STRESS DEGRADATION STUDIES AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF ANTI-VIRAL DRUGS SOFOSBUVIR AND LEDIPASVIR IN BULK AND PHARMACEUTICAL DOSAGEFORMS BY RP-HPLC

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Abstract

Sofosbuvir and Ledipasvir are antiviral drugswidely used in the treatment of hepatitis C virus infections. The same combinations of drugs also used in the treatment of patients with mild to moderate COVID-19. The aim of the studies was to evaluate the stability of the drugs under stress.The drugs were subjected to stress degradation studies as per the conditions prescribed in ICH Q1 (R2) guideline. Sofosbuvir and Ledipasvir are subjected under stress conditions acid, alkaline,neutral,oxidative, thermal and photolytic conditions. The drugs were found to be highest stability under oxidative, thermal and photolytic conditions. The method also validated according to the ICH guidelines, for the simultaneous determination of Sofosbuvir and Ledipasvir in pure and market formulations. Separation was carried out using column Hypersil BDS (250mm x4.6 mm,5µm particle size) in isocratic mode using mobile phase composition was 40:60 phosphate buffer: Acetonitrile and pH was adjusted to 3 with sodium hydroxide and UV detection at 265 nm. The compounds were eluted at a flow rate of 1.0mL min−1.The average retention times for Sofosbuvir and Ledipasvir were 2.511and 3.127 respectively. The method was linear over the concentration of 100-600 µg/ml and 22.5- 135 µg/ml forSofosbuvir and Ledipasvir. Correlation coefficient was found to be 0.9992 &0.9993 for Sofosbuvir and Ledipasvir respectively. The LOD and LOQ of Sofosbuvir were found to be 1.8231µg/mL and 5.52µg/mL and of were found to Ledipasvir be 0.378µg/mL and 1.145

µg/ml.The % RSD of all validation parameters found to be less than 2% indicating high degree of accuracy and precision of the proposed HPLC method. The validated HPLC method was successfully used to analyze the abovementioned drugs in their pure and dosage forms without interference from common excipients present in commercial formulations.

Keywords: Sofosbuvir, Ledipasvir, stress degradation studies, RP-HPLC, ICH Guidelines

INTRODUCTION

Sofosbuvir [1] is a prodrug of 2′-deoxy-2′-fluoro-2′-C-methyluridine monophosphate that is phosphorylated intracellular to the active triphosphate form (Fig-1). SFV is a white crystalline solid(Molecular formula $C_{22}H_{29}FN_3O_9P$ and molecular weight of 529.458 g/mol) has two pKa values, pKa_1 9.38 (amide), pKa_2 10.30; slightly soluble in water, freely soluble in ethanol and acetone. Sofosbuvir is a nucleotide analog inhibitor of hepatitis C virus NS5B polymerase—the key enzyme mediating HCV RNA replication andis commonly used for the treatment of chronic HCV infection [2-7].

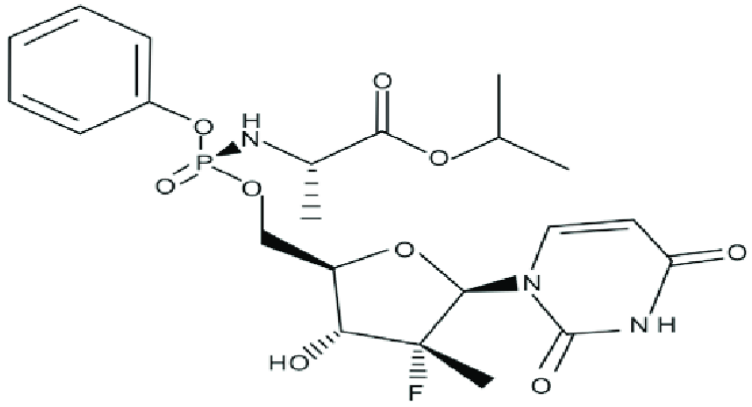

Fig-1 Sofosbuvir

IUPAC Name: propan-2-yl (2*S*)-2-[[[(2*R*,3*R*,4*R*,5*S*)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy-phenoxyphosphoryl]amino]propanoate

Ledipasvir [8] is a potent inhibitor of HCV NS5A, a viral phosphoprotein that plays an important role in viral replication, assembly, and secretion. LDV is a pale yellow colour solid,(Molecular formula $C_{49}H_{54}F_2N_8O_6$, molecular weight = 889.01 g/mol) has a pKa_1 value

of 11.33, soluble in organic solvents like ethanol,DMF and DMSO and is a novel HCV NS5A inhibitor that has shown potent antiviral activity (Fig-2). LDV affects the HCV NS5A protein, which is involved in both RNA replication and aggregation of HCV virus. A fixed-dose combination of SFV/LDV (under the trade name Harvoni) is currently recommended for the treatment of patients infected with the genotype 1HCV [4,5]. A randomized clinical trial was also done with the same combination of drugs in the treatment of patients with mild to moderate COVID-19[9]

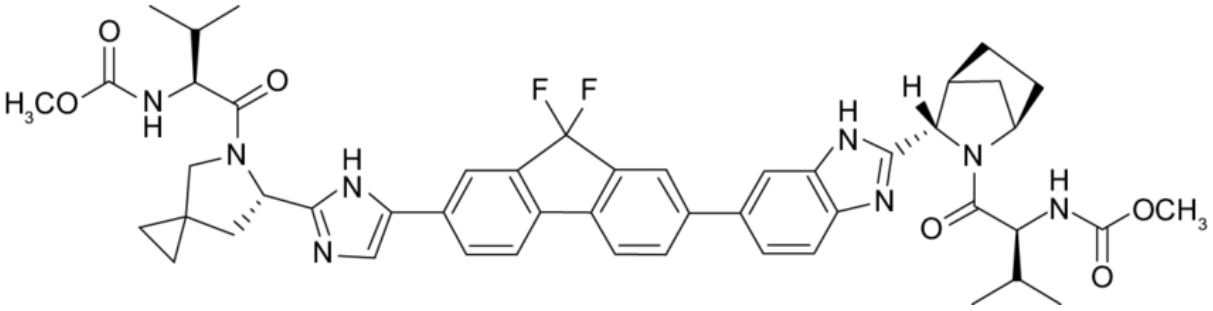

Fig-2 Ledipasvir

IUPAC Name:Methyl[(2S)-1-{(1R,3S,4S)-3-[5-(9,9-difluoro-7-{2-[(6S)-5-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl}-5-azaspiro[2.4]hept-6-yl]-1H-imidazol-4-yl}-9H-fluoren-2-yl)-1H-benzimidazol-2-yl]-2-azabi cyclo[2.2.1]hept-2-yl}-3-methyl-1-oxo-2-butanyl]carbamate.

Literature survey revealed thatthe quantification of SFV alone or with its metabolite in human plasma has also been achieved by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [10].The content ofSofosbuvirin its pure form, in tablet dosage form, or in the presence of its degradation products under various stress conditionswas determined by reversed-phase high-performance liquid chromatography (RP-HPLC)[11-13]. Ledipasvir content has been determined in rat plasma by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS) [14-16]. Several methods for the simultaneous determination of SFV either with LDV or with other drugs were reported in human plasma[16-21].Earlier reports on RP-HPLC based determination of stability of the combination of drugs were noted and we developed a novel combination of mobile phase constituents that gave results with better precision and sensitivity.

However, there are few methods established for the stability indicating RP-HPLC under stress degradation studies for this combination[22-32]. The present work describes the development of stability indicating RP-HPLC method, which can quantify these components simultaneously from a combined dosage form. The present RP-HPLC method was validated [33-34] and applied under stressed conditions according to ICH guidelines. ICH has made the mandatory need of developing stability indicating assay methods for every drug candidate. Stability indicating assay methods helps in establishing the inherent stability of the drug which provides assurance on detection changes in identity, purity and potency of the product on exposure to various conditions [35]. So an attempt has been made to develop a method under stress conditions like acidic, basic, neutral thermal, photolytic and oxidative, which in turn can help in establishing the degradation pathways and the intrinsic stability of the molecules [36, 37]. The objective of the present work was to develop a stability indicating method for the simultaneous determination of Sofosbuvir, Ledipasvir in bulk powder and pharmaceutical dosage forms.

MATERIALS AND METHODS

EXPERIMENTAL DETAILS OF SOFOSBUVIR AND LEDIPASVIR

2.1 Instruments and columns

Waters HPLC model 2695 Series quaternary Pump, auto sampler equipped with UV Visible detector synchronized against waters alliance empower2 software was used for the present study. The column is maintained in a constant temperature column oven that can maintain 5ºC to 60ºC column temperature. Other equipment used were Power Sonicator, model no: 405, Hwashin Technology, Korea, The column used in the development for determination of the drugs is Hypersil BDS C18 (250 mm× 4.6mm, 5µm).

2.2 Chemicals used

HPLC grade acetonitrile, hydrochloric acid hydrogen peroxide, water and methanol were purchased from Merck, Mumbai, India, sodium dihydrogen phosphate and sodium hydroxide AR grade purchased from SD Fine Chem, Mumbai, India.The reference samples were supplied by Mylan labs, Hyderabad, Telangana, India and branded formulation was purchased from local market.

2.3 Selection of chromatographic method

Selection of chromatographic method in general was donetaking into consideration of several parameters like thenature of the drugs, molecular weight and solubility.Since the drugs selected were polar in nature, RP-HPLCwas selected for initial chromatographic conditionbecause of its simplicity and suitability.

2.4 Selection of wave length (λ max)

An ideal wavelength is one that uses good response forthe drugs to be detected in diluent the spectraswere scanned on UV- visible spectrophotometer in therange of 200 nm to 400 nm against diluents as blank. The max absorbance for the drugs Sofosbuvir and Ledipasvir found to be 265 nm

OPTIMIZED METHOD

Table:I Chromatographic conditions

Method parameters	**Optimization conditions**
Flow rate	**1**ml/min
Column	BDS(250mm x 4.6 mm, 5µm)
Detector wave length	265nm
Column temperature	30°C
Injection volume	10µL
Run time	6min
Mobile phase	Sodium dihydrogen phosphate: Acetonitrile40:60v/v
Run time	6 min
Volume of injection	10µl
pH	3
Diluent	Water and ACN (50:50)
Elution type	Isocratic

PREPARATION OF SOLUTIONS

Preparation buffer Solution

2.5gms of sodium dihydrogen phosphate was taken in a 1000ml of volumetric flask to which about 900ml of milli-Q water was added and degassed to sonicate and remaining volume is finally made up with water. pH was adjusted to 3 with 1M dil. NaOH

Preparation of mobile phase

Buffer and Acetonitrile were taken in the ratio of 40:60v/vwas filtered through 0.05µ membrane filter and sonicated by using Power Sonicator (model no: 405, Hwashin Technology, Korea) before use. Acetonitrile was selected because it is medium polar solvent,

miscible with water and organic solvents,dissolves wide range of ionic and non-polar compounds and possess good elution strength. The flow rate of the mobile phase was maintained at 1mL/min. The column temperature was maintained at 30ºC and the detection of the drugs was carried out at 265 nm.

Preparation of Stock Solution(400µg/ml Sofosbuvir & 90µg/ml Ledipasvir)

Accurately weighed and transferred 40 mg &9 mg of Sofosbuvir and Ledipasvir working Standards into a 10ml clean dry volumetric flask respectively, added 7ml of diluent, sonicated for 30 minutes and made up to the final volume with diluents.

Preparation of Standard Solutions

From the above stock solution, 1ml was pipetted out in to a 10ml volumetric flask and then made up to the final volume with diluent.

Preparation of samplesolution

10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 7ml of diluent added and sonicated for 30 min, further the volume was made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent. (1 tablet was equivalent to 490 mg)

Label Claim:400mg Sofosbuvir + 90mg of Ledipasvir

VALIDATED RP-HPLC METHOD FOR SOFOSBUVIR AND LEDIPASVIR

Validation of the optimized method was performed according tothe ICH Guidelines

A linear relationship was evaluated by the analytical procedure with a minimum of six concentrations. A series of standard dilutions of Sofosbuvir and Ledipasvir were prepared over a concentration range of 100-600 µg/ml and 22.5- 135 µg/ml from stock solution injected. Linearity was evaluated by a plot of peak areas as a function of analyte concentration, and the results were evaluated by using the statistical parameters like slope, intercept and regression (R^2) correlation coefficients (R) Table-II,III&V; (Fig-III&IV)

Table: IILinearity data for Sofosbuvir

S.NO.	LinearSolutions(%)	Concentration of SFV µg/ml	Peak area
1	0	0	0
2	25	100	706564
3	50	200	1524941
4	75	300	2210355

5	100	400	3015636
6	125	500	3635802
7	150	600	4382311

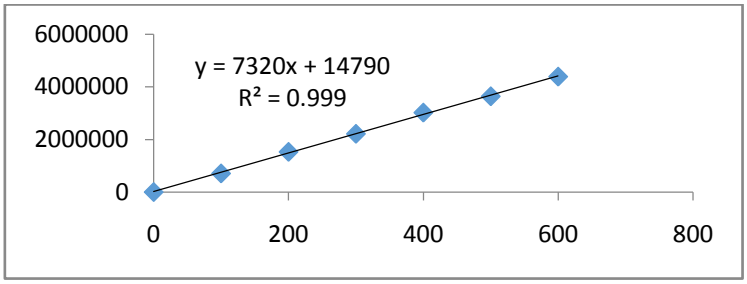

Fig-3: Calibration curve for Sofosbuvir

Table:III Linearity data for Ledipasvir

S.NO	Linearsolutions (%)	Concentration of LDV µg/ml	Peak area
1	0	0	0
2	25	22.5	277978
3	50	45	603986
4	75	67.5	900132
5	100	90	1206816
6	125	112.5	1489239
7	150	135	1750943

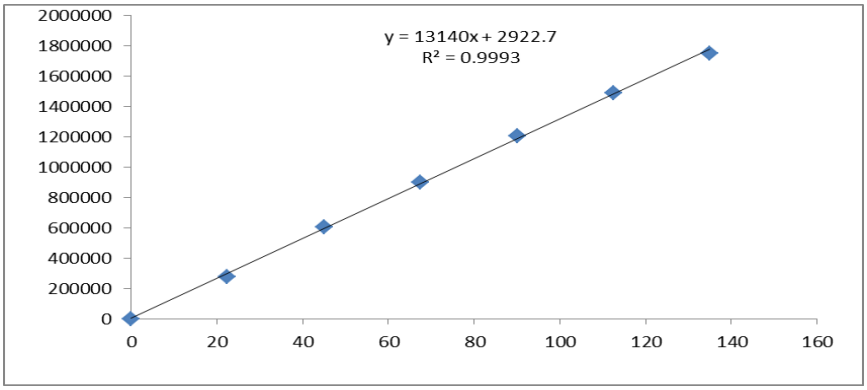

Fig-4: Calibration curve for Ledipasvir

Precision

Repeatability expresses the precision under the same operating conditions over a short interval of time. The six repeated homogenous injections of standard solutions were made of

about 40µg/mL Sofosbuvir and 9µg/mL Ledipasvirand the response factor of drug peaks, mean, standard deviation and % RSD were calculated Table-V.

Method Precision

Method precision was determined by injecting six sample solutions in Single batch and analysed as per test method. The mean, standard deviation and % RSD for peak areas of sample solutions were calculated Sofosbuvir and Ledipasvir Table-V.

Accuracy

For accuracy determination, three different concentrations were prepared separately i.e.50%, 100%, and 150% of analytes and the chromatograms were recorded for the same. The results obtained for recovery were found to be within the limits(Table-IV).

Table:IV Results for Accuracy

Accuracy	Amount added (µg/ml)		Amount recovered(µg/ml)		Percentage recovered	
	SFV	LDV	SFV	LDV	SFV	LDV
50%	200	45	200.67	45.09	100.22	100.22
100%	400	90	397.08	89.99	99.27	99.99
150%	600	135	600.3	134.37	100.05	99.53
Overall mean of three levels % recovery					99.84	99.91

Table: V Summary of Validation Parameters

S.No	Validation Parameter	SFV	LDV
1	Accuracy	99.84	99.91
2	Detection of wave length	265nm	
3	Linearity Calibration range (µg/ml)	100 -600	22.5 -135 µg/ml
4	Regression Equation (y = mx + c)	y = 7320x + 14790	y =13140x+ 2922.7
5	Slope (m)	7320.03	13140
6	Intercept (C)	4043.94	2922.7
7	Correlation coefficient (r^2)	0.9992	0.9993
8	System Precision (n=6) %RSD	0.9	0.94
9	Method precision (n=6) %RSD	0.71	0.99
10	Specificity Interference from mobile phase, diluents, placebo and degradants	No interference at the retention time of both drug peaks	
11	LOD(µg/mL)	1.8231	0.378
12	LOQ(µg/mL)	5.52	1.145
13	USP Plate count	3429	4024
14	USP Tailing factor	1.38	1.32
15	USP Resolution	-	3.2

Robustness

To evaluate the robustness small deliberate variations inColumn oven temperature (±5^{0}C), flow rate (±10%), Buffer (±5%) were madein the method and the samples were analyzed in triplicate.The system suitability was evaluated in each condition and compared withthe results of method precision (Table-VI).

Table: VI Robustness data for Sofosbuvir and Ledipasvir

S No	Chromatographic condition	SFV		LDV	
		RT (min)	Area (μV^2 Sec)	RT (min)	Area (μV^2 Sec)
1.	Flow rate at 0.9 mL/min	2.492	2871047	3.126	1209473
2.	Flow rate at 1.1mL/min	2.487	2876205	3.122	1217615
3.	Column oven temperature at 25^{0}C	2.479	2933725	3.113	1240452
4.	Column oven temperature at 35^{0}C	2.487	2872937	3.122	1215228
5.	Buffer variation at 35:65	2.479	2935381	3.113	1240068

6.	Buffer variation at 45:55	2.473	3159484	3.111	1302718

Stability of the analytical method

The stability of standard solution during analysis was determined over a period of 24h at room temperature. For all the solutions tested no change in the retention time and peak areas for SFV and LDV were observed. Further for the two drugs %RSD was 0.6& 0.5 respectively this indicates that there was no significant degradation during period of study were observed, i.e. both solutions were stable for 24h. The results were displayed in (Table VII).

Table: VII Stability data for Sofosbuvir and Ledipasvir

	Sofosbuvir				**Ledipasvir**			
Stability parameter	Rt value	Peak Area	USP Plate count	Tailing factor	Rt value	Peak area	USP Plate count	Tailing factor
Solution stability at room temp (0 hrs)	2.508	2853212	3426	1.35	3.123	1198575	4025	1.34
Solution stability at room temp (24 hrs)	2.515	3107481	3347	1.39	3.131	130885	4014	1.33

Specificity

Specificity shall be established by demonstrating that the procedure is unaffected by the presence of interference at the retention time of the Sofosbuvir and Ledipavir with respect to mobile phase, diluents, placebo and degradants. The specificity studies include deliberate degradation of the tablet sample by exposure to stress conditions. Specificity studies also include blank, placebo solution, and sample solution (control sample), Sofosbuvir and Ledipasvir standard solution were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of the peaks. Peak purity data reveals that Sofosbuvir and Ledipasvir were homogeneous and there was no interference at the retention time of both drug peaks(Fig-5-7)

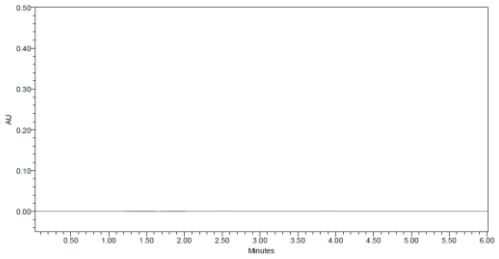

Fig-5:Blank Chromatogram of SFV and LDV

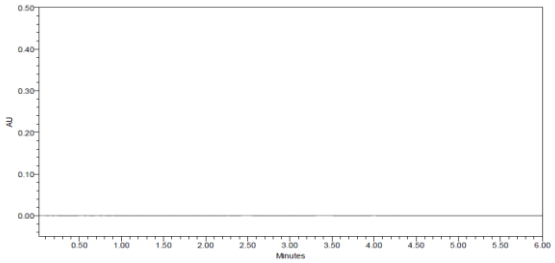

Fig-6: Placebo Chromatogram of SFV and LDV

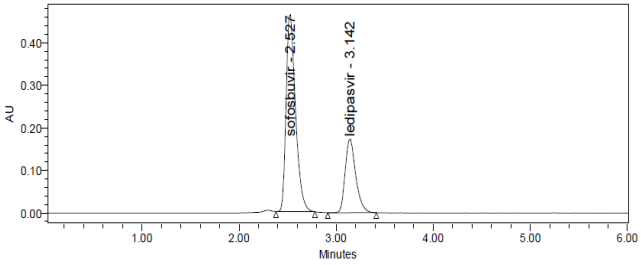

Fig-7: Standard Chromatogram of SFV and LDV

Stress Degradation Studies

Forced degradation or stress test is undertaken to demonstrate specificity. When developing stability- indicating methods, particularly very little information is available about potential degradation products. These studies also provide information about the degradation pathways and degradation products that could form during storage. Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing and packaging in which knowledge of chemical behavior can be used to improve a drug product.

Forced Degradation study was carried out by treating the sample under the following conditions [35-37]. Ten tablets were weighed and average weight was determined and finally powdered. Tablet powder equivalent to 40 mg Sofosbuvir and 9 mg Ledipasvir was

accurately weighed and transfer to 10 mL volumetric flask. The contents were sonicated for about 15 min for complete solubility of the drug after adding 10 mL of mobile phase and the volume was made up to the mark with the diluent. Then the mixture was filtered through a 0.45µ membrane filter. From the above solution 1 mL aliquot was taken into a separate 10 mL volumetric flask and diluted up to the volume with the diluent and mixed well.

AcidDegradation

To 1 ml of stock solution Sofosbuvir and Ledipasvir, 1 ml of 2N Hydrochloricacidwasadded and refluxed for 30mins at 60^0C. The resultant solutionwasdilutedtoobtain400 µg/ml& 90µg/ml solution and10µl solutionswereinjectedintothesystemandthe chromatogramswererecordedtoassessthestability ofsample(Fig-8).

AlkaliDegradation

To 1 ml of stock solutionof Sofosbuvir and Ledipasvir, 1 ml of 2 N sodium hydroxidewasadded and refluxed for 30mins at 60^0C. Theresultantsolutionwas diluted to obtain 400µg/ml& 90µg/ml solution and 10µl were injected into the system and the chromatograms were recordedtoassessthestabilityofsample(Fig-9).

Oxidation

To 1 ml of stock solution of Sofosbuvir and Ledipasvir 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For PLC study, the resultant solution was diluted to obtain 400 µg/ml & 90 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. (Fig-10).

DryHeatDegradation

Thestandarddrug solution was placedinovenat105^0C for6h,tostudydryheat degradation.ForHPLCstudy,the resultant solution was diluted to 400µg/ml & 90µg/ml solution and10µl were injected into the system and the chromatogramswererecordedtoassessthestability ofthesample (Fig-11).

PhotoStabilitystudies

The photochemical stability of the drug was also studied by exposing the 400µg/ml & 90µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber.For HPLC study, the resultant solution was diluted to obtain 90µg/ml&400µg/ml solutions and 10µl were injected into the system and the

chromatograms were recordedtoassessthestabilityofsample(Fig-12).

NeutralDegradation

Stress testing under neutral conditions was studied by refluxingthedruginwaterfor6hrs atatemperature of 60ºC. For HPLC study, the resultant solution was diluted to 400µg/ml& 90µg/ml solution and 10µl were injected intothesystemandthechromatogramswererecorded toassessthestabilityofthesample(Fig-13).

Table: VIII Stress degradation data for Sofosbuvir and Ledipasvir

Condition	Retention time (min)		Area(μV^2 Sec)		% of Active drug Present after Degradation	
	SFV	LDV	SFV	LDV	SFV	LDV
Control sample	2.511	3.127	3064775	1296134	70.19	29.81
Acid Degradation	2.479	3.111	2965068	1252924	66.73	27.89
Alkaline Degradation	2.479	3.111	2981272	1259305	67.20	27.71
Peroxide degradation	2.479	3.111	3011367	1276262	66.60	29.37
Thermal degradation	2.485	3.117	3042585	1289125	70.29	29.71
Photolytic degradation	2.480	3.109	3055341	1290794	70.25	29.75
Neutral degradation	2.485	3.116	3057840	1293445	70.22	29.78

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

The LOD and LOQ of the developed method were determined by analysing progressively low concentration of the standard solutions using the developed methods(Table-IV).

LOD= 3.3 σ/ S and LOQ = 10 σ/ S

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

Analysis of marketed formulations

The fixed chromatographic conditions were applied for the estimation ofSofosbuvir and Ledipasvir (Harvoni tablet:formulation 400 mg of Sofosbuvir and 90mg Ledipasvir) formulation by RP-HPLC method. Ten tablets were weighed and average weight was determined and finally powdered. Tablet powder equivalent to 400mgSofosbuvir and 90mg Ledipasvirwas accurately weighed and transfer to 10 mL volumetric flask. The contents were sonicated for about 15 min for complete solubility of the drug after adding 10 mL of mobile phase and the volume was made up to the mark with mobile phase. Then the mixture was filtered through a 0.45µ membrane filter. From the above solution, 1 mL aliquot was taken into a separate 10 mL volumetric flask and diluted up to the volume with the diluent and mixed well. Initially,inject 10 µL of blank, placebo, sample solution and standard solution Discarded peaks due to blank and placebo if any (Table-IX); (Fig-8)

Table: IXMarketed formulations (Assay) data for Sofosbuvir and Ledipasvir

Drug	Quantity claim (mg/tablet)	*Quantity found (mg/tablet) ± SD	*% Assay found ± SD

Sofosbuvir	**400**	**400 ±0.009**	**100.05 ± 0.60**
Ledipasvir	**90**	**90± 0.008**	**101.56± 0.92**

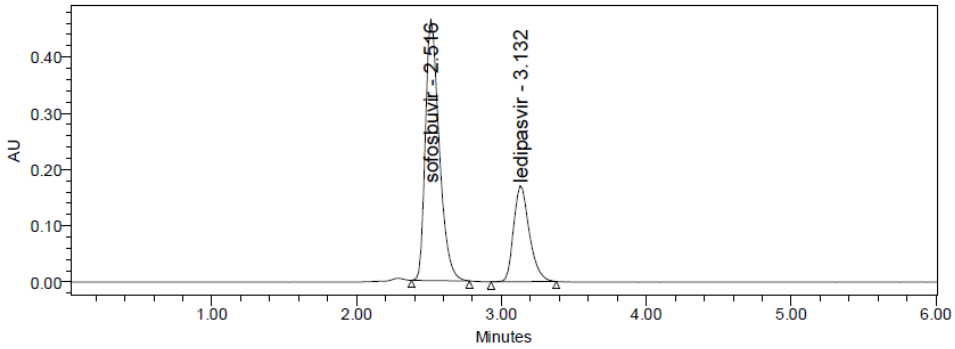

Fig-8: Analysis of marketed formulations (Assay) for Sofosbuvir and Ledipasvir

Recording of chromatograms

The standard solutions were tested for the system until stability was obtained. Initially the blank solution and placebo were injected. The standard chromatograms were recorded by injected standard solutions and the peak areas of standard chromatograms were noted. Calibration graph was plotted using peak area versus concentration. Then the sample solution was injected and the amount of Sofosbuvir and Ledipasvir present in the formulation was calculated from the calibration curve. The amount of Sofosbuvir and Ledipasvir present per tablet was found to be 400 ± 0.009mg and 90± 0.008mg. Total label claim for (Harvoni: 400mg of Sofosbuvir and 90mg Ledipasvir) formulation(Fig.VIII).

Results and discussion

The goal of the study is the development of simple, rapid, sensitive, specific and accurate HPLC method for the routine quantitative determination of API. Hypersil C_{18} BDS Column (250 mm x 4.6 mm; 5µm) was used a stationary phase. The mobile phase includedsodium dihydrogen phosphate buffer, Acetonitrile in the ratio of 40:60 and pHadjusted to 3 with dilutesodium hydroxide. A good linear relationship (r^2 = 0.9992 & r^2 = 0.9993) was observed in the range of 100-600 µg/mL & 22.5-135µg/mL for Sofosbuvir and Ledipasvir. The recovery values for linearity obtained by the proposed method are accurate.

The system precision was established by six replicate injections of the standard solutions containing analyte of interest. The value of relative standard deviation of Sofosbuvir and Ledipasvir was found to be 0.9 and 0.94 within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analysis six times using the proposed method. The relative standard deviation of Sofosbuvir and Ledipasvir was found to be 0.9 and 0.7 within the limit, indicating the injection repeatability of the method (Table-V)

Six samples of the same batch were prepared on different days. Calculated %RSD for two different days in six samples for ruggedness results with the method precision within the limits was measured. The system suitability was evaluated in each condition and the results were compared with method precision results. The method is robust for change in wave length, mobile phase composition and column oven temperature.

The specificity studies include deliberate degradation of the tablet sample by exposure to stress conditions. Specificity studies also include blank, placebo solution and sample solution (control sample),Sofosbuvir and Ledipasvir standard solution were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of the peaks. Peak purity data reveals that they were homogeneous.

Degradation studies showed that both the drugs were highly stable under photolytic, thermal and neutral conditions.But a slight decrease in RT value, peak area and an additional peak in oxidative condition was observed. In acidic and alkaline conditionstwo additional peaks were observed with slight decrease in RT values and peak areawhen compared to standard (Fig-9-14).In the present work on Sofosbuvir and Ledipasvir, highest degradation was observed in acidic and alkaline conditions, whereas relative stability wasnoticed when they were exposed to oxidative, thermal, photolytic and neutral conditions.Thus the developed RP-HPLC method was found to be simple, rapid, sensitive, accurate, precise and specific for the simultaneous estimation of the two drugs in bulk and pharmaceutical dosage forms and for routine analysis in stability studies of these drugs.

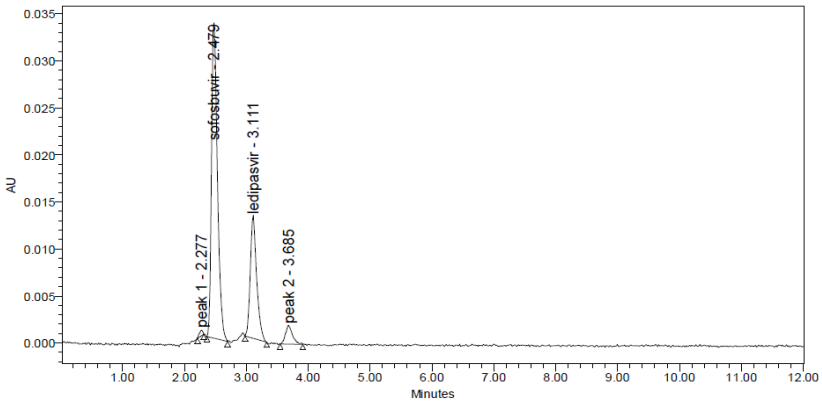

Fig-9: Chromatogram of acid degradation of SFV and LDV

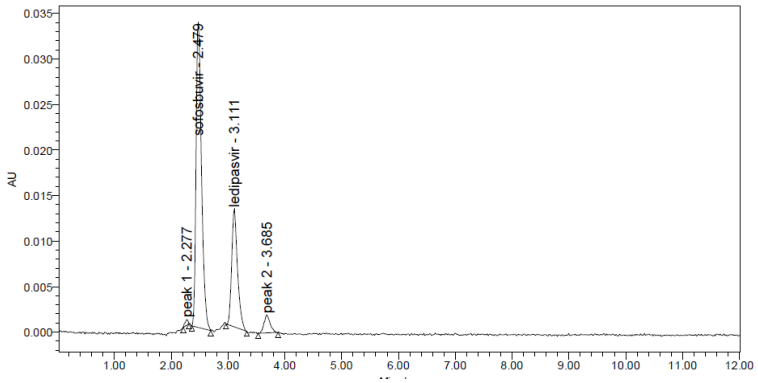

Fig-10: Chromatogram of alkaline degradation of SFV and LDV

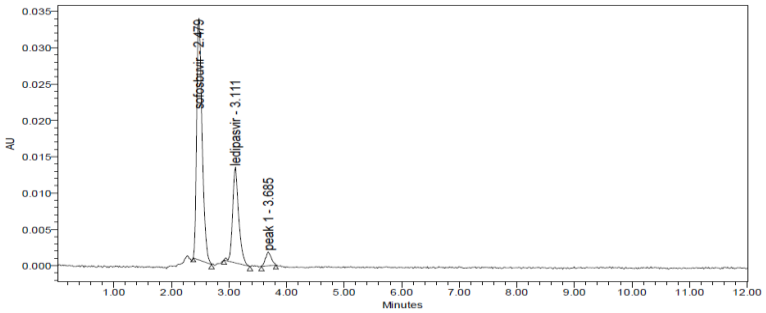

Fig-11: Chromatogram of oxidative degradation of SFV and LDV

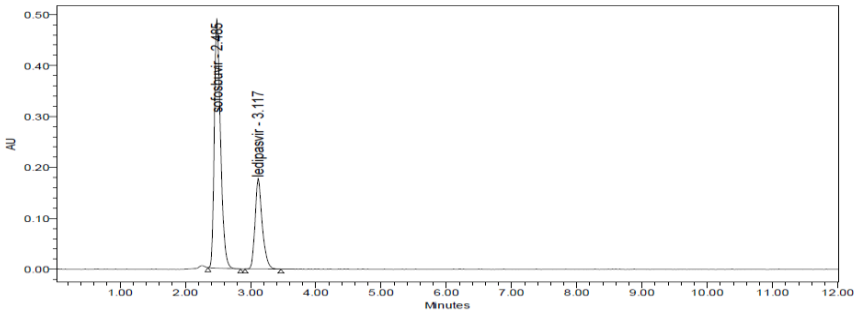

Fig-12: Chromatogram of Thermal degradation of SFV and LDV

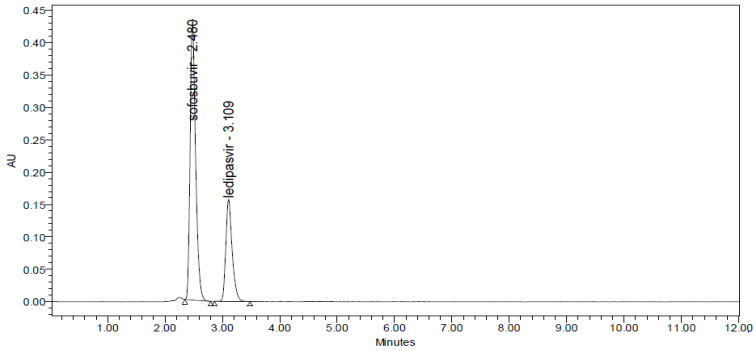

Fig-13: Chromatogram of Photolytic degradation of SFV and LDV

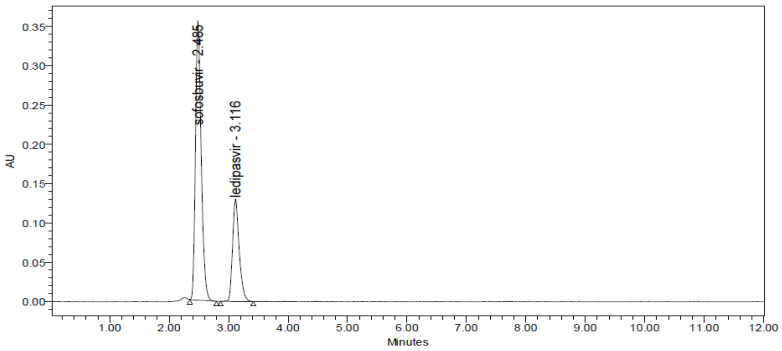

Fig-14: Chromatogram of Neutral degradation of SFV and LDV

CONCLUSION

The chosen drugs are recent and have a wide usage among the world population in the treatment of viral diseases like hepatitis C infections and also used with the same combination of drugs in treatment of novel corona virus. Therefore, fast, simple and reliable methods for their regular analysis in pharmaceutical industries are highly desired. Forced degradation studies in pharmaceutical development are aimed at exploring the condition that increase the drug degradation after its function in the body and there by avoid drug toxicity to

the subject. The results of the present study can be taken as inputs for further investigation on various parameters of drug metabolism by contemporary researchers. The present investigation facilitates the drug development and degradation products for metabolic studies. In future studies,there is more scope for the characterisation of the degradants.

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