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Research Article

A new analytical method development and validation for the simultaneus estimation of ledipasvir and sofosbuvir using RP-HPLC

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ABSTRACT

A simple and selective LC method is described for the determination of LEDIPASVIR and SOFOSBUVIR in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Mixed Phosphate Buffer:ACN (55:45) with detection of 213 nm. Linearity was observed in the range 60-140 µg/ml for LEDIPASVIR oxalate (r^2 =0.999) and 6-14 µg /ml for SOFOSBUVIR (r^2 =0.996) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Liquid chromatography(LC),RSD Relative standard deviation,r^2 correlation coefficient.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books [1].

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis.Qualitative analysis is performed to establish composition of a substance.

It is done to determine the presence of a compound or substance in a given sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half life etc.

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the simultaneous estimation of Ledipasvir And Sofosbuvir pharmaceutical dosage form [2].

Plan of Work

Solubility determination of Ledipasvir and Sofosbuvir various solvents and buffers [3].

- ✓ Determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- ✓ Optimize the mobile phase and flow rates for proper resolution and retention times.
- ✓ Validate the developed method as per ICH guidelines.

METHODOLOGY

Mobile Phase

A mixture of Mixed Phosphate Buffer:ACN were prepared [10]. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately [5].

Preparation of standard stock solution of LEDIPASVIR

5 mg of LEDIPASVIR was weighed and transferred in to 100ml volumetric flask and dissolved in water and then make up to the mark with water and prepare 100 µg /ml of solution by diluting 1ml to 10ml with water [7-9].

Preparation of standard stock solution of SOFOSBUVIR

10 mg of SOFOSBUVIR was weighed in to 100ml volumetric flask and dissolved in water and then dilute up to the mark with water and prepare 100 µg /ml of solution by diluting 1ml to 10ml with water [4].

RESULTS AND DISCUSSIONS

Solubility Studies

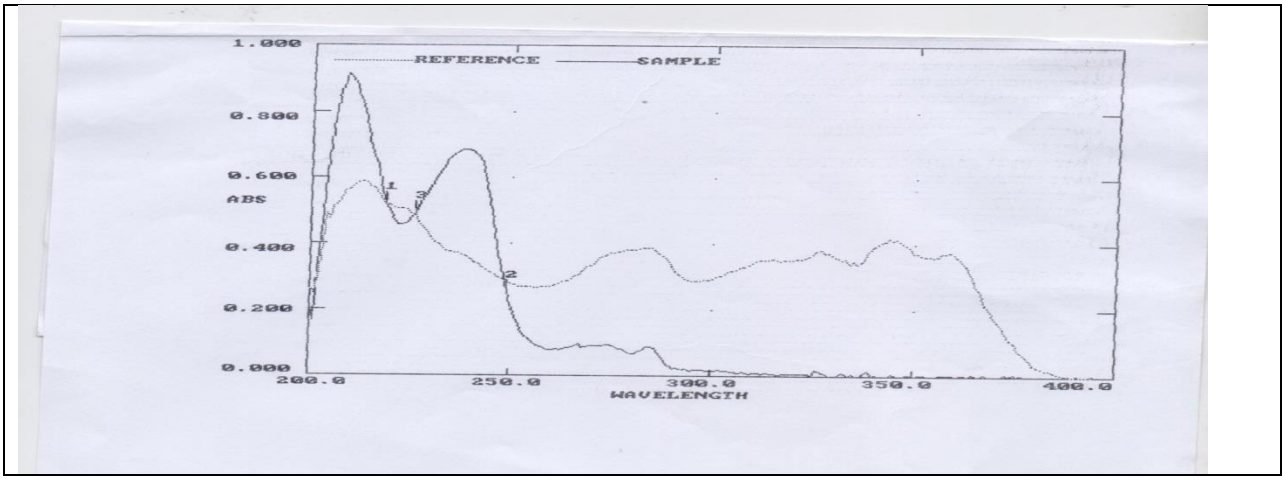
These studies are carried out at 25 ^{0} C

Ledipasvir

Freely soluble in ethanol and methanol, and slightly soluble in acetone and isopropanol and very slightly soluble in water [6].

Sofosbuvir

Freely soluble in methanol and water.



RESULTS

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra and the absorption curve shows the isobestic point was found to be 239 nm for the combination.

METHOD DEVELOPMENT OF LEDIPASVIR AND SOFOSBUVIR

Trial- 1

Chromatographic conditions

Mobile phase : Mixed phosphate buffer:ACN
pH : 6.8
Ratio : 55:45
Column : Inertsil ODS , (250×4.6× 5µ)
Wavelength : 213 nm
Flow rate : 1ml/min

Preparation of mixed standard solution

Weigh accurately 10 mg of LEDIPASVIR and SOFOSBUVIR in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.From above stock solution 10µg/ml of LEDIPASVIR and SOFOSBUVIR is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

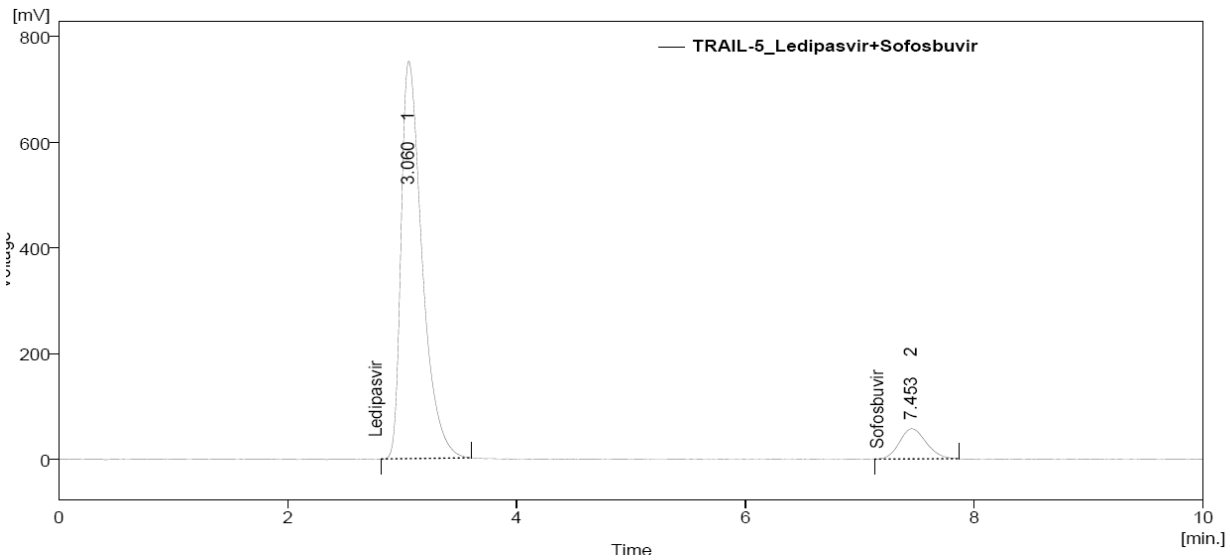


Fig. 8.3.5: Chromatogram of SOFOSBUVIR and LEDIPASVIR by using mobile phase

Observation

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for both SOFOSBUVIR and LEDIPASVIR.
- The efficiency was more than 2000 SOFOSBUVIR and LEDIPASVIR.
- Resolution between two peaks >1.5.
- Thedetails are given in the figure 8.3.8, hence this method was for optimized.

Table: Optimized chromatographic conditions

Mobile phase	Mixed phosphate buffer:ACN(55:45)
Ph	6.8
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	213
Injection volume	20 µl
Run time	10min
Retention time	About 3.060 min for LEDIPASVIR and 7.453 min for SOFOSBUVIR.

Assay

Preparation of mixed standard solution

weigh accurately 10mg of LEDIPASVIR and 10 mg of SOFOSBUVIR in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of LEDIPASVIR and SOFOSBUVIR is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains SOFOSBUVIR-400 mg LEDIPASVIR-90 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of SOFOSBUVIR and LEDIPASVIR (µg/ml) were prepared by dissolving weight equivalent to 10 mg of SOFOSBUVIR and LEDIPASVIR and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of SOFOSBUVIR and LEDIPASVIRwas made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of SOFOSBUVIR and LEDIPASVIRpresent in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

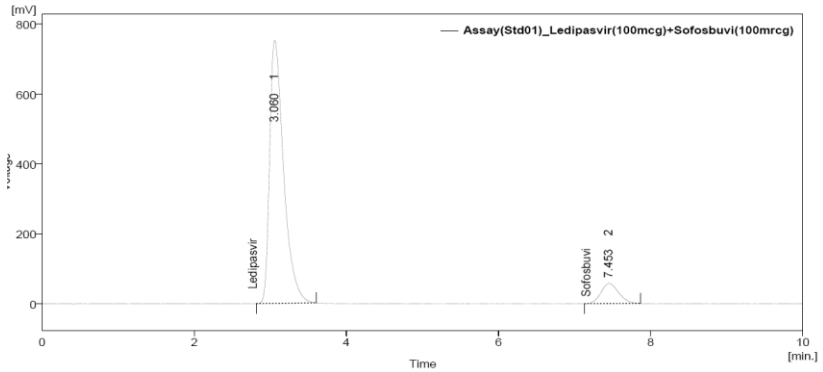


Fig: Chromatogram of Assay standard preparation-1

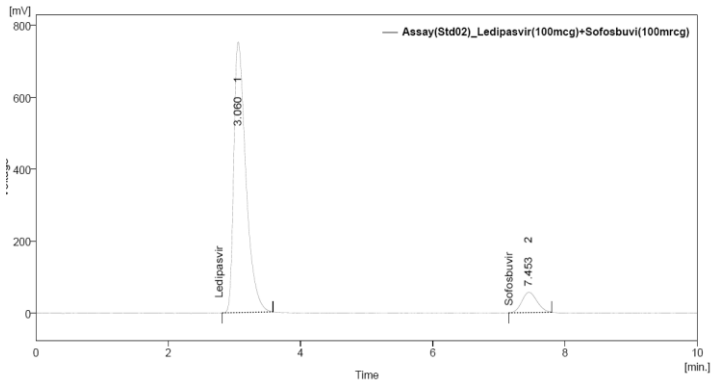


Fig: Chromatogram of Assay standard preparation-2

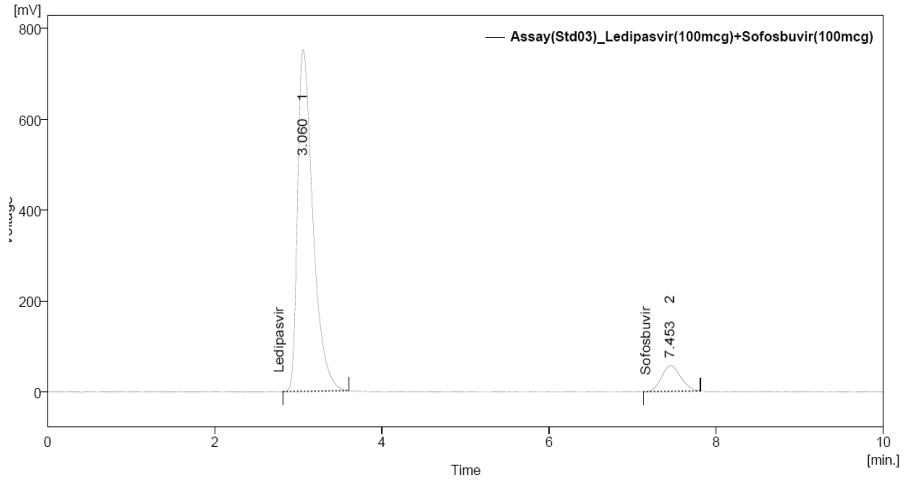


Fig: Chromatogram of Assay standard preparation-3

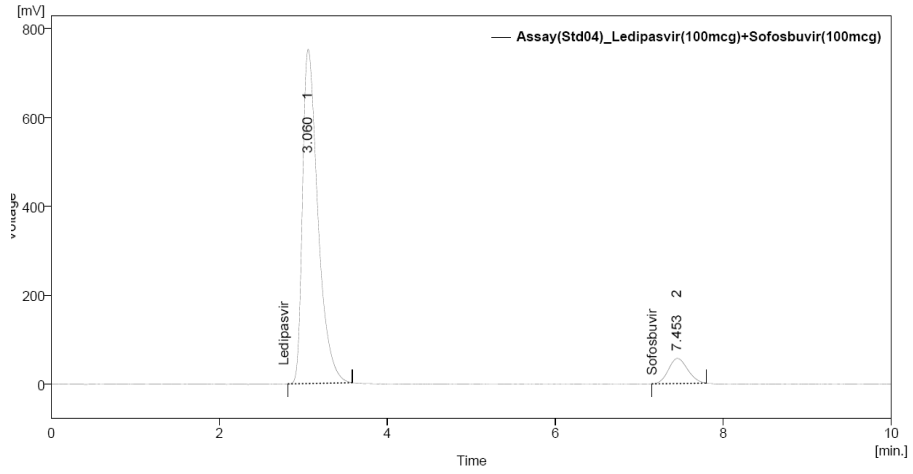


Fig: Chromatogram of Assay standard preparation-4

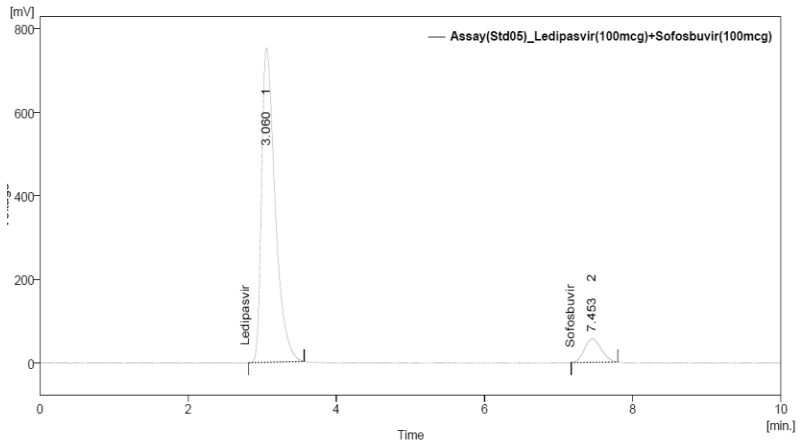


Fig: Chromatogram of Assay standard preparation-5

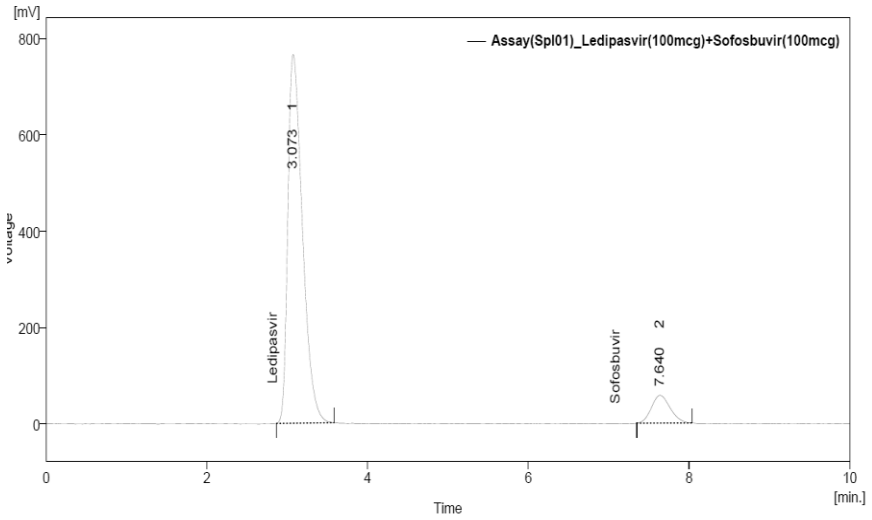


Fig: Chromatogram of Assay sample preparation-1

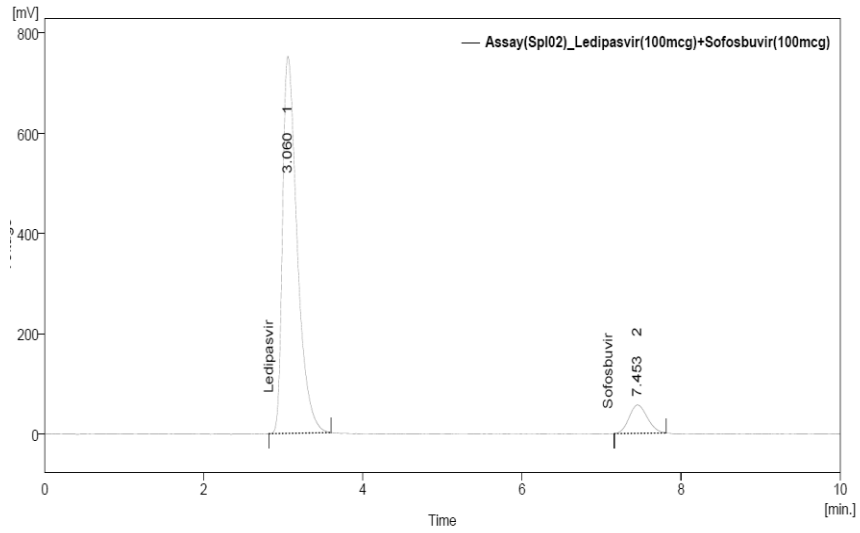


Fig: Chromatogram of Assay sample preparation-2

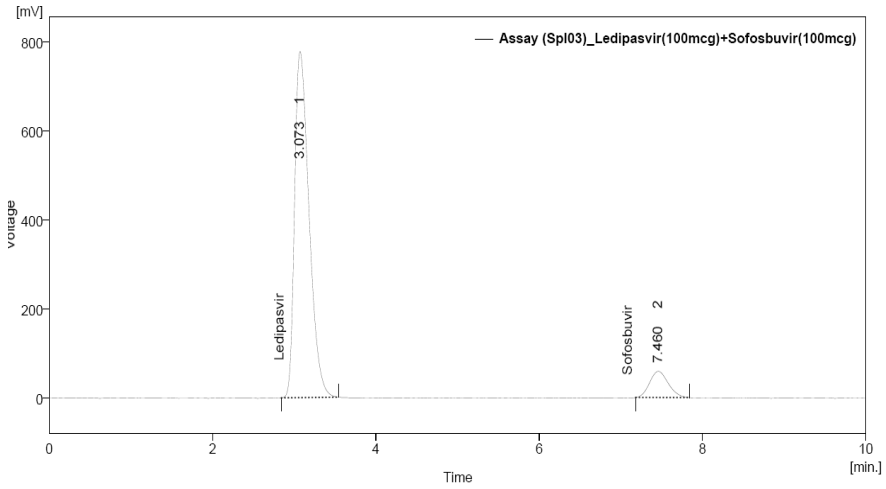


Fig: Chromatogram of Assay sample preparation-3

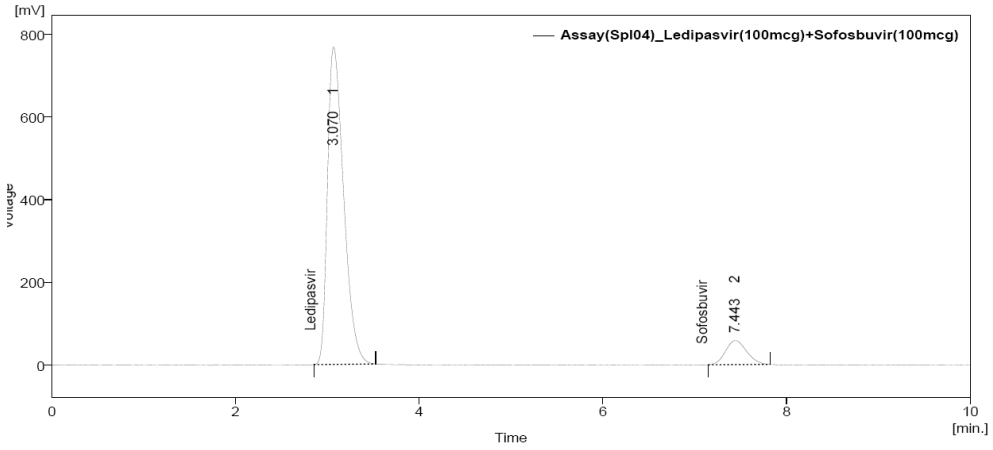


Fig: Chromatogram of Assay sample preparation-4

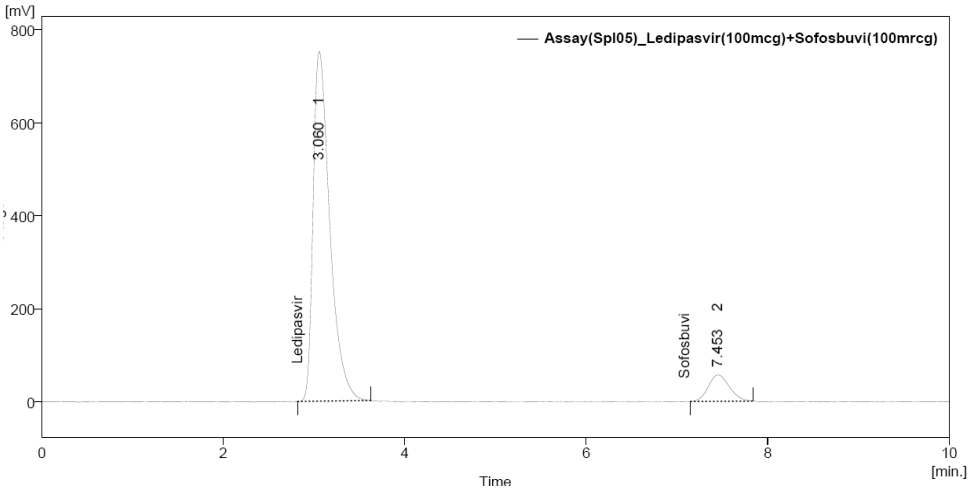


Fig: Chromatogram of Assay sample preparation-5

Table : Assay Results

LEDIPASVIR			SOFOSBUVIR	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	9610.218	9618.037	929.107	933.278
Injection-2	9596.321	9610.218	898.860	902.356
Injection-3	9610.218	9545.801	909.64	928.769
Injection-4	9596.321	9394.586	898.86	934.043
Injection-5	9578.389	9612.063	891.613	918.958
Average Area	9598.293	9556.141	578.6002	923.4808
Assay(%purity)	99.5608344		101.97266	

Observation

The amount of LEDIPASVIR and SOFOSBUVIR present in the taken dosage form was found to be 99.56 % and 101.97 % respectively.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

weigh accurately 10mg of LEDIPASVIR and 10 mg of SOFOSBUVIR in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of LEDIPASVIR and SOFOSBUVIR is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains SOFOSBUVIR–90 mg LEDIPASVIR -400 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of SOFOSBUVIR and LEDIPASVIR (100µg/ml) were prepared by dissolving weight equivalent to 10 mg of SOFOSBUVIR and LEDIPASVIR and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of SOFOSBUVIRand LEDIPASVIR was made by adding 1 ml of stock solution to 10 ml of mobile phase.

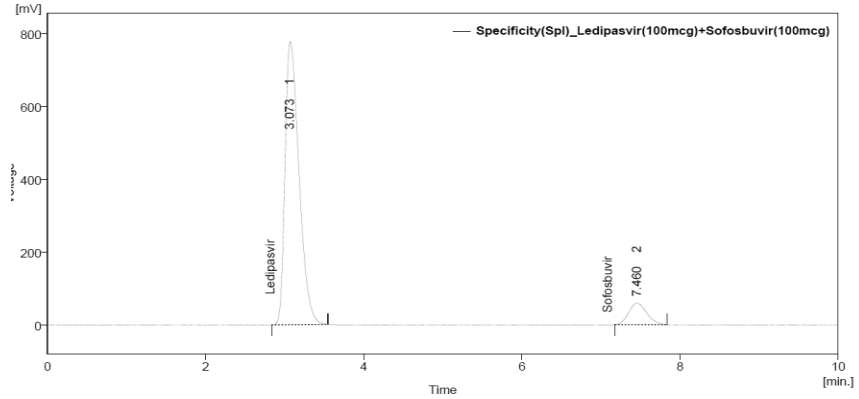


Fig: Chromatogram for specificity of LEDIPASVIR and SOFOSBUVIR sample

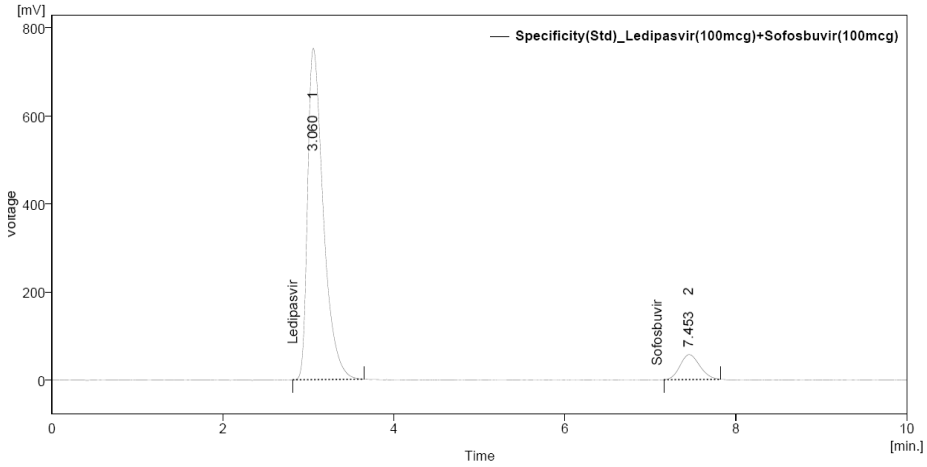


Fig: Chromatogram for Specificity of LEDIPASVIR and SOFOSBUVIR standard

Observation

It is observed from the above data, diluent or excipient peaks are not interfering with the LEDIPASVIR and SOFOSBUVIR peaks.

Linearity and range

Preparation of standard stock solution

Preparation of standard stock solution

Standard stock solutions of LEDIPASVIR and SOFOSBUVIR (microgram/ml) were prepared by dissolving 10 mg of LEDIPASVIR and SOFOSBUVIR dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase.

Table: Linearity Preparations

Preparations	Volume from standard stock transferred in ml		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
				LEDIPASVIR	SOFOSBUVIR
Preparation 1	0.5	0.5	10	50	50
Preparation 2	0.75	0.75	10	75	75
Preparation 3	1.0	1.0	10	100	100
Preparation 4	1.5	1.5	10	150	150
Preparation 5	1.75	1.75	10	175	175

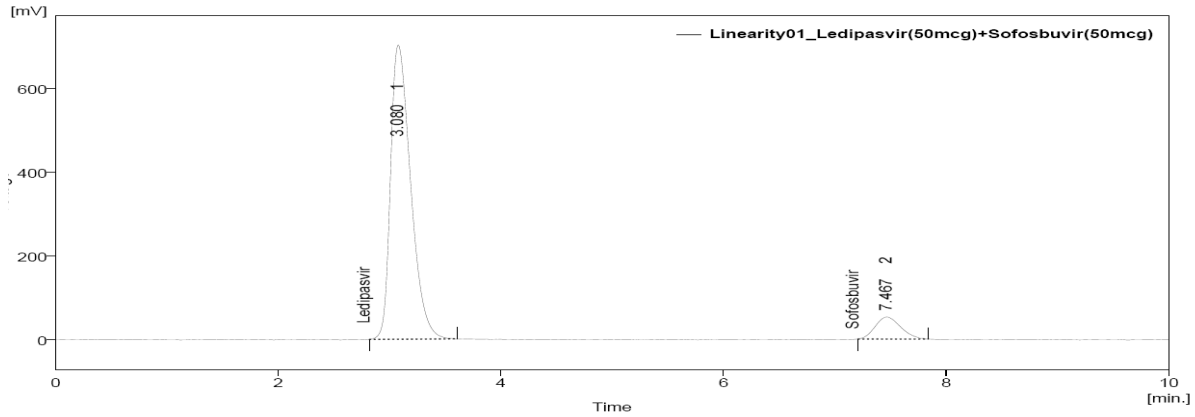


Fig. : Chromatogram of LEDIPASVIR and SOFOSBUVIR preparation-1

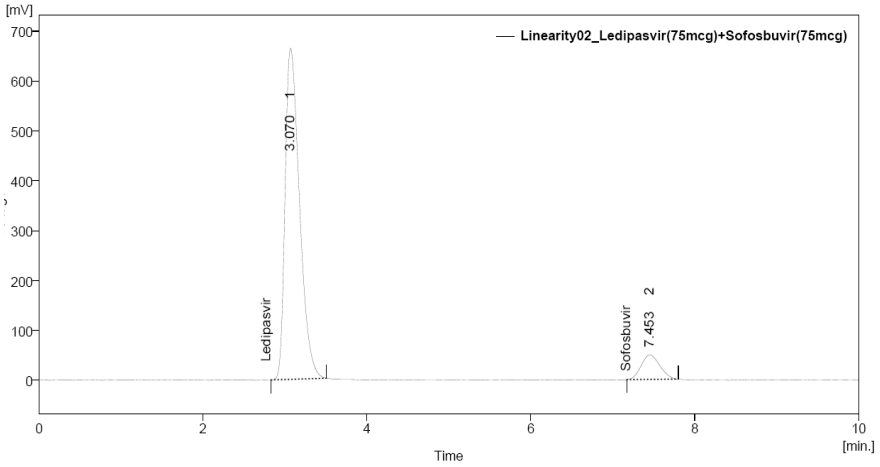


Fig. Chromatogram of LEDIPASVIR and SOFOSBUVIR preparation-2

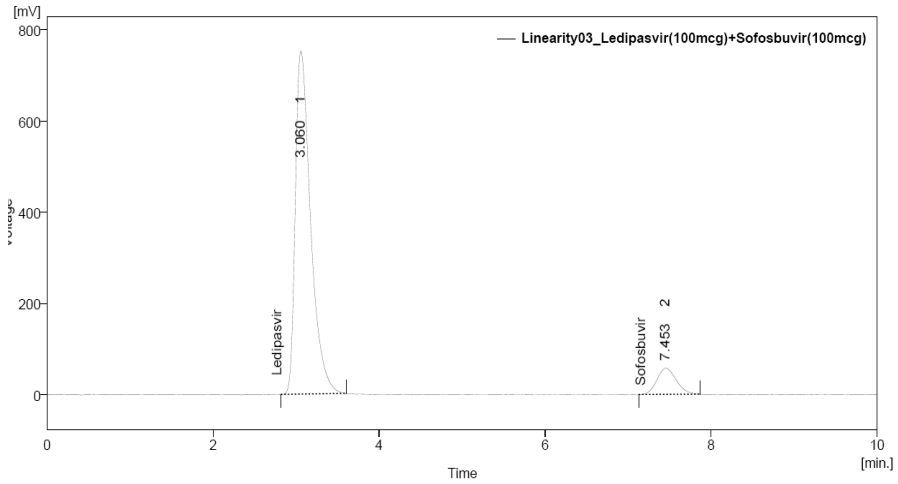


Fig. Chromatogram of LEDIPASVIR and SOFOSBUVIR preparation-3

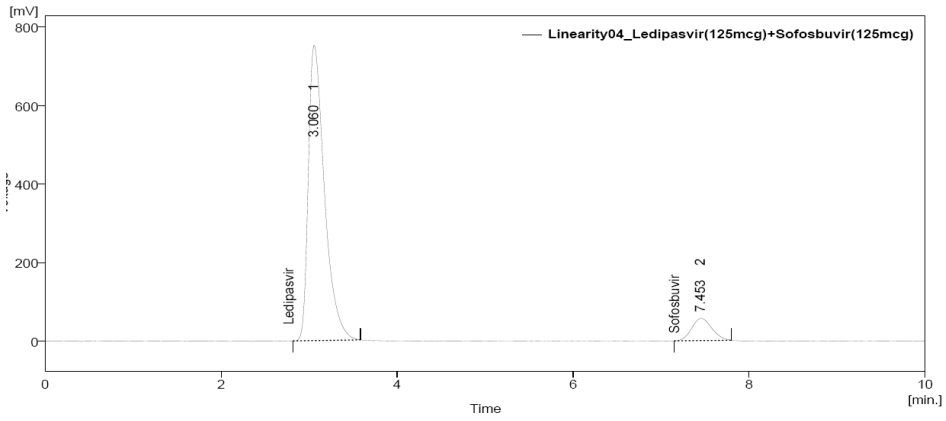


Fig: Chromatogram of LEDIPASVIR and SOFOSBUVIR preparation-4

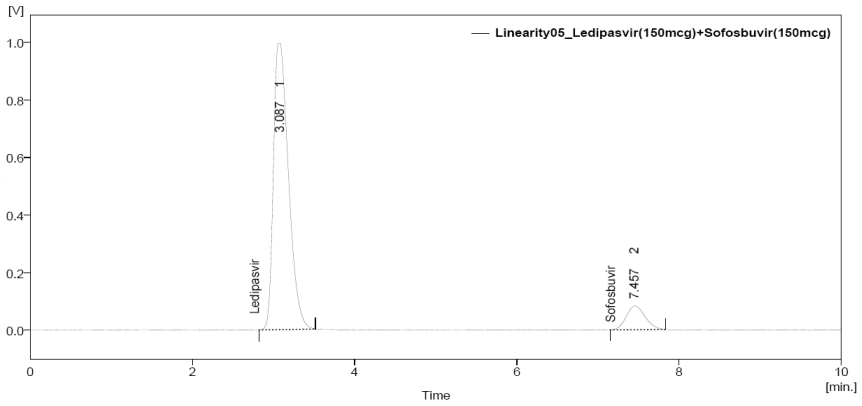


Fig. Chromatogram of LEDIPASVIR and SOFOSBUVIR for preparation-5

Table linearity of LEDIPASVIR

S .No.	Conc.(µg/ml)	Area
1	50	8118.069
2	75	8859.874
3	100	9690.218
4	150	10999.32
5	175	11897.59

Table: linearity of SOFOSBUVIR

S.No.	Conc.(µg/ml)	Area
1	50	774.04
2	75	876.449
3	100	999.107
4	150	1199.86
5	175	1298.846

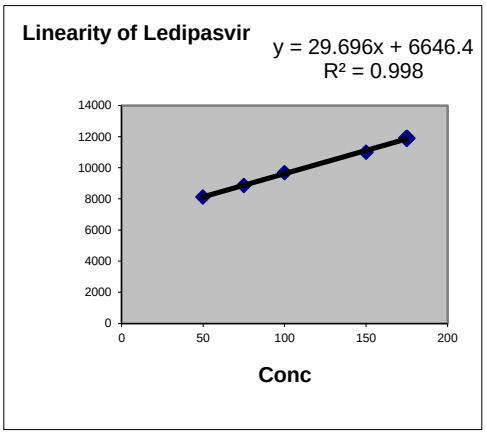


Fig. 9.3.9: Linearity graph of LEDIPASVIR

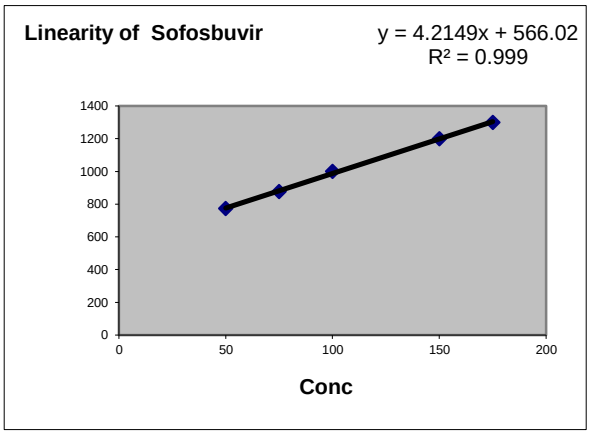


Fig. 9.3.9.1: Linearity graph of SOFOSBUVIR

Acceptance criteria

The relationship between the concentration of LEDIPASVIR and SOFOSBUVIR and area of LEDIPASVIR and SOFOSBUVIR should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of LEDIPASVIR and SOFOSBUVIR is 0.999 and 0.996. The relationship between the concentration of LEDIPASVIR and SOFOSBUVIR and area of LEDIPASVIR and SOFOSBUVIR is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

ACCURACY

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 100%, 120%, 140%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 100%, 120%, 140%.

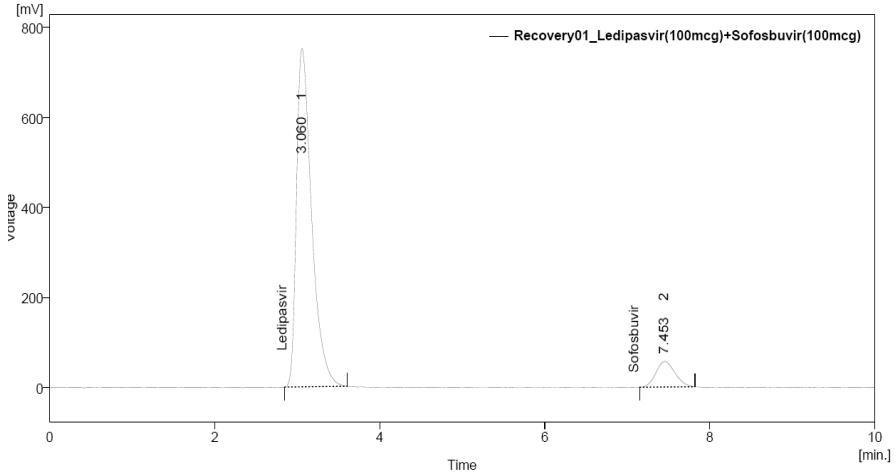


Fig. 9.4.1: Chromatogram of 50% recovery (injection 1)

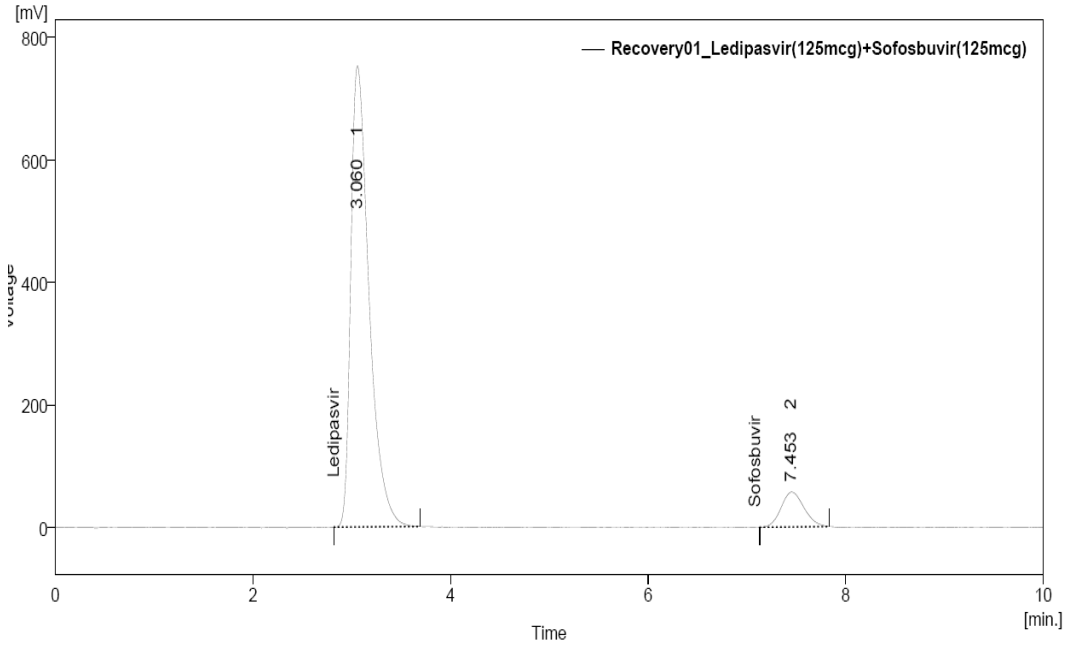


Fig. 9.4.2: Chromatogram of 100% recovery (injection 2)

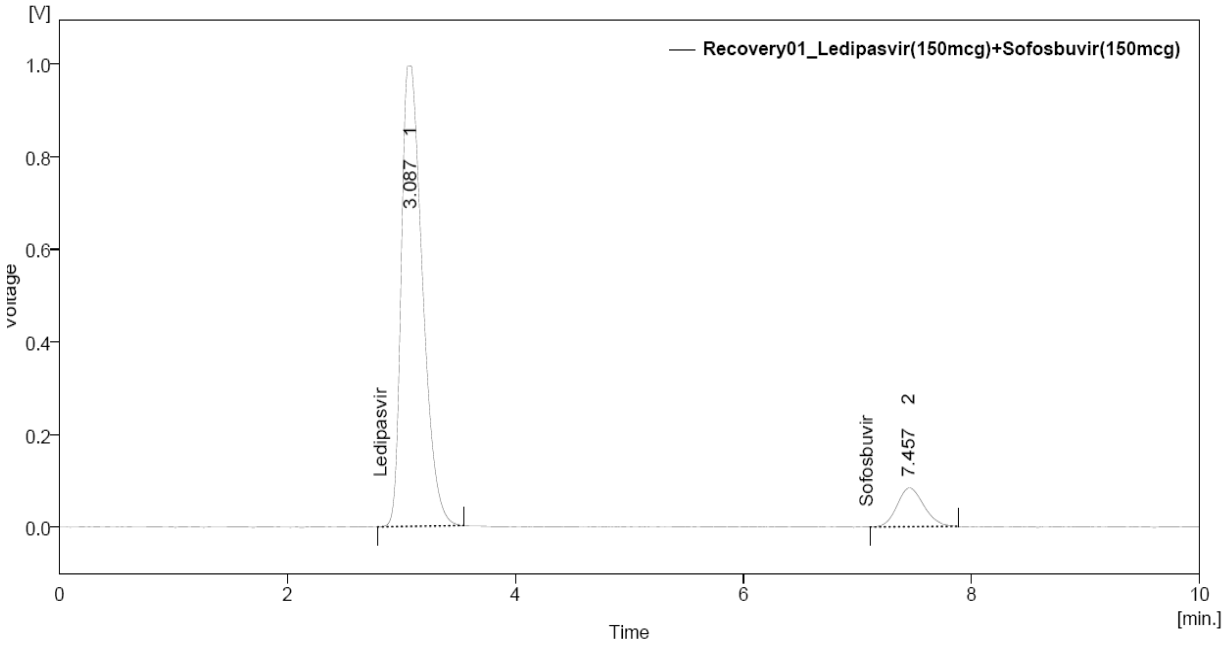


Fig. 9.4.3: Chromatogram of 150% recovery (injection 3)

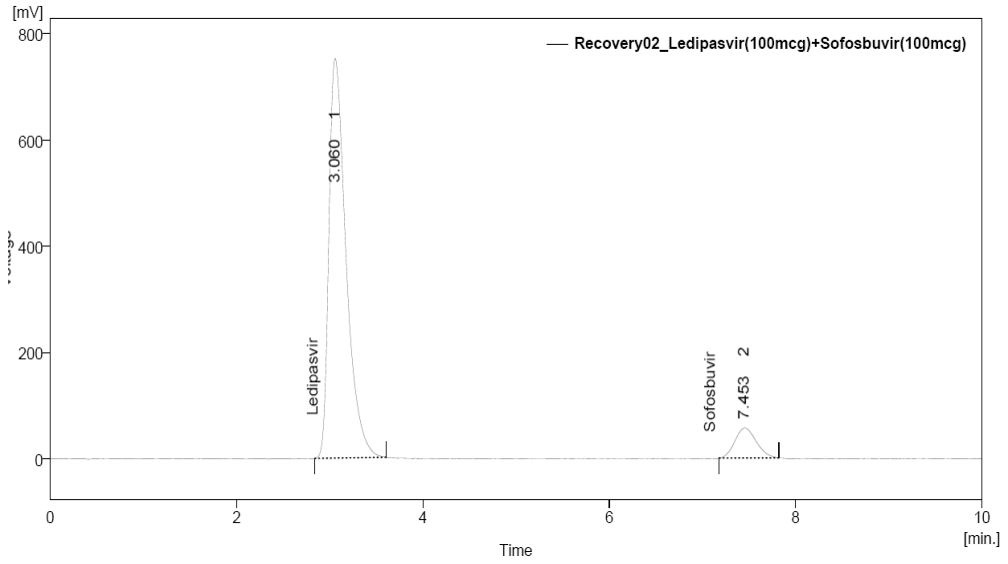


Fig. 9.4.4: Chromatogram of 50% recovery (injection 1)

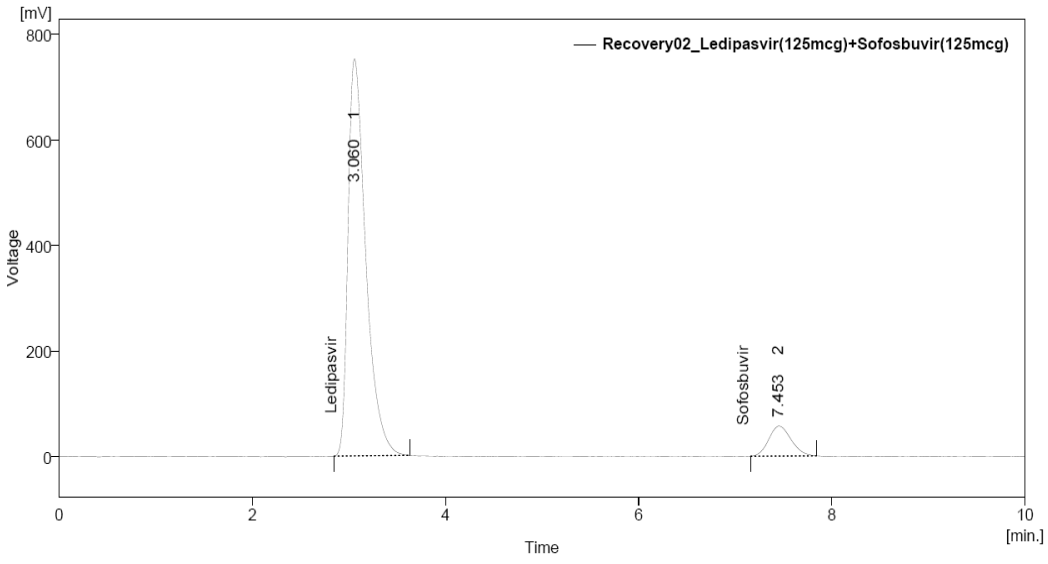


Fig. 9.4.5: Chromatogram of 100% recovery (injection 2)

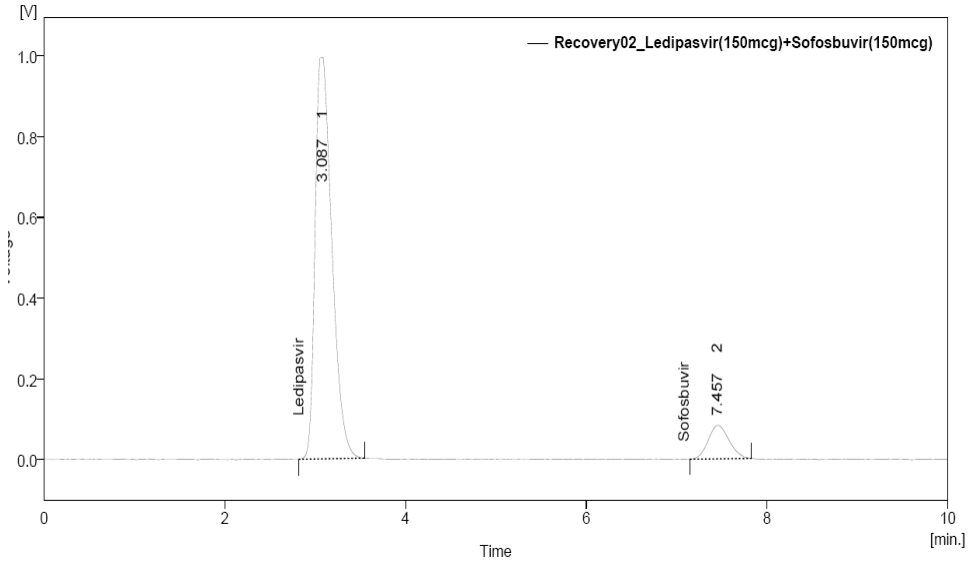


Fig. 9.4.6: Chromatogram of 150% recovery (injection 3)

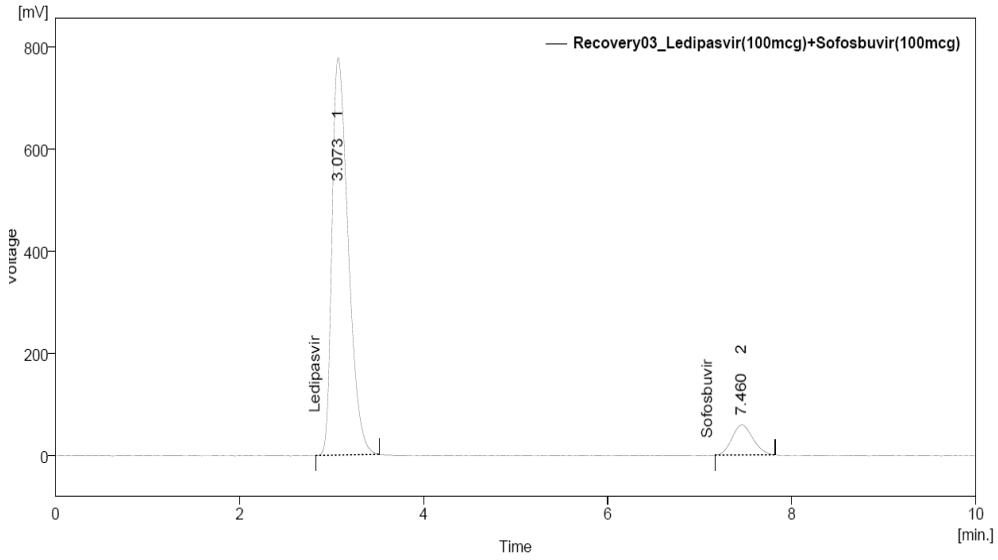


Fig. 9.4.7: Chromatogram of 50% recovery (injection 1)

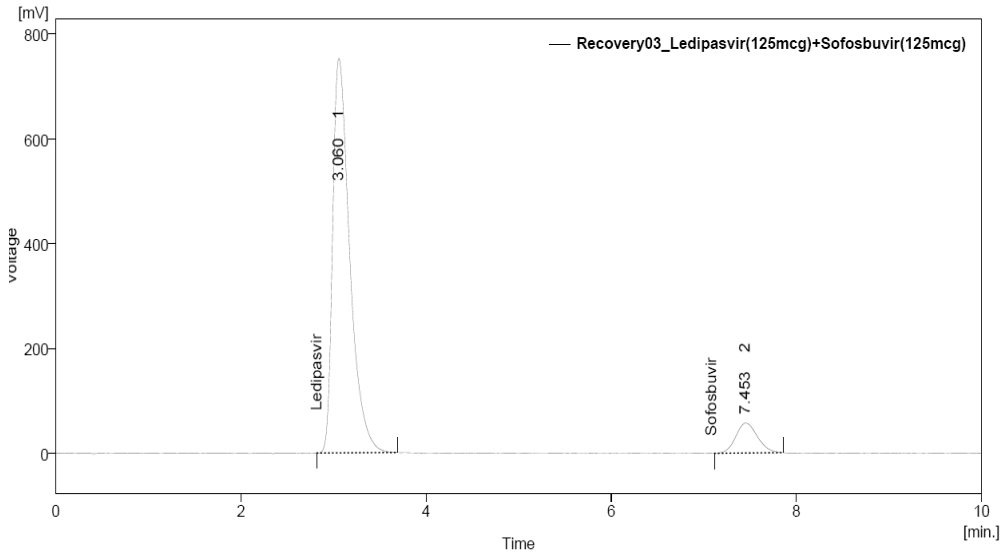


Fig. 9.4.8: Chromatogram of 100% recovery (injection 2)

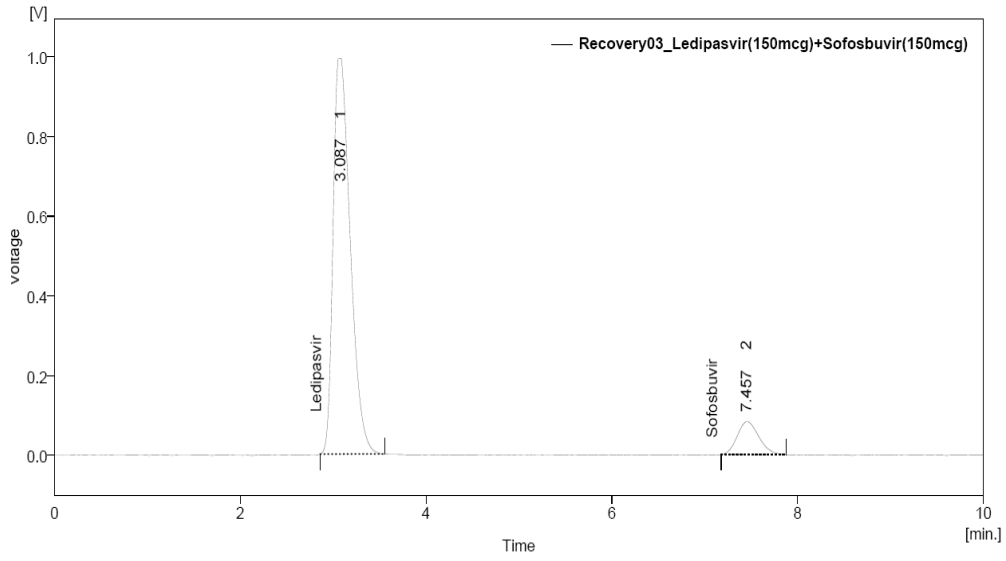


Fig: Chromatogram of 150% recovery (injection 3)

Table 9.4.9.1 : Recovery results for LEDIPASVIR

Recovery level	**Accuracy LEDIPASVIR**		Average % Recovery
	Amount taken(mcg/ml)	Area	%Recovery	
100	**75**	**9603.072**	**99.38428878**	**99.15405317**
	75	**9605.652**		
	75	**9533.752**	**99.41098976**	

			98.66688096	**99.02230106**
120	**100**	**10641.469**	**99.11779744**	
	100	**10610.711**		
	100	**10641.469**	**98.83130831**	**100.4409523**
			99.11779744	
140	**150**	**11814.282**		
	150	**11810.235**	**97.81486708**	
	150	**12769.881**	**97.78136045**	
			105.7266292	

Table 9.4.9.2 : Recovery results for SOFOSBUVIR

Recovery level	**Accuracy SOFOSBUVIR**			Average % Recovery
	Amount taken(mcg/ml)	Area	%Recovery	
100	75	**913.199**		**102.83**
	75	**901.016**	**191.8314016**	
	75	**929.723**	**189.2721763**	
			195.3025203	**99.36**
120	100	**921.96**	**96.83589175**	
	100	**915.556**		**99.103**
	100	**931.069**	**96.16326273**	
			97.79263406	
140	150	**1370.413**		
	150	**1331.217**	**86.36285626**	
	150	**1336.835**	**83.89274067**	
			84.24678469	

Observation

The percentage mean recovery of LEDIPASVIR and SOFOSBUVIR is 99.59 % and 100.43% respectively.

Precision

Method precision

Method precision

Prepared sample preparations of SOFOSBUVIRand LEDIPASVIR as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of SOFOSBUVIR and LEDIPASVIR should be not more than 2.0%.

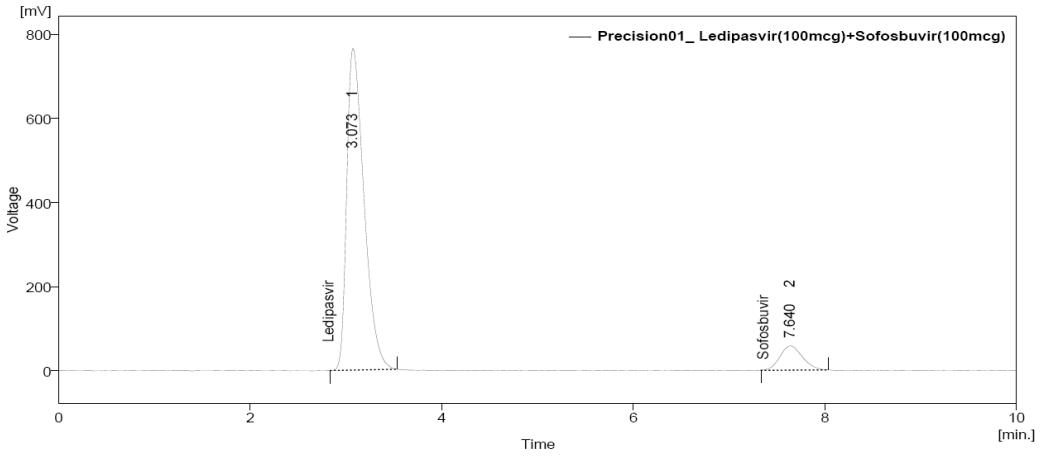


Fig. 9.5. 1: Chromatogram of precision injection 1

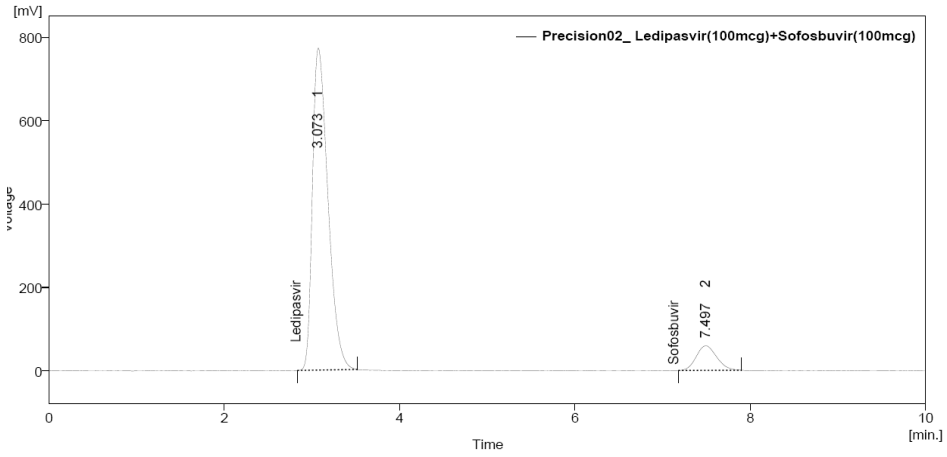


Fig. 9.5.2: Chromatogram of precision injection 2

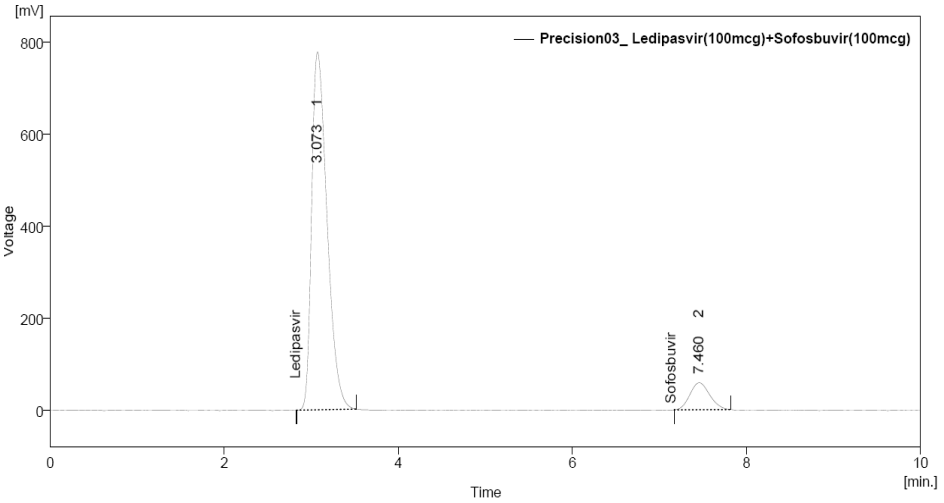


Fig. 9.5.3: Chromatogram of precision injection 3

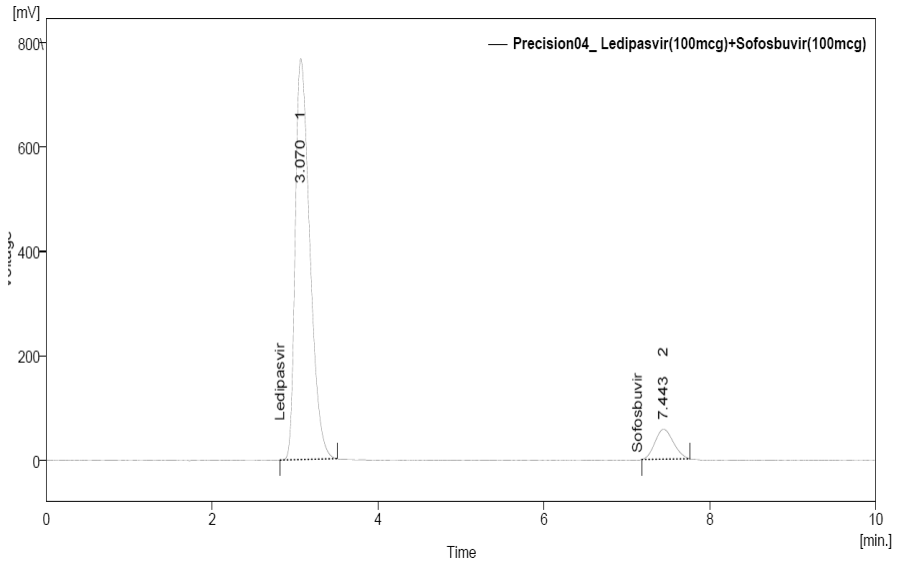


Fig. 9.5.4: Chromatogram of precision injection 4

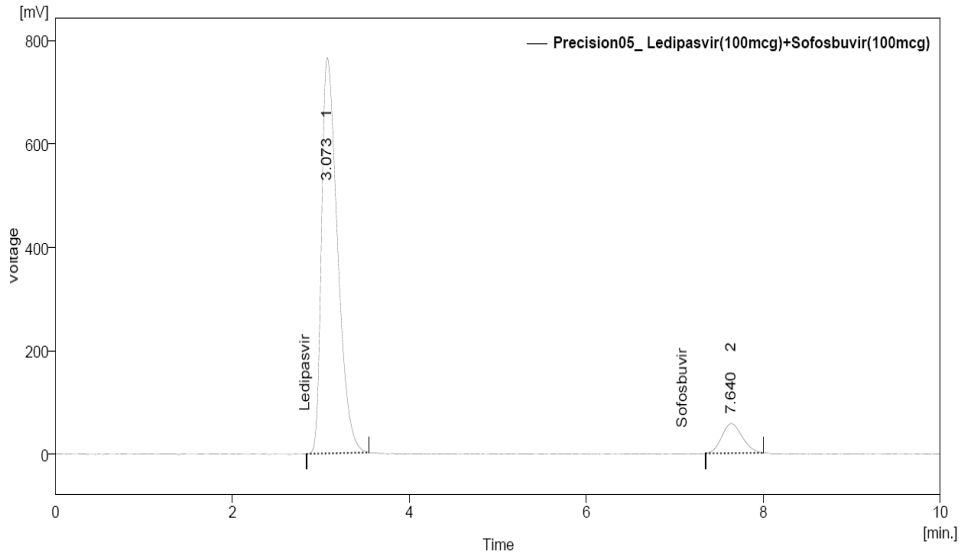


Fig. 9.5.5: Chromatogram of precision injection 5

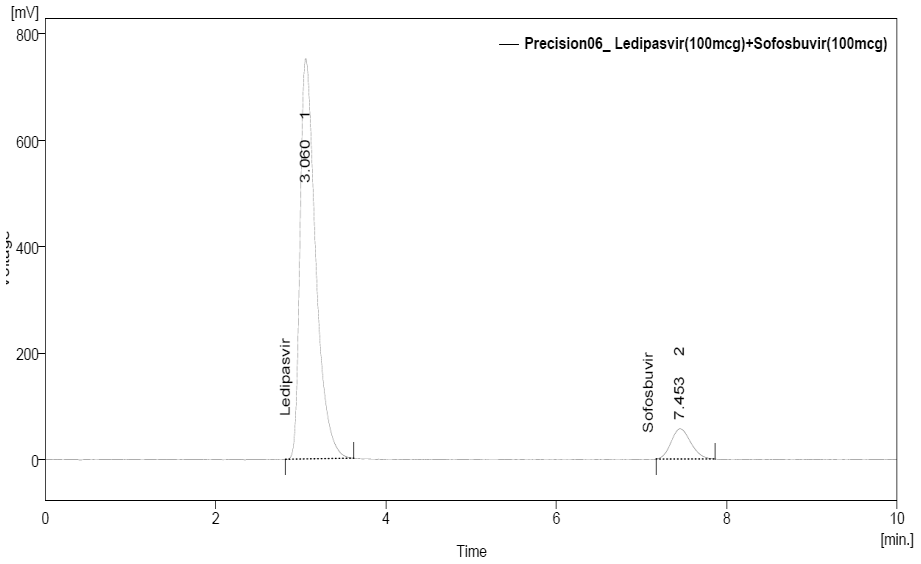


Fig. 9.5.6: Chromatogram of precision injection 6

Table: Results for Method precision of LEDIPASVIR and SOFOSBUVIR

LEDIPASVIR			**SOFOSBUVIR**		
S.No.	Rt	Area	S.No.	Rt	Area
1	2.397	3898.811	1	5.630	591.352
2	2.403	3954.691	2	5.650	605.193
3	2.393	3996.327	3	5.603	610.259
4	2.387	3941.128	4	5.597	620.470
5	2.347	3902.778	5	5.523	610.067
6	2.403	3948.922	6	5.650	614.702
avg	2.3883	3940.443	**avg**	5.609	608.674
stdev	0.0212	36.195	**stdev**	0.048	9.921
%RSD	**0.89**	**0.92**	**%RSD**	**0.85**	**1.63**

Observation

Test results for SOFOSBUVIR and LEDIPASVIR are showing that the %RSD of Assay results are within limits.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

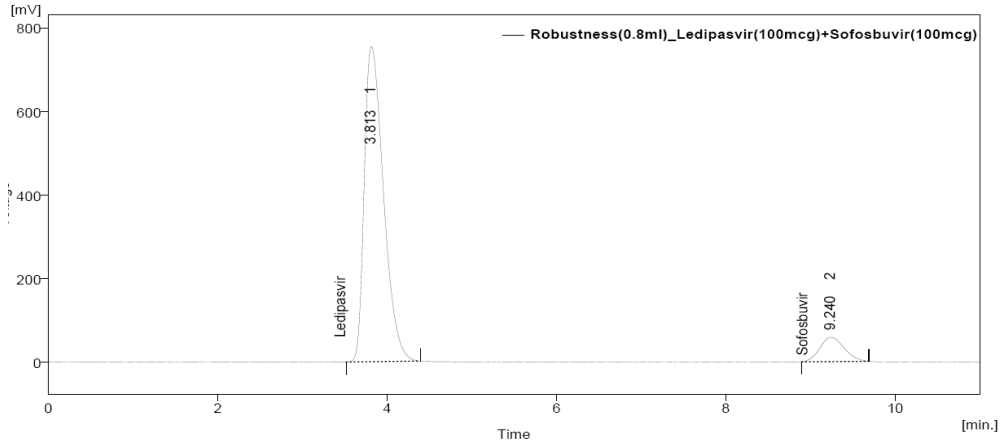


Fig. : Chromatogram of LEDIPASVIR and SOFOSBUVIR Robustness (0.8 ml/min)

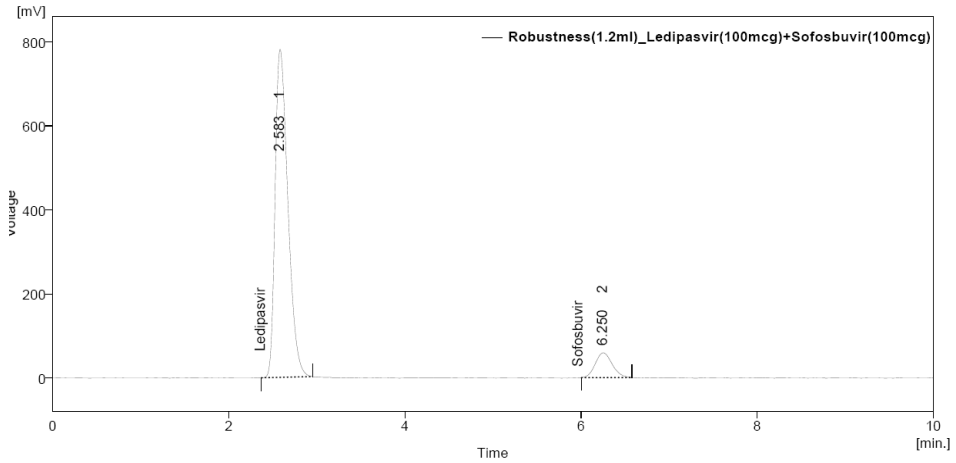


Fig. : Chromatogram of LEDIPASVIR and SOFOSBUVIR Robustness (1.2 ml/min)

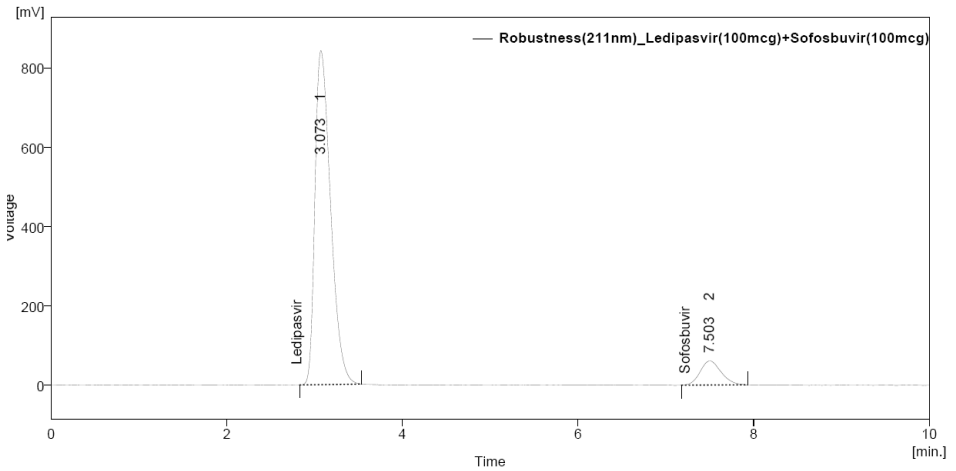


Fig: Chromatogram of LEDIPASVIR and SOFOSBUVIR for Robustness (211nm)

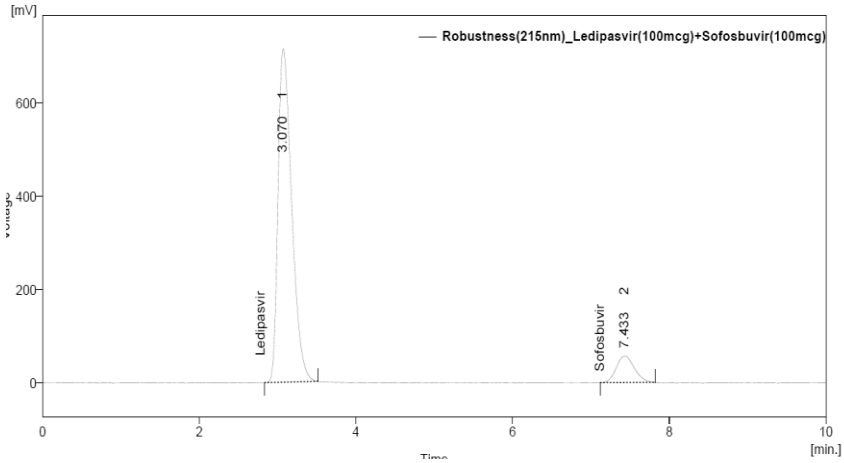


Fig: Chromatogram of LEDIPASVIR and SOFOSBUVIR for Robustness (215nm)

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

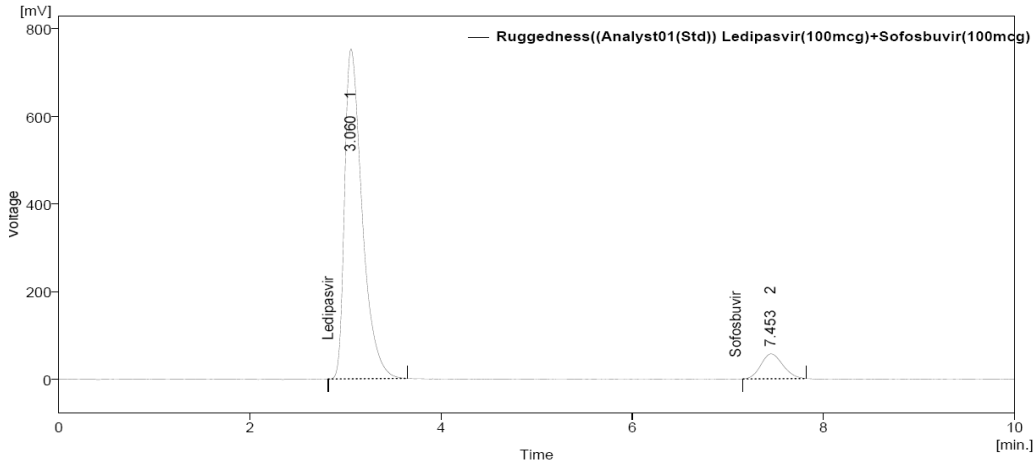


Fig. 9.9.1: Chromatogram of Analyst 01 standard preparation

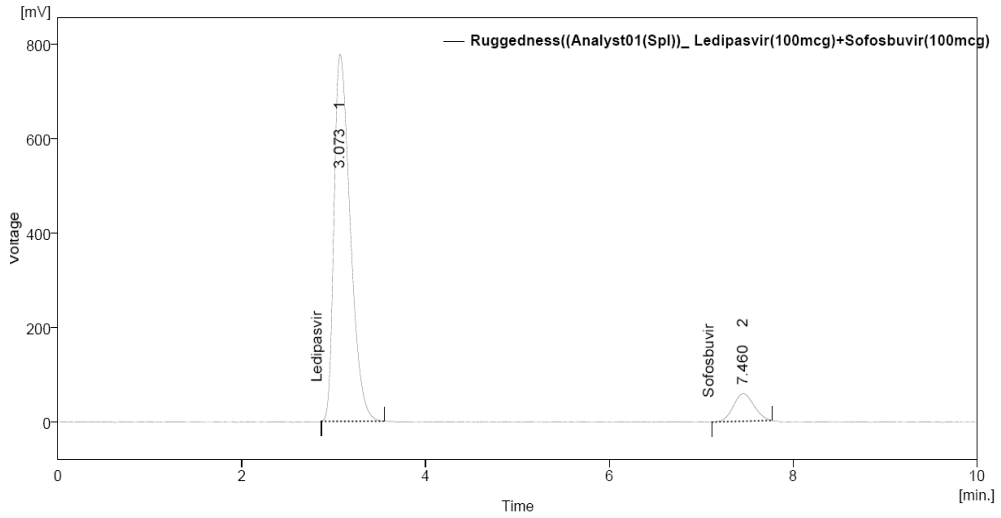


Fig. 9.9.2: Chromatogram of Analyst 01 sample preparation

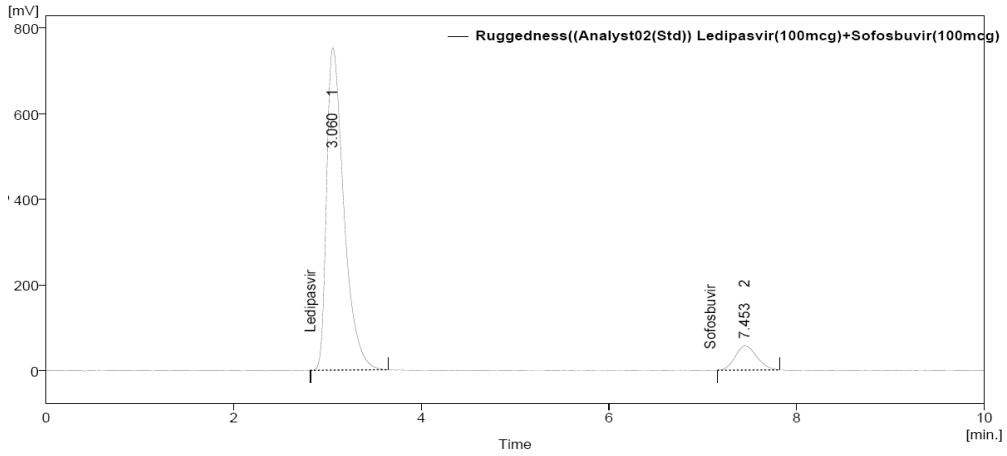


Fig. 9.9.3: Chromatogram of Analyst 02 standard preparation

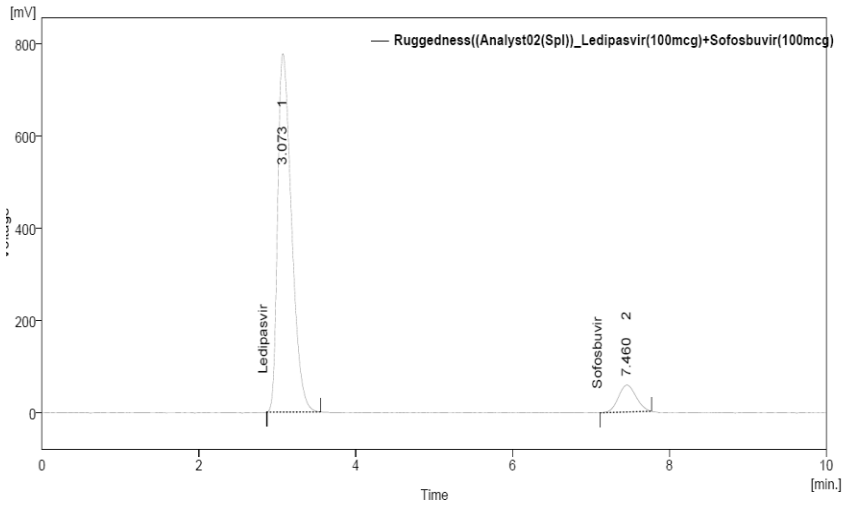


Fig. 9.9.4: Chromatogram of Analyst 02 sample preparation

Observation

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

BIBLIOGRAPHY

[1]. "Ledipasvir" (PDF). United States Adopted Name.
[2]. "Ledipasvir-submitted-to-FDA".
[3]. "GS-5885". Gilead Sciences.
[4]. ELECTRON: 100% Suppression of Viral Load through 4 Weeks' Post-treatment for Sofosbuvir + Ledipasvir (GS-5885) + Ribavirin for 12 Weeks in Treatment-naïve and -experienced Hepatitis C Virus GT 1 Patients. Gane, Edward et al. 20th Conference on Retroviruses and Opportunistic Infections. March 3–6, 2013. Abstract 41LB.
[5]. CROI Sofosbuvir + Ledipasvir + Ribavirin Combo for HCV Produces 100% Sustained Response. Highleyman, Liz. HIVandHepatitis.com. 2013.
[6]. "Gilead Files for U.S. Approval of Ledipasvir/Sofosbuvir Fixed-Dose Combination Tablet for Genotype 1 Hepatitis C". Gilead Sciences. 2014.
[7]. "U.S. Food and Drug Administration Approves Gilead's Harvoni (Ledipasvir/Sofosbuvir), the First Once-Daily Single Tablet Regimen for the Treatment of Genotype 1 Chronic Hepatitis C". 2014.
[8]. Afdhal, N; Zeuzem, S; Kwo, P; Chojkier, M; Gitlin, N; Puoti, M; Romero-Gomez, M; Zarski, J. P.; Agarwal, K; Buggisch, P; Foster, G. R.; Bräu, N; Buti, M; Jacobson, I. M.; Subramanian, G. M.; Ding, X; Mo, H; Yang, J. C.; Pang, P. S.; Symonds, W. T.; McHutchison, J. G.; Muir, A. J.; Mangia, A; Marcellin, P; Ion-1, Investigators. "Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection". New England Journal of Medicine. 370 (20): 1889–98. doi:10.1056/NEJMoa1402454. PMID 24725239. 2014.
[9]. http://www.gilead.com/~/media/Files/pdfs/medicines/liver-disease/harvoni/harvoni_pi.pdf
[10]. http://www.hepatitisc.uw.edu/page/treatment/drugs/ledipasvir-sofosbuvir