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Research Article

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A Novel HPLC Method for the Estimation of Sofosbuvir and Ledipasvir in Pharmaceutical Dosage Form

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Abstract The developed RP-HPLC method was used for the estimation of Sofosbuvir and Ledipasvir. The developed method was successfully validated as per ICH Q2 (R1), and from the results, it was concluded that the present method might be used for the routine estimation of the raw materials and in the pharmaceutical formulations. The linearity study reveals that the proposed method gave the linear results in the range of 40–200 µg/m Land 9–45µg/mL for Sofosbuvir and Ledipasvir respectively. From the results of precision data (intra-day and inter-day precision) and low levels of RSD, it was concluded that the proposed method was precise. The LOD and LOQ values were established for the Sofosbuvir and Ledipasvir. The % recovery for Sofosbuvir was found to be 99.56 %, 99.23% and 99.71% and for Ledipasvir it was found to be 98.19%, 97.54 % and 99.16%. The low values of RSD reveal that the method was accurate in the established range. The standard deviation of the retention time was calculated for each parameter to check the robustness of the method and the RSD was found to be less than 2 % for Sofosbuvir and Ledipasvir.

Keywords Sofosbuvir, Ledipasvir, RP-HPLC, linearity

Introduction

Viruses are the ultimate expression of parasitism. They not only take the nutrition from the host cell but also direct its metabolic machinery to synthesize new virus particles. Viral chemotherapy was therefore considered impossible as it would require interference with cellular metabolism in the host. However in the past 50 years virus directed enzymes have been identified in the infected cell and some viruses have few enzymes of their own which may have higher affinities for some antimetabolites or inhibitors then the regular cellular enzymes. In addition, drugs have been developed which direct target specific steps like cell penetration, uncoating, reverse transcription, virus assembly or maturation [1-8].

Viruses consist of nucleic acid and a protein coat. The nucleic acid of the virusinstructs the host cell to produce viral components, which leads to an infectious virus. Many factors account for the difficulty in developing antiviral agents. The structure of each virus differs, and specific therapy is often unsuccessful because of periodic changes in the antigenic proteins of the virus. The need for a host cell to support the multiplication of the virus makes treatment difficult because the agent must be able to inhibit the virus without seriously affecting the host cells. An antiviral agent must act at one of five basic steps in the viral replication cycle in order to inhibit the virus [9-11].

Virus, infectious agent of small size and simple composition that can multiply only in living cells of animals, plants or bacteria. The name is from a Latinword meaning slimy liquid or poison. A typical virus consists of a protective protein coat, known as a capsid. The capsid shape varies from simple helical and icosahedra forms to more complex

structures with tails. The capsid provides protection for the viral genome against the environment and functions in receptor recognition, targeting the virus to a susceptible host and cell type.

Some viruses have a phospholipids envelope, from the infected host's cell membrane, that surrounds the protein capsid. Inserted into the lipid envelope there are usually viral encoded proteins known as spike projections, is typically glycoprotein and are also involved in receptor recognition and viral tropism [12].

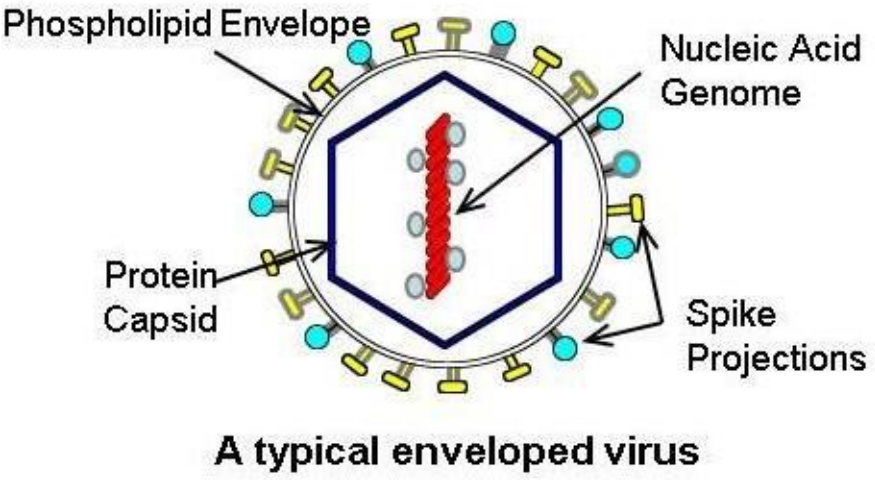


Figure 1: Diagrammatic Representation of an enveloped virus [3]

The greatest success against virus infections has been by increasing immunity through vaccination with live attenuated or killed viruses. Some virus groups contain 50 or more different viruses, making effective vaccination difficult. Passive immunization with serum or globulin from immune persons has been used to prevent viral infections. Immunoglobulins such as those used against hepatitis and respiratory syncytial virus, are effective only for prevention, not for treatment [13-14].

Materials and Methods

Active Pharmaceutical Ingredient

Pure Sofosbuvir and Ledipasvir were obtained as gift samples from Zydus Cadila (Cadila Healthcare Ltd.), Kundaim Goa.

Identification of Drugs

Physicochemical properties of all the drugs were determined by description, state, odour and measuring solubility in different solvents like Water, Acetone, Chloroform, and Methanol. Identification of Drugs was carried out by melting point range determination by using melting point apparatus and spectral analysis by comparing the IR spectra of the individual drug with their standard.

Method Development and estimation of Sofosbuvir and Ledipasvir

Optimized Chromatographic Condition

The HPLC experimental conditions were optimized on the Cosmosil C 18, (250 mmx4.6 mm, internal diameter, 5 µm particle size) analytical column.

Table 1: Optimized Chromatographic Conditions

Parameters	Conditions
Column	C_{18} COSMOSIL, (250mm x4.6mm,5µm)
Mobile Phase	Acetonitrile:0.1%OPA, (55:45)% V/V
Flow rate	0.7 mL/min
Column oven temperature	30±0.3°C
Auto sampler temperature	15±3°C

Volume of injection	20µL
Detector	PDA detector
Detection Wavelength	283 nm
Runtime	10.0minutes

Preparation of Solutions Orthophosphoric acid in water (0.1%V/V)

Pipette out 0.5mL of orthophosphoric acid in to measuring cylinder (500mL capacity) containing 250 mL HPLC grade water and made up the volume upto 500mL with HPLC grade water. Transferred into a reagent bottle and mixed the contents thoroughly. Stored at ambient temperature. This solution was used within 3 days from the date of preparation.

Mobile phase Acetonitrile: 0.1%OPA (55:45%V/V)

In measuring cylinder 550 mL of Acetonitrile and 450 mL of 0.1% OPA was taken, then transferred into a reagent bottle and mixed the contents thoroughly. Stored at ambient temperature. This solution was used within 3 days from the date of preparation. The same was used as the diluent.

Auto sampler Rinsing Solution

In measuring cylinder 500 mL of Methanol and 500 mL of water was taken, then transferred into a reagent bottle and mixed the contents thoroughly. Stored at ambient temperature. This solution was used within 3 days from the date of preparation.

Sofosbuvir Stock Solution, 4000 µg/mL

Accurately weighed 40 mg of standard Sofosbuvir was transferred to a 10 mL volumetric flask and appropriate volume of Methanol was added to make final concentration of Sofosbuvir equivalent to 4000 µg /mL. The solution was stored in refrigerator at 5±3°C and used within solution within 7 days from date of preparation.

Ledipasvir Stock Solution, 900µg/ mL

Accurately weighed 9 mg of standard Ledipasvir was transferred to a 10 mL volumetric flask and appropriate volume of Methanol was added to make final concentration of Ledipasvir equivalent to 900 µg/ mL. The solution was stored in refrigerator at 5±3°C and used within solution within 7 days from date of preparation.

Mix Stock solution, (Sofosbuvir 40 µg/m Land Ledipasvir 9 µg/mL):

0.1 mL of Stock Solution of Sofosbuvir 4000µg/ mL and Ledipasvir 900µg/mL was transferred in 10.0 mL volumetric flask. The solution was made up to the mark using diluent to obtain a solution containing concentration of Sofosbuvir 40 µg/mL and Ledipasvir 9 µg/mL. The solution was stored in refrigerator at 5±3°C and used within solution within 7 days from date of preparation.

System Suitability

This was carried out to verify that the chromatographic system was suitable for intended application. Some of the parameters which can be checked using system suitability are Precision Requirement, Theoretical Plates and Tailing Factor.

Acceptance criteria

Relative standard deviation for peak area less than 2 %. Theoretical plates more than 2000. Tailing Factor between 0.85 to 2.0

Method Validation

The developed method was validated for various parameters as required by ICH Q2 (R1) guideline.

Specificity

Specificity was demonstrated by the resolution of the two compounds, Sofosbuvir and Ledipasvir. The sample was injected six times wherein it was observed that the two sharp peaks for Sofosbuvir and Ledipasvir were obtained and the sample matrix did not show any interference with the analyte peaks.

Linearity

The linearity of the method was determined at different concentration levels two times. The calibration curve was constructed by plotting response factor against the concentration of drugs. Sofosbuvir and Ledipasvir exhibited linearity of the concentration range of 40-200 µg/mL for Sofosbuvir and 9-45 µg/mL for Ledipasvir respectively were injected and chromatograms were recorded.

Precision

System Repeatability (Intra-Day Precision)

The system repeatability was determined by six replicates of the prepared sample solutions.

The repeatability of the sample application and measurement of peak area for the drugs were calculated by assay six times at concentration level of 80 µg/mL for Sofosbuvir and 18 µg/mL for Ledipasvir in the same day at 2 hours' time interval for intra-day precision.

Intermediate Precision (Inter-Day Precision)

- The intermediate precision was determined by six replicates of the prepared sample solutions.
- The intermediate precision of the sample application and measurement of peak area was obtained by the assay of six sample sets on different days at concentration levels of 80 µg/mL for Sofosbuvir and 18 µg/mL for Ledipasvir for inter-day precision.

Accuracy

Accuracy studies were carried out by spiking the sample three times with known concentration of the standard drug. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets with Sofosbuvir 40 µg/mL and Ledipasvir 9 µg/mL with three different concentrations of standards Sofosbuvir 32, 40, 48 µg/mL and Ledipasvir 7.2,9, 10.8 µg/mL respectively. The good recoveries with the standard addition method prove the good accuracy of the proposed methods.

Robustness

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as Flow Rate, pH and wavelength. The sample was injected three times wherein it was observed that there were no marked changes in the analytical performance of the method.

Flow rate: ± 0.1 mL/min Wavelength:±2 nanometer pH:± 0.1

Limit of Detection (LOD) and Limit of Quantization (LOQ)

The limit of detection (LOD) and quantization (LOQ) for SOF and LED were determined according to ICH Guidelines. LOD was defined as 3.3σ/S, and LOQ was 10σ/S based on "standard deviation of the response and slope" of the calibration curve specially constructed in a low region of 0.05 to 1% of the target analyte concentration. The standard deviation of the y-intercepts of the regression lines was used as σ (the standard deviation of the response), and S is the slope of the calibration curve.

Assay

% Assay determination of Sofosbuvir and Ledipasvir in Combined Dosage Formulation

20 tablets were weighed accurately and powdered. 188.3g of the powder was accurately weighed and diluted with 10 mL of methanol and sonicated for 15 minutes. This was then filtered to give a solution containing 4000 µg/mL of Sofosbuvir and 900 µg/mL of Ledipasvir From the above solution a final concentration of 80 µg/mL of Sofosbuvir and 18 µg/mL of Ledipasvir was prepared.

Results and Discussion

Characterization and Identification of Drugs

Physical Properties of Drugs Used in Project Work

Table 2: Physical Properties of Sofosbuvir and Ledipasvir

Physical Property	Sofosbuvir		Ledipasvir	
	Observed	**Standard**	**Observed**	**Standard**
Appearance	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder

Solubility

Sofosbuvir was freely soluble in Methanol while Ledipasvir was freely soluble in Ethanol and Methanol which was complied with its standard for solubility.

Melting Point determination

The melting points of both API were taken and compared with the reported melting point. The melting points were found to be in the range of reported melting points.

Table 3: Melting Point Study of Drug Samples

Drug Sample	Observed Melting Point	Reported Melting Point
Sofosbuvir	100-106ºC	105ºC
Ledipasvir	170-225ºC	178ºC

IR Identification

IR Spectral Analysis of Sofosbuvir

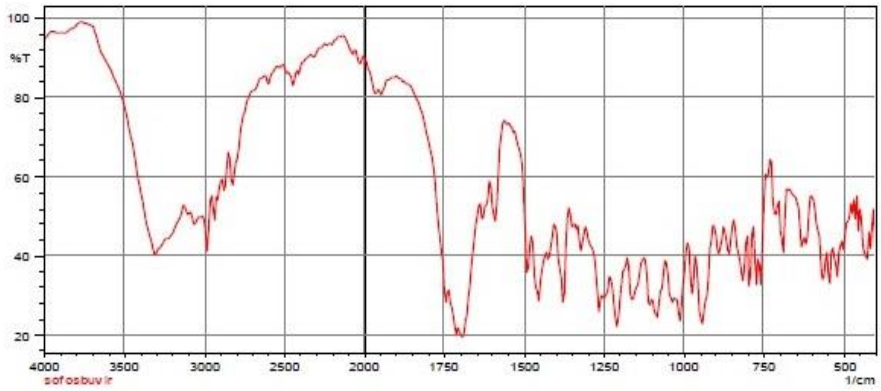


Figure 2: IR Spectra of Sofosbuvir

IR Spectral Analysis of Ledipasvir

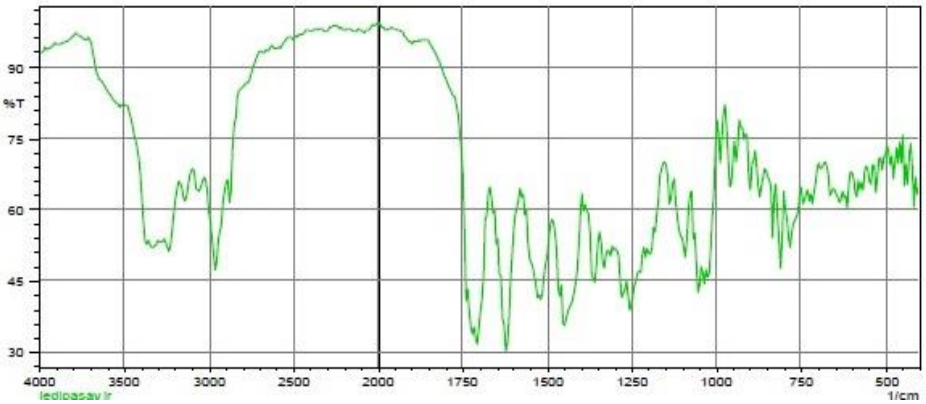


Figure 3: IR Spectra of Ledipasvir

Table 4: IR Spectral Interpretation of Ledipasvir

Sr. No.	Functional group	Observed Frequency (cm^{-1})
1	C=O(Ester)	1715
2	C=O(Anilide)	1645
3	NH	3350

Method Development

Selection of stationary phase

Both the drugs Sofosbuvir and Ledipasvir are polar, hence carried out using C18 column.

Selection of wavelength

Selectivity of HPLC method depends on the wavelength selected hence a selection of the wavelength was done in such a way that both the drugs gave good response, 283 nm was selected as a detection wavelength for Sofosbuvir and Ledipasvir.

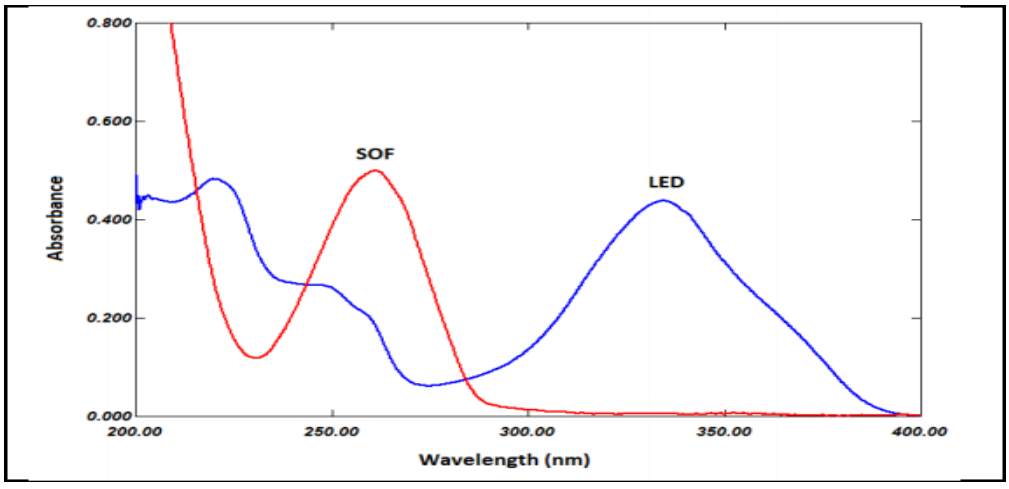


Figure 4: Wavelength Selection - Overlay UV spectra of SOF (20 µg/mL) and LED (4.5 µg/mL)

Selection of mobile phase

Based on the literature survey, a mixture of Acetonitrile and 0.1% Ortho phosphoric acid in different ratio was selected as the mobile phase and trials were carried out. From the trials carried, finally a mixture of Acetonitrile:

0.1% OPA in the ratio of 55:45 %V/V was selected and further trials were carried out to get optimized chromatograms by changing the other parameters.

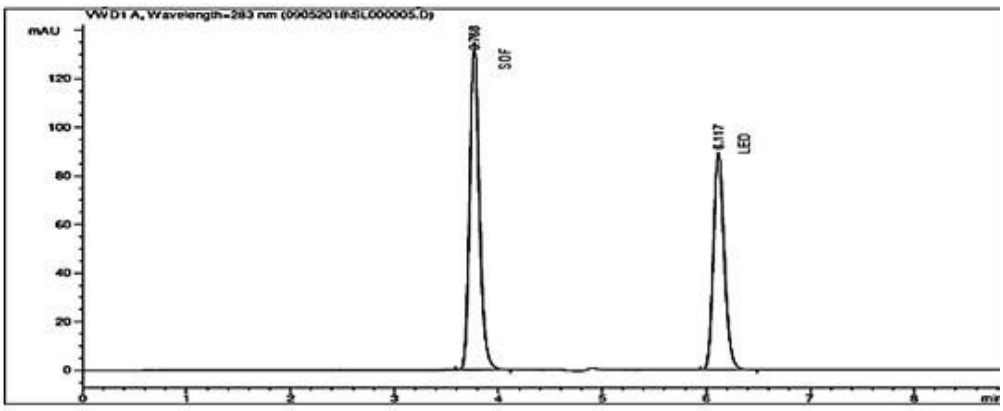
Trials taken during the selection of mobile phase and other parameters.

The conditions selected for trial are depicted in table.

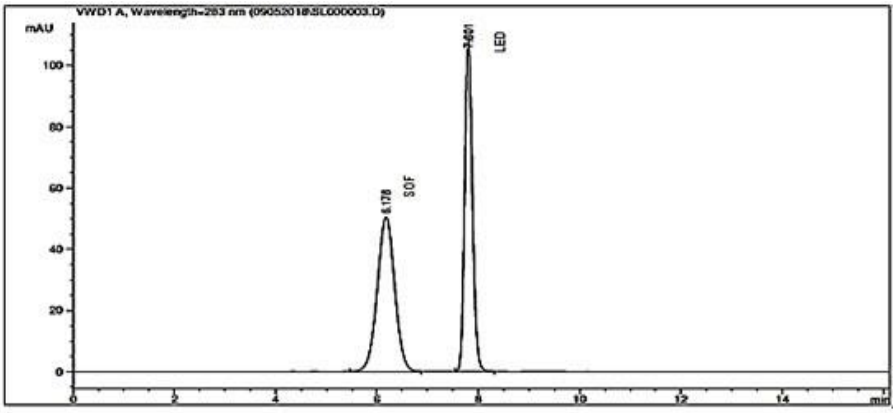
Trials taken for the selection of mobile phase

Table 5: Trials taken for the selection of mobile phase

Sr. No.	Column	Mobile phase	Flow rate mL/min	Observation
1	C18 COSMOSIL, (250mmx 4.6mm, 5microns)	MeOH: 0.1% OPA(80:20 %V/V)	0.7 mL	Poor Chromatogram was observed
2	C18 COSMOSIL, (250mmx 4.6mm, 5microns)	MeOH: 0.1% OPA(70:30 %V/V)	0.7 mL	Poor Chromatogram and less resolution was observed
3	C18 COSMOSIL, (250mmx 4.6mm, 5microns)	ACN:0.1%OPA (80:20 %V/V)	0.7 mL	Better resolution and slight tailing was observed
4	C18 COSMOSIL, (250mmx 4.6mm, 5microns)	ACN:0.1%OPA (75:25 %V/V)	0.7 mL	Better resolution and slight tailing was observed
5	C18 COSMOSIL, (250mmx 4.6mm, 5microns)	ACN:0.1%OPA (50:50 %V/V)	1.0 mL	Chromatogram was Good but still slight resolution modification was required for better result
6	C18 COSMOSIL, (250mmx 4.6mm, 5microns)	ACN:0.1%OPA (50:50 %V/V)	0.7 mL	Better resolution and chromatography was observed



Trial No 1



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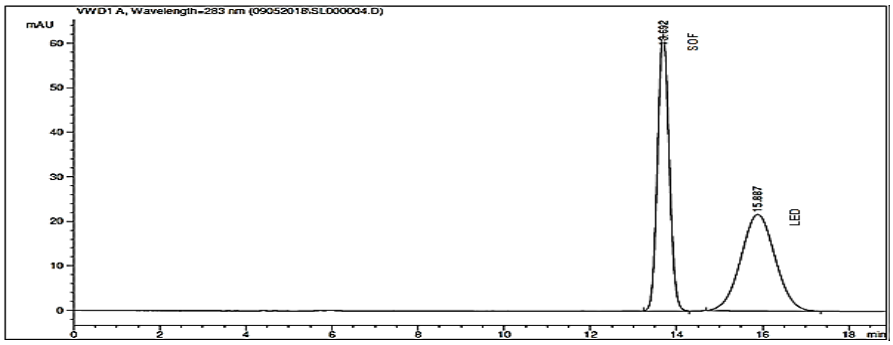

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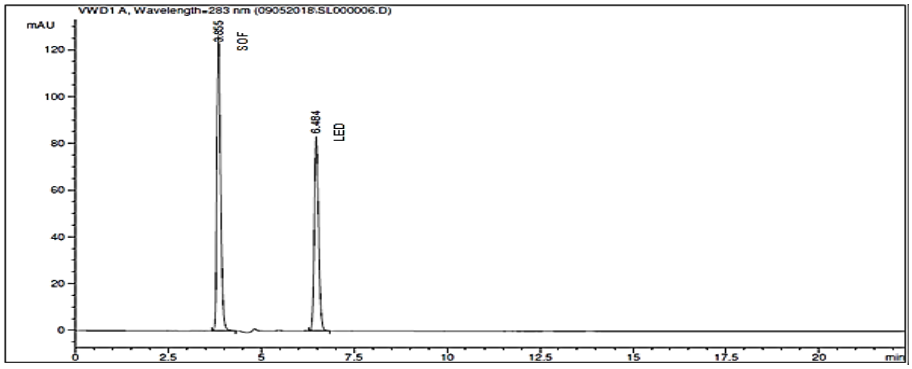

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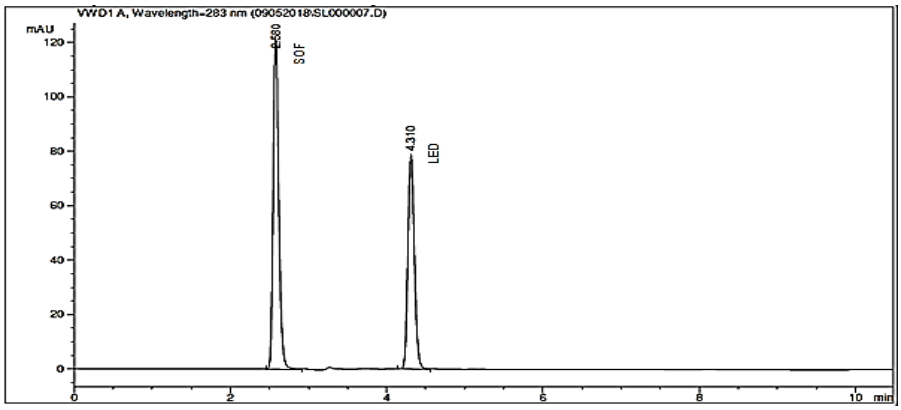

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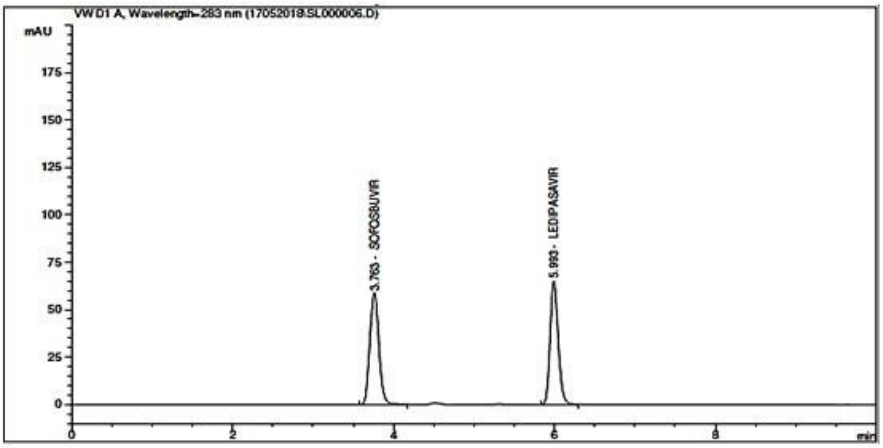

Trial No 6

Figure 5: Trial Chromatogram of Sofosbuvir and Ledipasvir

Establishment of the Retention time of individual drugs

The diluted mixed standard of the drugs was injected into the system to check for the separation. Slight changes were done in flow rate so as to achieve greater resolution and higher number of theoretical plates and finally the following conditions were established to give an optimized chromatogram.

- HPLC make: Agilent Technologies 1100 gradient system
- Particle size packing: 5µm
- Stationary phase: C_{18} COSMOSIL, (250mmx4.6mm, 5microns)
- Detection wavelength: 283nm
- Flowrate: 0.7mL/min
- Mobile Phase: Acetonitrile:0.1 % OPA, in the ratio of 55:45 % V/V
- Temperature: Ambient
- Size: 20 µL

Standard drug mixture separation

The retention time, Theoretical Plates of the two drugs is shown in table and the chromatogram is shown in Figure.

Table 6: Retention time of SOF and LED

Parameters	Sofosbuvir	Ledipasvir
Retention time (min)	3.669	5.926
Theoretical plates	5535	17825
Tailing factor	0.925	0.810
Resolution		11.80

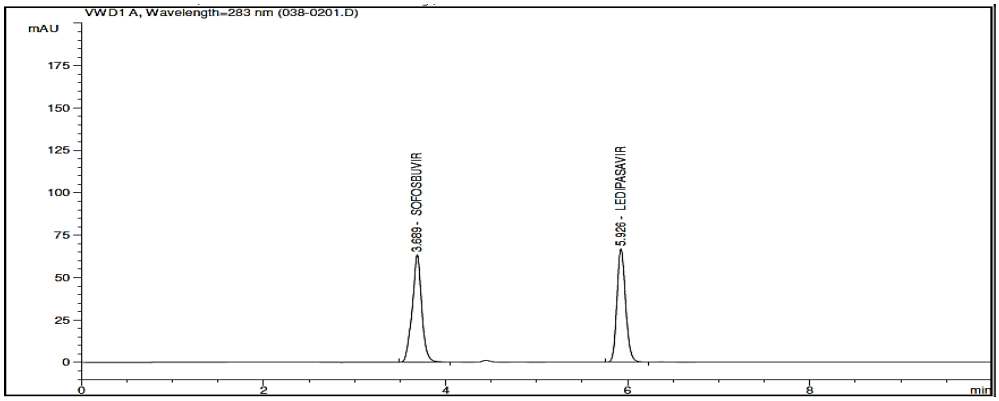

Figure 6: Chromatogram for separation of Sofosbuvir and Ledipasvir

System Suitability

The column efficiency, resolution and tailing factor were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of the selected drug combinations. System suitability parameters may fall within 2 % relative standard deviation range during routine performance of the method. The chromatogram is in figure and comparative results of system suitability parameter are in table.

Table 7: System Suitability

Results for SOF

n =6	Peak Area	Retention Time	Tailing Factor	Theoretical Plates
1	461.385	3.783	0.930	5531.000
2	461.812	3.776	0.920	5513.000
3	462.082	3.763	0.910	5474.000
4	462.580	3.740	0.930	5556.000
5	463.860	3.739	0.910	5552.000
6	464.060	3.728	0.930	5520.000
AVG	462.630	3.750	0.920	5524.330
SD	1.100	0.020	0.010	27.370
RSD	0.240	0.540	0.970	0.500

Table 8: System Suitability Results for LED

n =6	Peak Area	Retention Time	Tailing Factor	Theoretical Plates
1	452.568	5.992	0.800	17849.000
2	453.035	6.000	0.810	17925.000
3	452.296	5.990	0.810	17855.000
4	450.560	5.980	0.810	17750.000
5	451.250	5.990	0.800	17827.000
6	451.870	5.970	0.810	17739.000
AVG	451.930	5.990	0.810	17824.170
SD	0.910	0.010	0.004	63.896
RSD	0.200	0.179	0.584	0.358

Table 9: Comparative results of both the drugs

Parameters	Sofosbuvir	Ledipasvir
Retention time (min)	3.750	5.990
Theoretical plate	5524.330	17824.170
Tailing factor	0.920	0.810
Resolution	11.80	

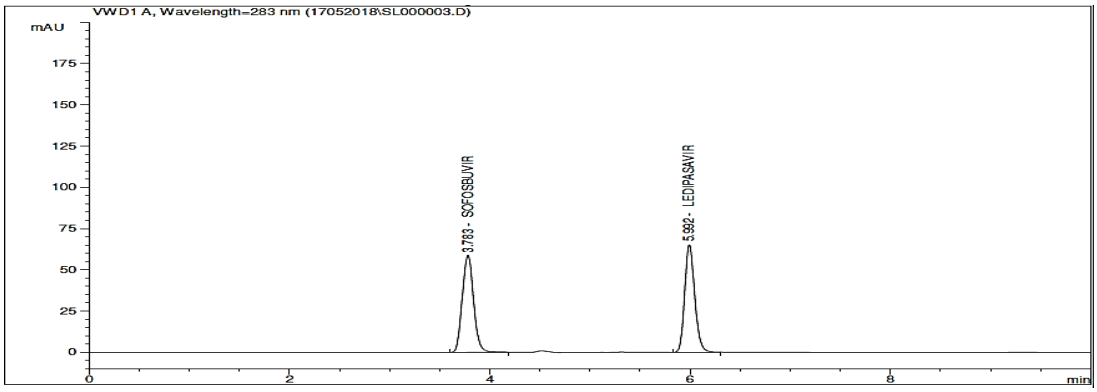

Figure 7: All system suitability variables of the developed method complied with its standard

Method Validation

Specificity

The selectivity of the method was checked by injecting solutions of both drugs. It was observed that two sharp peaks for Sofosbuvir and Ledipasvir were obtained at retention times 3.776 and 3.763, 6.004 and 5.993 min for standard and sample respectively. The retention times of the drug standards and the drugs from sample solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the tablets. (Fig shows Sofosbuvir and Ledipasvir Standard and Sample Chromatogram).

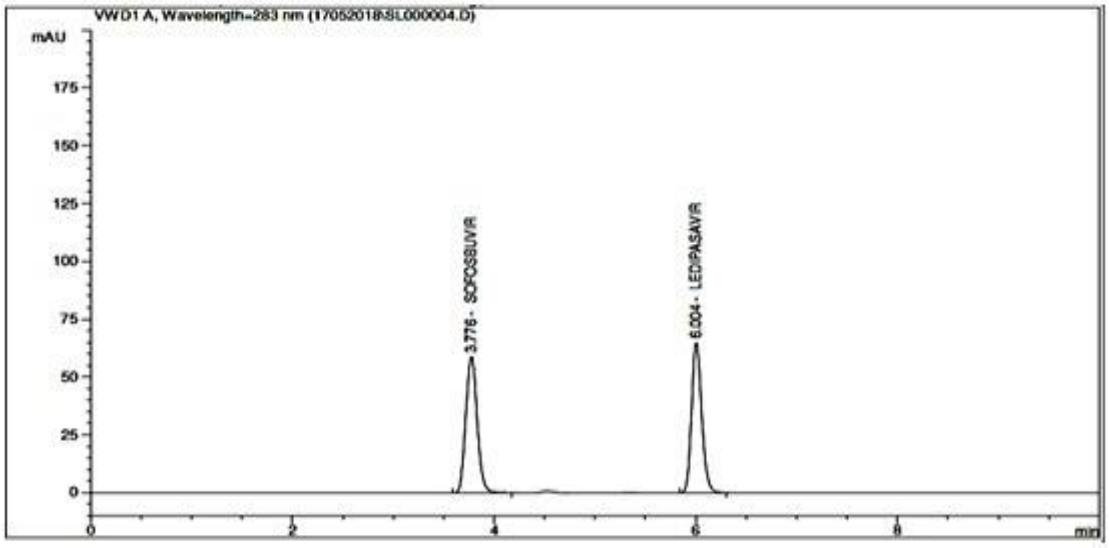

Figure 8: Chromatogram of Sofosbuvir and Ledipasvir Standard

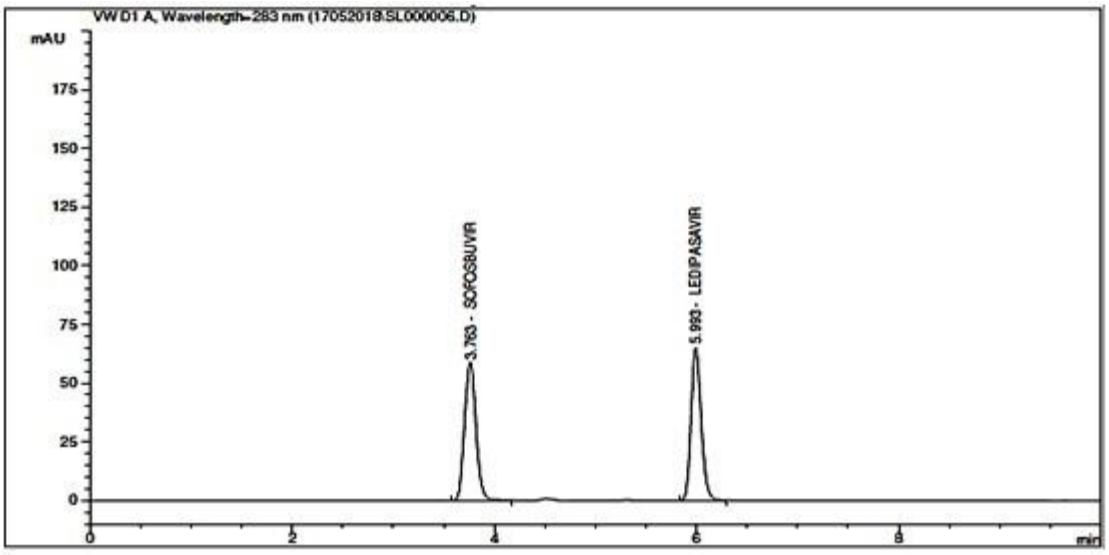

Figure 9: Chromatogram of Sofosbuvir and Ledipasvir Sample

Linearity

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against the concentration of drugs. Sofosbuvir and Ledipasvir exhibited linearity of the concentration range of 40-200 µg/mL and 9-45µg/mL. Plot the graph for Area vs. Concentration to get calibration curve. The linearity of Sofosbuvir is in Table and the calibration curve in Figure, and for the Ledipasvir the linearity is in Table and the calibration curve in Figure. The calibration curve was found linear.

Table 10: Linearity data of Sofosbuvir

Conc. (µg/mL)	Peak Area 001	Peak Area 002	Average Peak area (n=2)
40	238.994	237.907	238.450
80	468.212	471.527	469.870
120	715.665	713.112	714.389
160	942.344	937.266	939.805
200	1200.008	1205.240	1202.624

Table 11: Linearity data of Ledipasvir

Conc (µg/mL)	Peak Area 001	Peak Area 002	Average Peak area (n=2)
9	216.571	215.947	216.259
18	444.776	446.687	445.731
27	665.105	667.325	666.215
36	899.079	897.853	898.466
45	1139.157	1129.372	1134.264

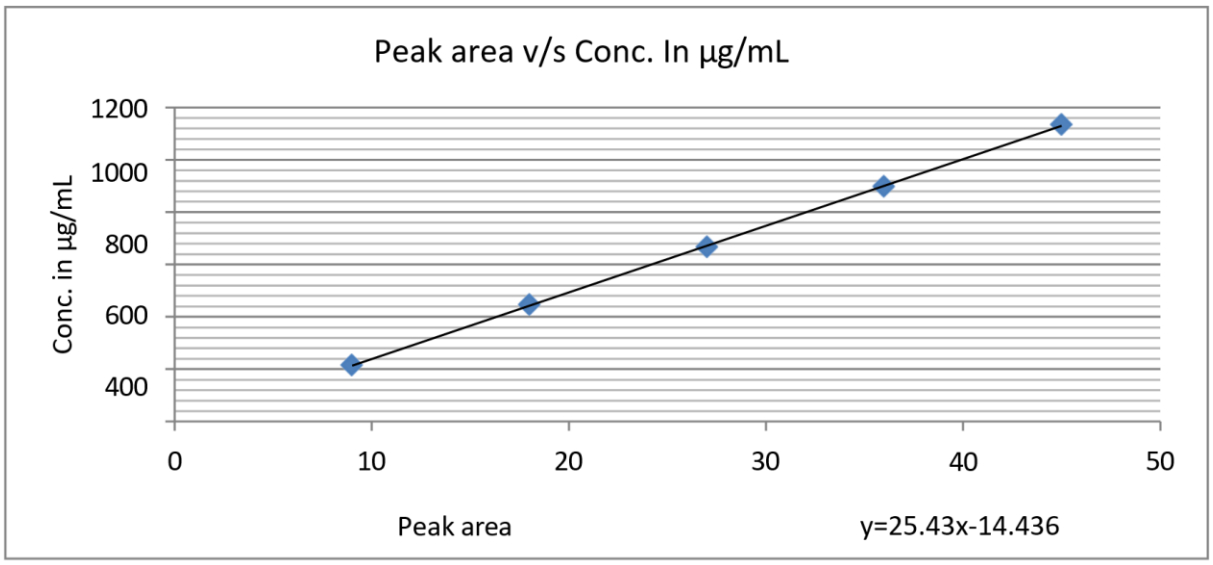

Figure 10: Calibration plot for Ledipasvir

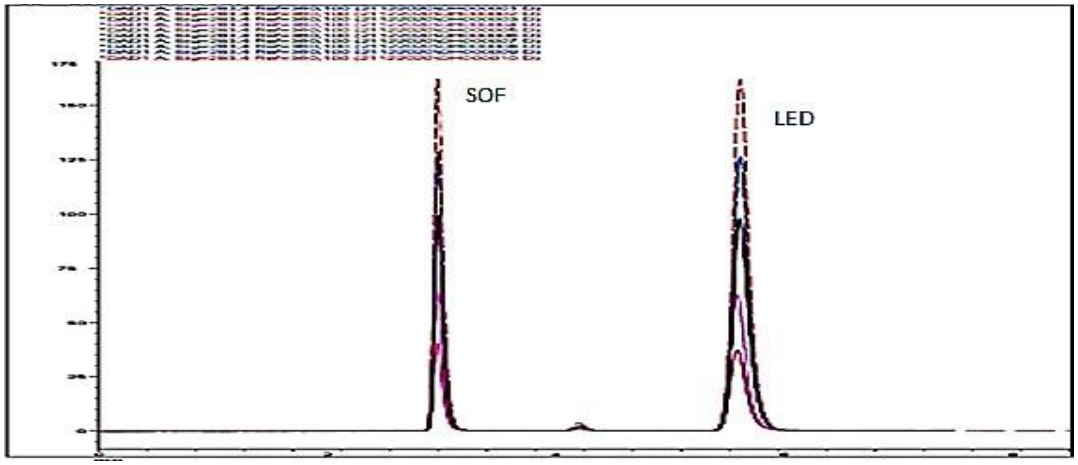

Figure 11: Overlay chromatogram of linearity data of Sofosbuvir and Ledipasvir

Precision

The precision of the method was demonstrated by system repeatability (intraday precision) and intermediate precision (inter-day precision) variation studies.

System Repeatability (Intra-Day precision)

The system repeatability was determined by six replicates of the prepared sample solutions. The repeatability of the sample application and measurement of peak area for the drugs were calculated by assay six times at concentration levels of 40, 80,120 µg/mL for Sofosbuvir and 9, 18, 27 µg/mL for Ledipasvir respectively for intra-day precision. Peak areas were determined and RSD was calculated and found to be less than 2% and it is presented in table.

Table 12: Results of Intraday precision

Sofosbuvir			Ledipasvir		
Conc. (µg/mL)	**Area Average (n=6)**	**RSD**	**Conc. (µg/mL)**	**Area average (n=6)**	**RSD**
40	238.994	0.320	9	216.259	0.200
80	468.630	0.590	18	449.229	0.260
120	715.665	0.250	27	666.215	0.240

Intermediate Precision (Inter-Day precision)

The intermediate precision was determined by three replicates of the prepared sample solutions. The intermediate precision of the sample application and measurement of peak area was obtained by the assay of three sample sets on different days at concentration levels of 80 µg/mL for Sofosbuvir and 18 µg/mL for Ledipasvir in different day time interval for inter- day precision. Peak areas were determined and RSD was calculated and found to be less than 2% and it is presented in table.

Table 13: Results of Inter-day precision

DAY	Sofosbuvir			Ledipasvir		
	Conc. (µg/mL)	**Area Average (n=3)**	**RSD**	**Conc. (µg/mL)**	**Area average (n=3)**	**RSD**
1	40	238.994	0.320	9	216.259	0.200
	80	468.630	0.590	18	449.229	0.260
	120	715.665	0.250	27	666.215	0.240
2	40	238.712	0.269	9	216.481	0.092
	80	465.070	0.460	18	450.070	0.200
	120	715.532	0.093	27	665.417	0.027
3	40	238.379	0.142	9	216.638	0.091
	80	466.660	0.320	18	451.750	0.210
	120	715.365	0.130	27	665.612	0.044

Accuracy

The accuracy of the methods was assured by the use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (Sofosbuvir 40 µg/mL and Ledipasvir 9µg/mL) with three different concentrations of standards (Sofosbuvir 32, 40, 48 µg/mL and Ledipasvir 7.2, 9, 10.8 µg/mL). The good recoveries with the standard addition method prove the good accuracy and the recovery studies were carried out according to ICH Guidelines, given in table.

Table 14: Results of Accuracy

Drug	Accuracy Level (%) (n=3)	Amount of drug taken (µg/mL)	Amt. of API added (µg/mL)	Total Amount found (µg/mL) ± S.D.(n=3)	% Recovery ± SD (n=3)
Sofosbuvir	80	40	32	71.540	98.56
	100	40	40	79.690	99.23
	120	40	48	87.870	99.71
Ledipasvir	80	9	7.2	16.070	98.19
	100	9	9	17.780	97.54
	120	9	10.8	19.710	99.16

Robustness

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as FlowRate, pH and wavelength. It was observed that there were no marked changes in the analytical performance of the method. The results presented indicate that the low values of % RSD (< 2) of % drug content obtained after introducing small changes in the method parameters were indicative of the robustness of the method as presented in Table.

Table 15: Robustness Data of Sofosbuvir and Ledipasvir (n=3)

Flow Rate (mL/min)		Area SOF	Rt SOF	Conc. (µg/mL)	Area LED	Rt LED	Conc. (µg/mL)
0.6	Average	459.860	**4.025**	80	478.550	**6.101**	18
	RSD	0.230	**0.068**		0.220	**0.028**	
0.7	Average	459.070	**3.712**	80	476.860	**5.907**	18
	RSD	0.352	**0.205**		0.193	**0.095**	
0.8	Average	451.340	**3.514**	80	469.070	**5.795**	18
	RSD	0.140	**0.139**		0.160	**0.182**	
pH		**Area SOF**	**Rt SOF**	**Conc. (µg/mL)**	**Area LED**	**Rt LED**	**Conc. (µg/mL)**
2.9	Average	454.120	**3.765**	80	473.800	**5.945**	18
	RSD	0.066	**0.013**		0.350	**0.117**	
3.0	Average	452.320	**3.698**	80	471.250	**5.962**	18
	RSD	0.135	**0.119**		0.261	**0.030**	
3.1	Average	453.250	**3.767**	80	474.130	**5.932**	18
	RSD	0.110	**0.109**		0.330	**0.025**	
Wavelength(nm)		**Area SOF**	**Rt SOF**	**Conc. (µg/mL)**	**Area LED**	**Rt LED**	**Conc. (µg/mL)**
281	Average	469.900	**3.711**	80	460.580	**5.965**	18
	RSD	0.030	**0.040**		0.090	**0.050**	
283	Average	457.350	**3.758**	80	458.800	**5.954**	18
	RSD	0.268	**0.518**		0.530	**0.035**	
285	Average	463.530	**3.681**	80	450.280	**5.928**	18
	RSD	0.400	**0.149**		0.490	**0.177**	

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and quantitation (LOQ) for both Sofosbuvir and Ledipasvir were determined according to ICH Guidelines. LOD was defined as 3.3σ/S, and LOQ was 10σ/S based on "standard deviation of the response and slope" of the calibration curve specially constructed in a low region of the target analyte concentration. The standard deviation of the y-intercepts of the regression lines was used as σ (the standard deviation of the response), and S is the slope of the calibration curve. The LOD and LOQ values for the Sofosbuvir and Ledipasvir are presented in Table.

Table 16: Result for LOD and LOQ

Sr. No.	DRUG	LOD (µg/mL)	LOQ (µg/mL)
1	Sofosbuvir	0.421	1.280
2	Ledipasvir	0.052	0.178

Assay

The developed HPLC method was applied for the analysis of marketed tablet formulation. The results obtained are seen in table 17.

Table 17: % Results of Assay of marketed formulation

Marketed Formulation	Ingredients	Conc. µg/mL	Area (n=3)	Amount Found (µg/mL)	%Assay
Harvoni	Sofosbuvir	80	461.860	79.360	99.20%
	Ledipasvir	18	453.285	18.284	101.58%

Summary and Conclusion

The developed RP-HPLC method was used for the estimation of Sofosbuvir and Ledipasvir. The developed method was successfully validated as per ICH Q2 (R1), and from the results, it was concluded that the present method might be used for the routine estimation of the raw materials and in the pharmaceutical formulations. The linearity study reveals that the proposed method gave the linear results in the range of 40—200µg/mL and 9—45µg/mL for Sofosbuvir and Ledipasvir respectively. From the results of precision data (intra-day and inter-day precision) and low levels of RSD, it was concluded that the proposed method was precise. The LOD and LOQ values were established for the Sofosbuvir and Ledipasvir. The % recovery for Sofosbuvir was found to be 99.56 %, 99.23% and 99.71% and for Ledipasvir it was found to be 98.19%, 97.54 % and 99.16%. The low values of RSD reveal that the method was accurate in the established range. The standard deviation of the retention time was calculated for each parameter to check the robustness of the method and the RSD was found to be less than 2 % for Sofosbuvir and Ledipasvir. From the successful completion of the validation study and the results found, it was concluded that the proposed method was linear, sensitive, precise, robust and accurate for the simultaneous estimation of Sofosbuvir and Ledipasvir in raw materials and pharmaceutical formulation. The standard deviation of the retention time was calculated for each parameter to check the robustness of the method and the RSD was found to be less than 2 % for Sofosbuvir and Ledipasvir. The results of the assay of the marketed formulation were 99.20% for Sofosbuvir and 101.58% for Ledipasvir respectively. From the successful completion of the validation study and the results found, it was concluded that the proposed method was linear, sensitive, precise, robust and accurate for the simultaneous estimation of Sofosbuvir and Ledipasvir in raw materials and pharmaceutical formulation.

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