and SOF

5.4.8 Robustness:

Acceptance Criteria: % RSD should not more than 2.0 % Results of change in flow rate of mobile phase, ratio of mobile phase composition and pH are shown.

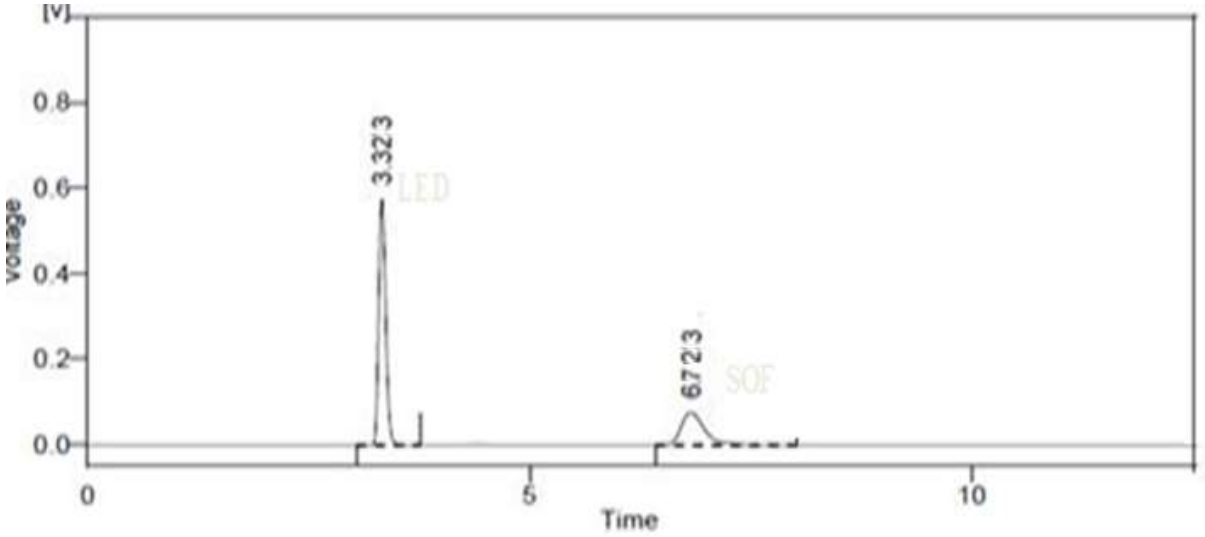

Figure 14: Robustness of LED and SOF

5.4.9 System Suitability

Acceptance Criteria: Asymmetry of both the analytes peak in standard should not be more than 2.0. Theoretical plates of both the analytes peak in standard should not be less than 2000. Relative Standard Deviation for three replicates injections of the standard preparation should not be more than 2.0 %.

As system suitability test is an integral part of chromatographic method development and is used to verify that the system is adequate for the analysis to be performed, the system suitability parameters for Sofosbuvir and ledispavir were evaluated.

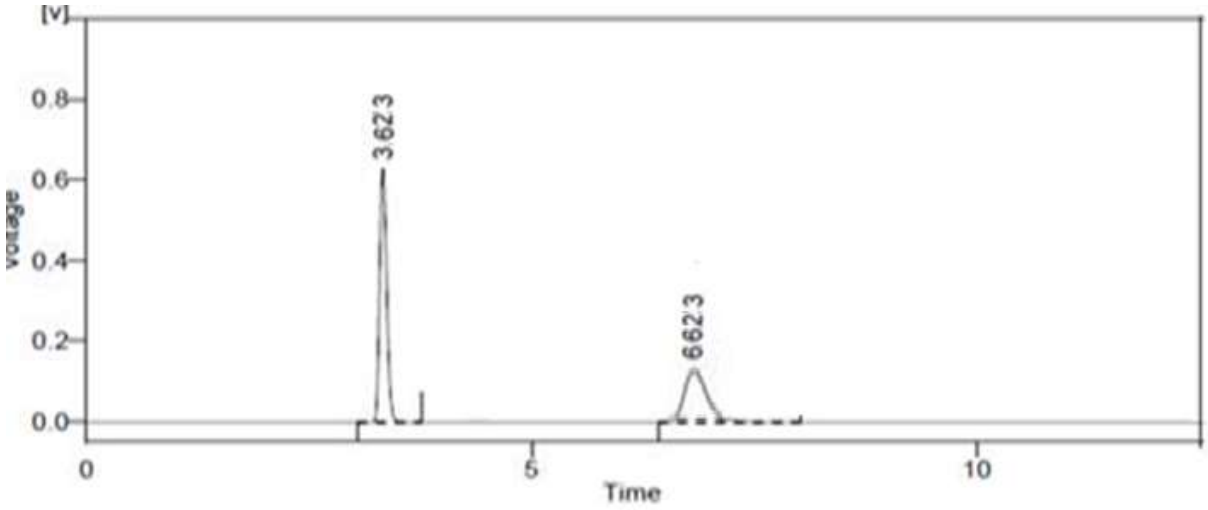

Figure 15: System Suitability of LED and SOF

5.4.10 Summary of Validation Parameters:

Table 4: Regression data analysis and summary of validation parameters of LED and SOF for proposed method

Validation Parameter				**Result**	
			LED		**SOF**
Linearity Range (µg/mL)			0.5 - 1.5		50 – 150
Correlation Coefficient (R^2)			0.9992		0.9989
Regression Equation (y = mx + c)			y = 1479.2x - 11.73		y = 30.146x + 78.959
Precision		Repeatability (n = 6)			
			1.56		0.88
		Intra-day Precision (n=3)	0.39	– 1.40	0.38 – 0.56
(%RSD)					
		Inter-day Precision (n=3)	1.12	– 1.77	0.53 – 1.09
Accuracy (% Recovery), (n = 3)			100.30	– 100.80	99.80 – 100.90
Limit of Detection (µg/mL)			0.13		14.88
Limit of Quantification (µg/mL)			0.38		45.10
Robustness			Complies		Complies
Solution Stability			Complies		Complies
System Suitability			Complies		Complies

6. CONCLUSION:

A validated stability-indicating RP-HPLC analytical method has been successfully applied for quantitative determination of LED and SOF in tablet dosage form. The stability-indicating RP-HPLC method developed meets the system suitability criteria and resolution of the parent drugs from its degraded products. Detection and quantification limits achieved, describe the sensitivity of developed method. High recovery and acceptable % RSD values confirms that the established RP-HPLC method is accurate and precise. The complete separation of the drug and its degradation product was accomplished within 10 minutes. The method has been successfully applied to perform accelerate stability study of LED and SOF. Hence, the method can be used for routine quality control analysis and pharmacokinetic study of SOF and LED.

REFERENCES:

1. Gurdeep R. Chatwal,& etal, “Instrumental Method of Chemical Analysis,” 1st Edition, PP- 2.149.
2. Gurdeep R. Chatwal, Sham K. Anand, “Instrumental Method of Chemical Analysis,” 1st Edition, PP- 2.149.
3. Gurdeep R. Chatwal, Sham K. Anand, “Instrumental Method of Chemical Analysis,” 1st Edition, PP- 2.167-2.172.
4. Sharma Y.R., “Elementary Organic Spectroscopy,” 1st Edition, PP- 52-54.
5. A.H. Beckett, J.B. Stenlake, “Practical Pharmaceutical Chemistry,” Part – II, 4th Edition, PP-269-273.
6. A.H. Beckett, J.B. Stenlake, “Practical Pharmaceutical Chemistry,” Part – II, 4th Edition, PP- 279-282.
7. A.H. Beckett, J.B. Stenlake, “Practical Pharmaceutical Chemistry,” Part – II, 4th Edition, PP- 287.
8. A.H. Beckett, J.B. Stenlake, “Practical Pharmaceutical Chemistry,” Part – II, 4th Edition, PP- 288.
9. Gurdeep R. Chatwal, Sham K. Anand, “Instrumental Method of Chemical Analysis,” 5st Edition, PP- 2.566.
10. Gurdeep R. Chatwal, Sham K. Anand, “Instrumental Method of Chemical Analysis,” 5st Edition, PP- 2.567.
11. Lippncott Williams and Wilkins, “Remington the Science and Practice of pharmacy,” Vol. - I, 21st Edition, PP- 667.
12. Gurdeep R. Chatwal, Sham K. Anand, “Instrumental Method of Chemical Analysis,” 5st Edition, PP- 2.624.
13. Gurdeep R. Chatwal, Sham K. Anand, “Instrumental Method of Chemical Analysis,” 5st Edition, PP- 2.570.
14. Ravi Shankar, “Instrumental Method of Analysis”, 2nd Edition, PP-18-6.
15. Ravi Shankar, “Instrumental Method of Analysis”, 2nd Edition, PP-18-3.
16. Lioud R. Synder et al, “Practical HPLC Method Development,” 2nd Edition, PP- 503.