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Development And Validation Of Stability Indicating RP-HPLC Method For Estimation Of Ledipasvir And Sofosbuvir

K.Nagaraju*, G.Tanuja[1], K.Divya[2]
[*]*Asst. Professor, Department Of Pharmaceutical Analysis,*
Sir C.R.Reddy College Of Pharmaceutical Sciences, Eluru-534 007, A.P., India.
[1]*Department of Pharmaceutical Analysis, Sir C.R.Reddy College Of Pharmaceutical Sciences, Eluru-534007, A.P., India.*
[2]*Department of Pharmaceutical Technology, Sir C.R.Reddy College Of Pharmaceutical Sciences, Eluru-534007, A.P., India.*

Abstract: *Ledipasvir and Sofosbuvir are the drugs used as anti-viral agents.The survey of literature reveals that good analytical methods are available for the drugs likeSofosbuvir&Ledipasvir. But the methods for estimation of related substances of anti-viral drugs were still emerging. The existing methods are inadequate to meet the requirements; hence it is proposed to improve the existing methods and to develop new methods for the estimation of related substances of Sofosbuvir&Ledipasvir in pharmaceutical dosage forms by using Shimadzu HPLC system consisting Zodiac C18 Column 250×4.6m, 5µm ; by using mobile phase Acetonitrile : Methanol: Water (60: 20: 20) with flow rate 1ml/min and detection was carried out at 270nm .Validation of the method was carried out in accordance with USP and ICH guideline for the estimation of related substances from active ingredients. The method was validated for parameters like accuracy, linearity, precision, specificity, repeatability, robustness, and system suitability.*

Keywords: *Anti-viral drugs, Validation, Method Development, RP-HPLC*

I. Introduction

1.1drug Profile Of Sofosbuvir:

CHEMICALNAME **:** Isopropyl (2s)-2-[[[2_R,3_R,4_R,5_R)-5-(2,4 DIOXO-3,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy-phosphoryl]amino]propanoate.

DESCRIPTION **:**Sofosbuvir inhibits the hepatitis C NS5B protein. Sofosbuvir appears to have a hidh barrier to the development of resistance.

MOLECULAR FORMULA**:**$C_{22}H_{29}FN_3O_9P$.

MOLECULAR WEIGHT **:** 529.45 g/mol.

SOLUBILITY **:** Slightly soluble in water, freely soluble in ethanol, acetone, 2-propanol.

1.2. Drug Profile Of Ledipasvir:

CHEMICALNAME **:**Methyl N-[(2s)-1-[(6s)-6-[5-[9,9-difluoro-7-[2-[(1S,2S,4R)-3-[(2S)-2(methoxycarbonylamino)3-methylbutanoyl]-3-azabicyclo[2.2.1]heptan-2yl]-3H-benzamidazol-5-yl]fluoren-2yl]1H-imidazol-2-yl]-5-azazaspiro[2.4]heptan-5-yl]3-methyl-1-oxobutan-2-yl]carbomate.

DESCRIPTION **:**A competitive serotonin type 3 receptor antagonist. It is effective in the treatment of nausea and vomiting caused by cytotoxic chemotherapy drugs, including cisplatin, and has reported anxiolytic and neuroleptic properties

MOLECULAR FORMULA **:** $C_{49}H_{54}F_2N_8O_6$.

MOLECULAR WEIGHT **:** 889.018 g/mol.

SOLUBILITY **:** partially insoluble in water and freely soluble in ethanol.

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP method with UV detection for the determination of Ledipasvir and Sofosbuvir in bulk form. The important features and novelty of the proposed method included simple sample treatment with sonicator of small amount of powder sample at ambient temperature, short elution time(less than 8min), good precision (R.S.D. less than 2%).

II. Materials And Methods

Material: Ledipasvir and Sofosbuvir were obtained from hetero pharmaceuticals.

Solvents: Acetonitrile (Hplc grade), Water (Hplc grade) ,Methanol (Hplc grade)

Preparation of mobile phase: Mix a mixture of above buffer solvents acetonitrile and water in 60:20:20 ratio and degas in ultrasonic water bath for 3minutes.Filter through 0.45µ filter under vacuum filtration.

Preparation of standard solution:Stock solution of LPS(1mg/mL) and SFV(1mg/ml) were prepared by weighing 10mg and dissolving in 10ml mobile phase. Standard solutions of LPS and SFV were prepared in the range of 10 µg/mL to 50 µg/mL by diluting the stock solution with mobile phase.

Selection of Analytical Wavelength

For HPLC method, analytical wavelength was determined from the absorption spectra ofLedipasvirSofosbuvir obtained by using UV-Visible spectrophotometer. From the solution of Ledipasvir and Sofosbuvir was scanned in the range of 200 – 400 nm. Wavelength of maximum absorption was determined for drug. Ledipasvirand Sofosbuvir showed maximum absorbance at 270 nm.

Instrument and chromatographic conditions: Schimadzu HPLC system with Discovery C18 (250mm x 4.6mm, 5µ) column, manual injector and PDA detection mode running on LC solutions software was used.

An isocratic mode with acetonitrile ,methanol and water in 60:20:20 as mobile phase at 1.0ml/min flow rate was used for separation of drugs. The detection of drugs was done at 270nm with column oven temperature maintained at 30ºC. The other instruments used were pH meter (EI), Digital Balance (Infra Instruments).

III. Method Validation

The developed method was validated for precision, specificity, accuracy (recovery), linearity and robustness as per the ICH guidelines

3.1. System suitability: The typical values for evaluating system suitability of a chromatographic procedure are relative standard deviation (RSD) <2%, tailing factor <1.5, and theoretical plates >1500. The retention time, peak area, theoretical plates, and tailing factor were evaluated for the system. The results were presented in table-2.

3.2. Linearity: Linearity was studied by analyzing five standard solutions covering the range of 10–50 µg/ml of Ledipasvir and Sofosbuvir. From the primary stock solution 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml of Ledipasvir and Sofosbuvir. A calibration curve (figure-5) with concentration versus peak areas was plotted by injecting the above concentrations. Linearity values were given in table-2

3.3. Accuracy : The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added. The accuracy is calculated from the test results as the triplicates sample preparation.

Procedure : The triplicates of stock solution equivalent to 50%,100%, and 150% were prepared by using standard stock solution. Each preparation was injected into the HPLC system. Accuracy values were shown in table-3.

3.4. Precision: The precision of the method was checked by repeated preparations. The measurement of peak areas of repeated solutions (n=6) for 10 µg/ml sample.precision values were given in table-4

3.5. Specificity: The specificity of the method was determined by injecting the placebo solution and comparing with standard solution for the interference with Ledipasvir and Sofosbuvir peak.

3.6.Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ are determined by standard deviation (SD) and slope of the calibration curve. The limiting values are calculated as per the following equations: LOD = (3.3 × SD)/ Slope and LOQ = (10 × SD)/ Slope

3.7. Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. E.g. flow rate, concentration, run time etc.

IV. Figures And Tables

4.1.Chemical structure of Ledipasvir:

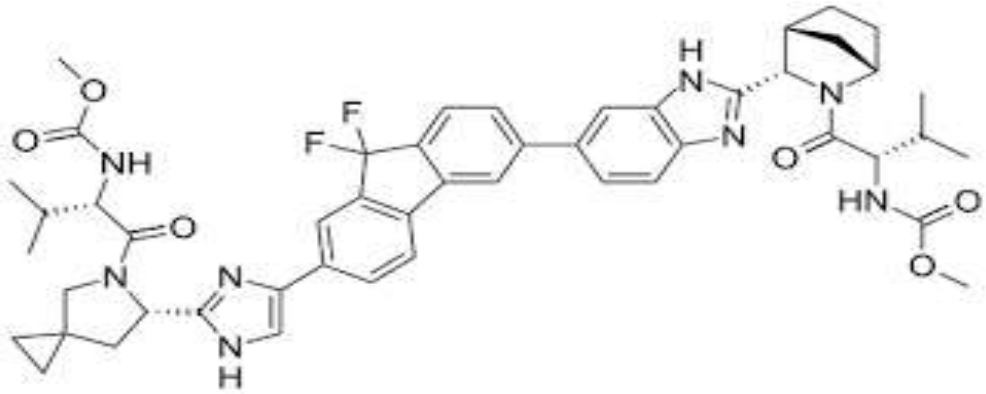


Figure-1: chemical structure of Ledipasvir

4.2. Chemical structure of Sofosbuvir:

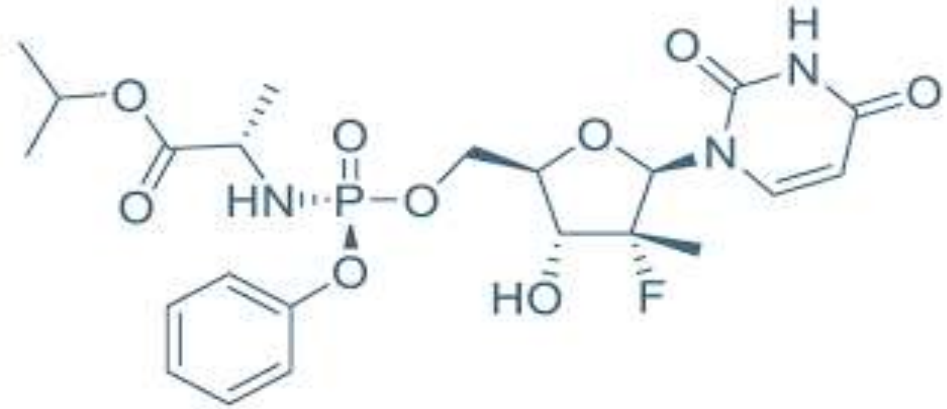


Figure-2: chemical structure of Sofosbuvir

4.3. Chromatograms

Figure.3: standard chromatogram of LedipasvirAndSofosbuvir

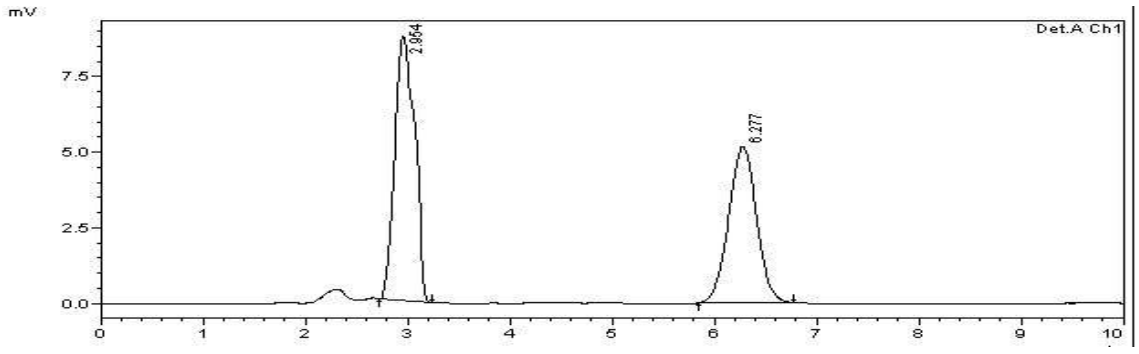
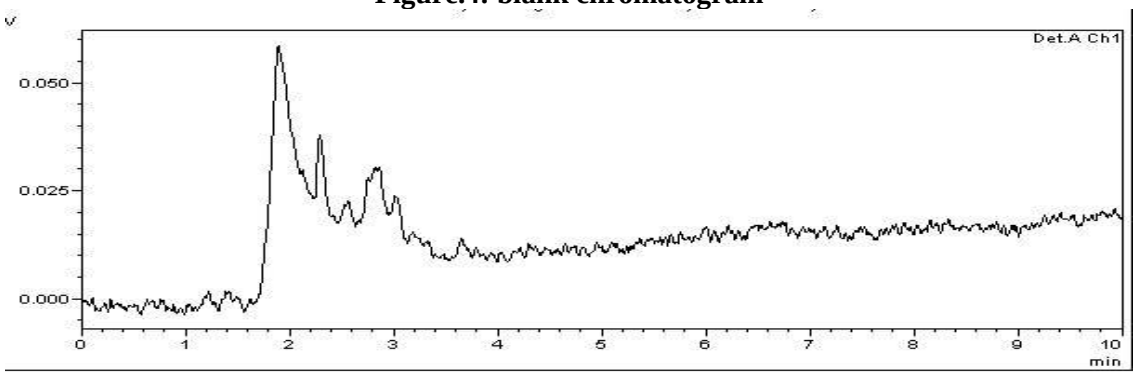
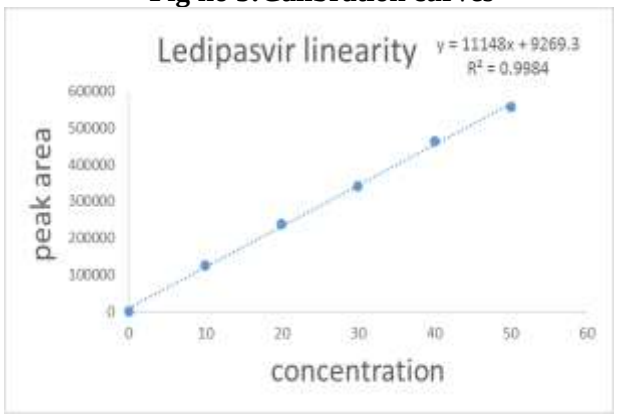


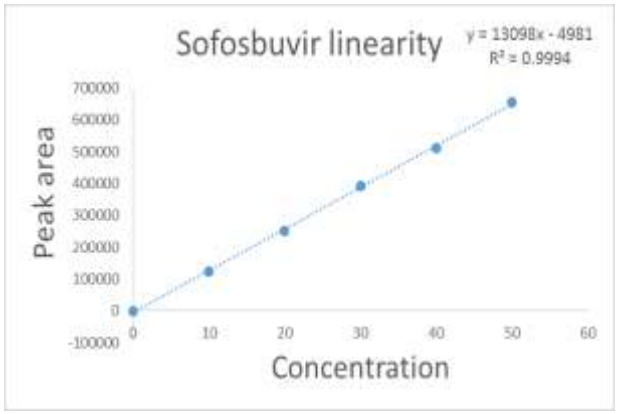
Figure.4: blank chromatogram



4.4. Calibration Curves:

Fig no 5:Calibration curves

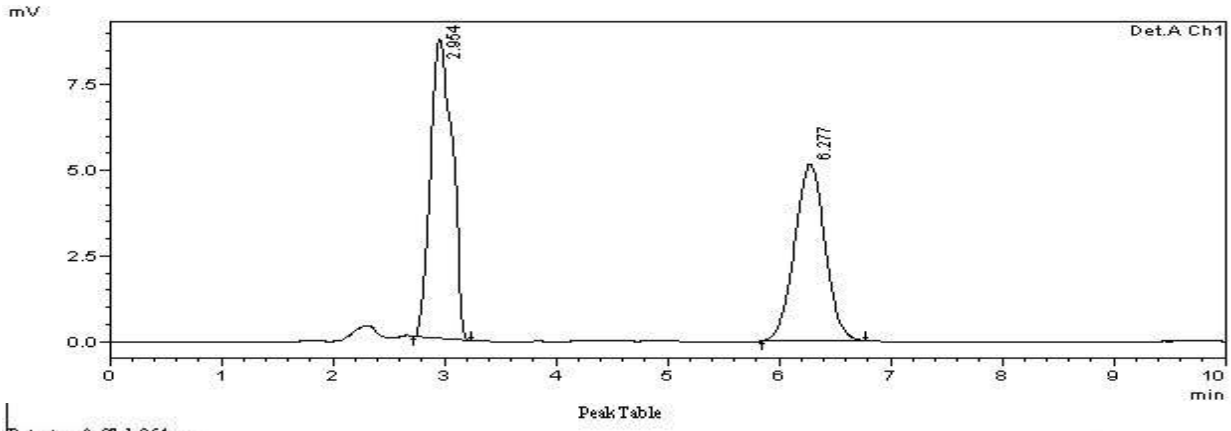




LINEARITY PLOT OF LEDIPASVIR **LINEARITY PLOT OF SOFOSBUVIR**

4.5. Optimized Method For Hplc Chromatographic Conditions:

Parameter	**Condition**
Stationary phase (column)	Zodiac C18 Column 250×4.6mm. 5µm
Mobile phase	Acetonitrile : Methanol: Water (60: 20: 20)
Flow rate	1 ml/min
Run time	10 min
Volume of injection	20µl
Detection wavelength	270



Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Theoretical Plates/meter	HETP	Tailing Factor
1	2.954	118736	6819.740	146.633	1.106
2	6.277	97904	16396.904	60.987	1.017
Total		216640			

Chromatogrm For Optimized Method

4.6. Results For System Suitability

S.NO	RETENTION TIME		AREA		PLATE COUNT		TAILING	
	(S)	(L)	(S)	(L)	(S)	(L)	(S)	(L)
1)	3.031	6.063	140611	104769	6199	18824	0.95	1.04
2)	3.022	6.075	143776	104499	6213	19036	1.00	1.04
3)	3.007	6.081	146726	105834	5561	19453	1.06	1.05
4)	3.038	6.627	145744	102160	6103	19622	0.96	1.07
5)	3.012	6.611	146929	102362	5483	19762	1.01	1.07
MEAN			147757	103924				
(σ)			2632.5	1600.3				
%RSD			1.7	1.5				
LIMITS			NMT 2.0%		NLT 2000		NMT 2.0	
(σ)= STANDARD DEVIATION, (S)= SOFOSBUVIR , (L)= LEDIPASVIR								

4.7. Results For Linearity

S.NO	**CONCENTRATION (µg/ml)**		**PEAK AREA**	
	SOFOSBUVIR	**LEDIPASVIR**	**SOFOSBUVIR**	**LEDIPASVIR**
1)	10	10	124005	126316
2)	20	20	251590	239510
3)	30	30	391440	340191
4)	40	40	511176	464528
5)	50	50	656574	557319
FOR SOFOSBUVIR CORRELATION CO-EFFICIENT = 0.999, Y= 13098x+4981			FOR LEDIPASVIR CORRELATION CO-EFFICIENT = 0.998, Y=11148x+9269	

4.8.Results Of System Precision

S.NO	RETENTION TIME		AREA REPRODUCIBILITY		PLATE COUNT		TAILING	
	(S)	(L)	(S)	(L)	(S)	(L)	(S)	(L)
1)	2.980	6.090	133355	169221	6932	14805	1.11	0.95
2)	3.031	6.063	132100	165813	7697	15103	0.94	1.00
3)	2.999	6.060	130824	163984	8301	14956	1.03	1.00
4)	3.007	6.075	131071	164715	7978	14998	1.09	1.00
5)	3.022	6.073	129109	165055	8733	15070	0.99	1.01
6)	3.022	6.081	132609	162218	7277	15041	0.93	1.02

MEAN		131511	165167		
STD. DEV(σ)		1509	2330		
%RSD		1.14	1.41		
LIMITS		NMT 2.0%		NLT 2000	NMT 2.0
(S)= SOFOSBUVIR , (L)= LEDIPASVIR					

4.9. Results Of Accuracy

CONC (µg/ml)	AMOUNT FOUND (µg/ml)		AMOUNT ADDED (µg/ml)		% RECOVERY		MEAN % RECOVERY	
	S	L	S	L	S	L	S	L
10	10.07	10.00	10	10	100.07	100.02	100.05	99.90
10	10.05	9.96	10	10	100.52	99.68		
10	9.95	10.00	10	10	99.56	100.01		
20	19.99	20	20	20	99.399	100	100	99.95
20	20.00	19.98	20	20	100.02	99.90		
20	19.99	19.99	20	20	99.99	99.96		
30	30.00	30.08	30	30	100.00	100.28	99.98	100.08
30	29.99	29.99	30	30	99.97	99.98		
30	29.99	29.99	30	30	99.99	99.99		

4.10. Loq Calculations

CALCULATION FOR SOFOSBUVIR
LOQ = 10 □ / Slope
10×1816 / 13098= 1.38

CALCULATION FOR LEDIPASVIR
LOQ = 10 □ / Slope
10×1195 / 11148= 1.07

4.11. LOD CALCULATIONS

CALCULATION FOR SOFOSBUVIR
LOQ = 3.3 □ / Slope
3.3×1816 / 13098= 0.45

CALCULATION FOR LEDIPASVIR
LOQ = 3.3 □ / Slope
3.3×1195 / 11148=0.30

V. Results And Discussion

In this paper we developing the reverse phased column procedure for a suitable method for the pharmaceutical analysis of Ledipasvir and Sofosbuvirdrugs. Atypical Chromatogram obtained by using the mobile phase .The precision and Accuracy of the method was determined.The method was validated for linearity, precision and accuracy parameters. Linearity of the method was studied by injecting six concentrations of drug prepared in the mobile phase in the range 10-50 µg/mL and solutions are analyzed through the high pressure liquid chromatographic technique .The peak area were plotted against concentration was subjected to linear plot and the results were placed in table (Table no.3). Precision of this method was studied in inter day and intraday variation . The precision of intraday studies was repeated on two consecutive days. The developed method was found to be precise as the percentage of RSD values for inter-day and intra – day precision studies were found to be less than 2%.

VI. Conclusion

A simple, rapid, accurate and precise RP-HPLC analytical method has been developed for the determination of Ledipasvir and Sofosbuvirin active pharmaceutical ingredient. The method was validated in accordance with ICH guidelines. First, the method goals are clarified based on process understanding. Here a better understanding of the factors influencing chromatographic separation and greater confidence in the ability of the methods to meet their intended purposes is done. Specificity of the method was determined by analyzing samples containing a mixture of the drug product and excipients. It givessymmetric peak shape, good resolution and reasonable retention for it. The method was validated accordance to ICH guidelines. All the validated parameters were found within acceptance criteria. The validated method is specific, linear, precise, accurate, robust for determination. RP-HPLC method resulted in more robust methods which can produce consistent, reliable, and quality data throughout the process and also save time and money.

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