An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF LEDIPASVIR AND SOFOSBUVIR IN TABLET DOSAGE FORM BY RP-HPLC

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ARTICLE INFO

***Key words**:*

Ledipasvir
Sofosbuvir,
RP-HPLC,
Gradient elution,
Validation, ICH guidelines

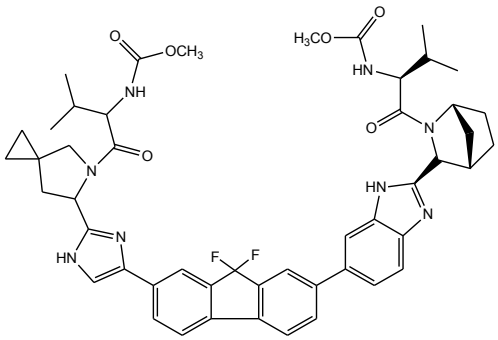
ABSTRACT

A new Reverse Phase High Performance Liquid chromatographic method was developed for the quantification of Ledipasvir and Sofosbuvir. The chromatographic separation was achieved on a Waters 2695, Inertsil - ODS C_{18} (250 x 4.6 mm, 5 µ) column within a runtime of 10 min under gradient elution Acetonitrile and methanol at a flow rate of 1.0ml/min. A photodiode array (PDA) detector set at 254nm was used for detection. The method was validated according to the ICH guidelines with respect to specificity, precision, accuracy and linearity. The proposed method was found to be reproducible and convenient for quantitative analysis of Ledipasvir and Sofosbuvir, in bulk and tablet dosage form.

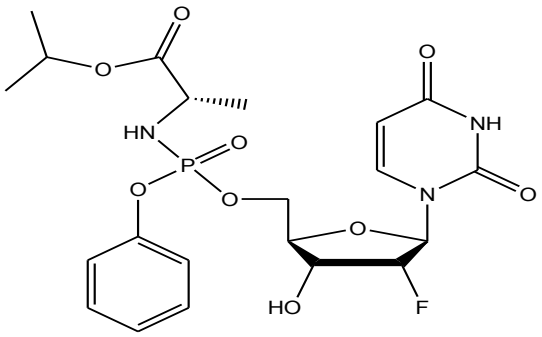
INTRODUCTION

Pharmaceutical Analysis plays a vital role in quality assurance and quality control of bulk drugs and their formulations. Pharmaceutical analysis is a particular branch of analytical chemistry, which includes isolating, identifying and determining the relative amounts of compounds in a sample matter[1]. It is concerned with chemical characterization of matter both quantitative and qualitative. In recent years many analytical techniques have been developed.[2,3] Pharmaceutical analysis derives its principles from different branches of science like Chemistry, Physics, Microbiology, Nuclear Science, Electronics etc. analytical method is a particular utilization of a procedure to solve a problem. Analytical instrumentation assumes an imperative part in the production and evaluation of new products and protection of Consumers and the environment[4]. This instrumentation provides the lower detection limits required to assure safe foods, medications, water and air[5]. Methods are developed for new products when no official methods are available. Alternate methods for existing (non-Pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness[6,7]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit / demerits are made available[8]. Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.[9]

There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality-control system. The second is that current good manufacturing practice regulation requires assay validation[10].



Ledipasvir structure



Sofasbuvir structure

Materials and Methods:

All chemicals and reagents used were of high quality, purity procured from various sources, Acetonitrile, Methanol Merck (HPLC-Grade), Ledipasvir and Sofosbuvir Reputed pharmaceutical company, Ledipasvir and Sofosbuvir tablets containing 90/400mg, are Purchased from local market Waters -2690/5, HPLC series with PDA, Inertsil -C18, BDS column, Detector wavelength 254nm, Colum Température is ambiant The Optimized chromatographic conditions are listed in Table No 1

Preparation of Ledipasvir Standard Solution:

Weigh down 10mg of Ledipasvir is dissolved in 10ml of Mobile phase taken in to 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from the solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

Preparation of Sofosbuvir Standard Solution:

Weigh down 10mg's of Sofosbuvir and dissolved in 10ml of Mobile phase taken in to 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from the solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

Validation of the Method

The method was validated in terms of system precision, linearity, precision, and specificity of the sample applications. The linearity of the method was investigated with correlation coefficient of Ledipasvir and Sofosbuvir was found to be 0.999 Precision was found to be lower than 1%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions, Placebo interference Sample was prepared by taking the placebo equivalent to about the weight in portion of test preparation as per the test method and blank interference mobile phase was prepared and injected and into the HPLC system, are in Fig No: 1-3

Accuracy

Accuracy of the method was expressed in terms of recovery of added compound at 50%, 100% and 150% level of sample. Mean % recovery and % RSD were calculated and were summarized in Table 2-3.The result shown that best recoveries (99.77±0.04) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Repeatability

The % Relative standard deviations of Ledipasvir and Sofosbuvir for Repeatability was found to be 0.143and 0.240.Hence the %RSD values indicate a good degree of precision within the specified range. The results are tabulated in Table No 4

Method precision

Precision of the assay method was determined by injecting, six (6) individual samples, in duplicate, of Ledipasvir and Sofosbuvir. The results are tabulated in Table No 5.

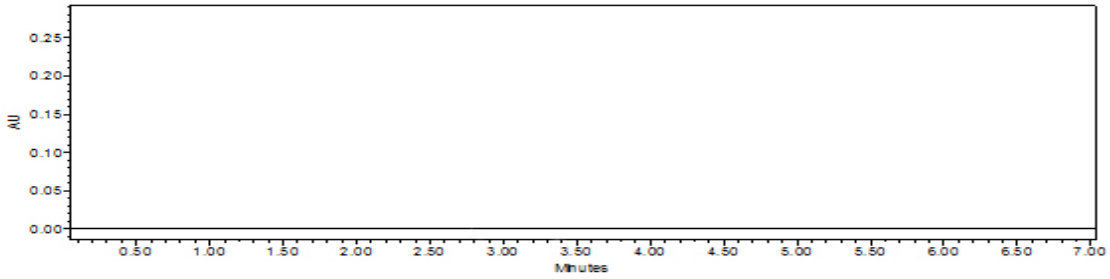


Fig No.1: Placebo chromatogram

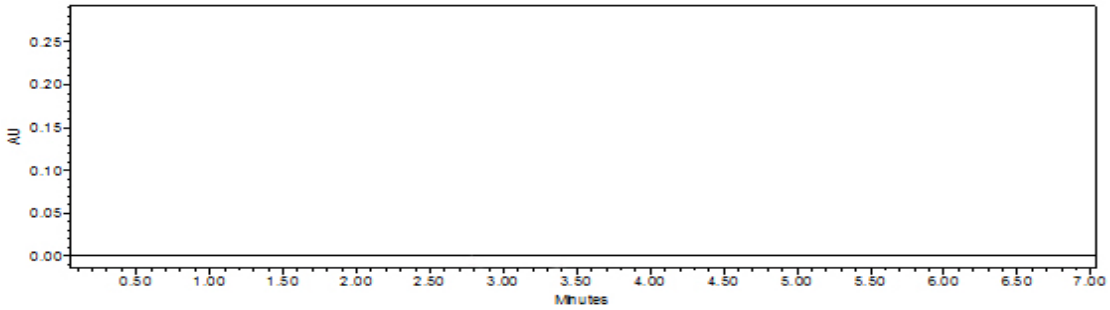


Fig No.2: Blank chromatogram

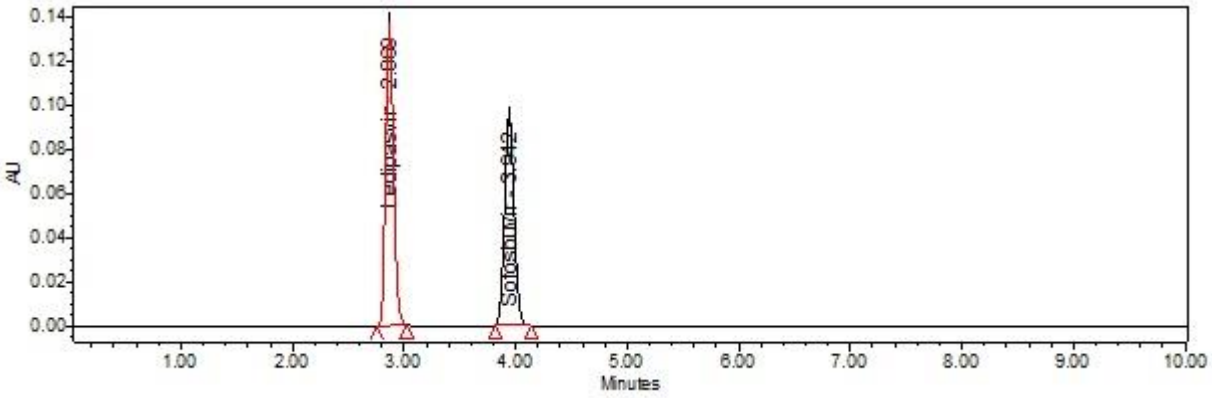


Fig No.3: chromatogram of Ledipasvir and Sofosbuvir

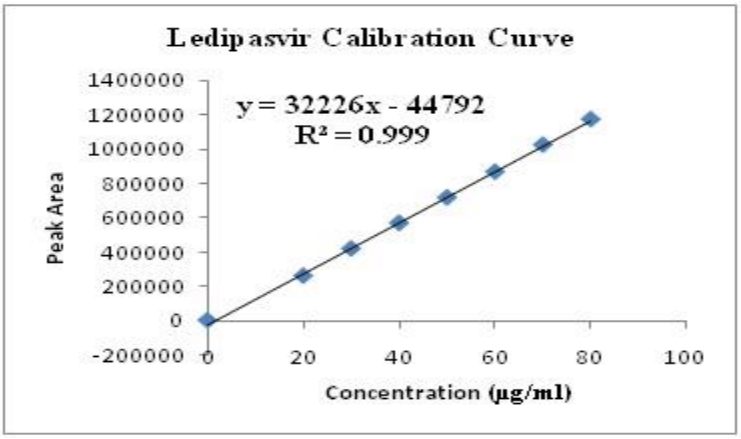


Fig No.4: Ledipasvir Calibration Curve

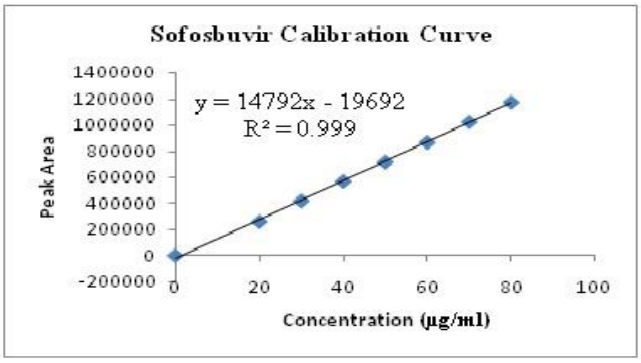


Fig No.5: Sofosbuvir Calibration Curve

Table No.1: Optimization method conditions

Parameters	Method
Stationary phase (column)	Inertsil -ODS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Acetonitrile : Methanol (60:40)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	254nm
Drug RT (min)	2.8 min for Ld and 3.9 min for Sb.

TABLE No.2: Accuracy Data for Ledipasvir

Concentration % of spiked level	Amount added (ppm)	Peak area	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
					Mean	%RSD
50% Injection 1	20	570656	19.95	99.75	99.81	0.55
50% Injection 2	20	568084	19.86	99.3		
50% Injection 3	20	2861594	20.08	100.4		
100 % Injection 1	40	1243701	40.14	100.35	99.91	0.42
100 % Injection 2	40	1238121	39.96	99.9		
100% Injection 3	40	1233165	39.80	99.5		
150% Injection 1	60	1866096	59.89	99.81	100.067	0.17
150% Injection 2	60	1870771	60.04	100.06		
150% Injection 3	60	1872326	60.09	100.15		

Table No.3: Accuracy data for Sofosbuvir

Concentration % of spiked level	Amount added (ppm)	Peak area	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
					MEAN	%RSD
50% Injection 1	20	259435	19.98	99.9	99.9	0.20
50% Injection 2	20	258914	19.94	99.7		
50% Injection 3	20	259956	20.02	100.1		
100 % Injection 1	40	565450	39.86	99.65	99.9	0.23
100 % Injection 2	40	568145	40.05	100.125		
100% Injection 3	40	567151	39.98	99.95		
150% Injection 1	60	864036	59.90	99.83	99.93	0.10
150% Injection 2	60	865044	59.97	99.95		
150% Injection 3	60	865768	60.02	100.03		

Table No. 4: Data of Repeatability (System precision) for Ledipasvir and Sofosbuvir

	Injection	Ledipasvir		Sofosbuvir	
		Peak area	%Assay	Peak area	%Assay
Concentration 40ppm	1	1239678	99.35	549407	99.98
	2	1243389	99.63	547265	99.30
	3	1264984	99.54	553482	99.60
	4	1248352	99.25	551981	99.84
	5	1256493	99.48	551495	99.72
Statistical Analysis	Mean	1250579	99.4	550726	99.71
	SD	10222.12	0.3546	6031.135	0.425
	% RSD	0.817391	0.143	0.439932	0.240

Table No.5: Data of Repeatability (Method Precision) for Ledipasvir and Sofosbuvir

	Injection	Ledipasvir		Sofosbuvir	
		Peak area	%Assay	Peak area	%Assay
Concentration 40ppm	1	1243389	98.6	547265	98.55
	2	1264984	99.02	553782	98.88
	3	1248352	98.12	551981	99.40
	4	1256493	98.31	551495	99.30
	5	1239664	98.81	547437	100.53
	6	1243411	98.36	549117	98.28
Statistical Analysis	Mean	1250579	98.48	550726	99.278
	SD	10222.12	0.352647	2422.819	0.827236
	% RSD	0.817391	0.35	0.439932	0.83

Table No.6: Data of Intermediate precision Ledipasvir and Sofosbuvir

	Injection	Ledipasvir		Sofosbuvir	
		Peak area	%Assay	Peak area	%Assay
Concentration 40ppm	1	1239364	99.78	547437	99.99
	2	1243411	99.95	549117	99.66
	3	1237979	100.00	546517	101.53
	4	1246482	98.55	550490	99.98
	5	1241537	99.91	547427	99.97
	6	1237979	99.38	549117	101.10
Statistical Analysis	Mean	1241755	99.86	548197.6	100.37
	SD	3358.178	1.105	1588.8	0.75354
	% RSD	0.2704	0.85	0.270438	0.75

Table No.7: Data of linearity (Ledipasvir)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0		
20	572087		
30	887800		
40	1239364	Slope	32226
50	1570861	y-Intercept	44792
60	1869524	Correlation Coefficient	0.999
70	2234112		
80	2546863		

Table No.8: Data of linearity (Sofosbuvir)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0		
20	259695		
30	418090		
40	567437	Slope	14792
50	715694	y-Intercept	19692
60	865479	Correlation Coefficient	0.999
70	1022457		
80	1170855		

Table No.9: Data of system to system variability

S. no.	Ledipasvir		Sofosbuvir	
	Peak area	Assay %	Peak area	Assay %
1	1243389	99.98	547265	99.35
2	1264984	99.30	553482	99.63
3	1248352	99.60	551981	99.54
4	1256493	99.84	551495	99.25
5	1239664	99.72	547437	99.48
6	1243411	98.89	549117	99.56
Mean	1249382	99.71	550129.5	99.46
%RSD	0.768595	0240244	0.4670	0.143228

Table No.10: Data for Effect of variation in flow rate (Ledipasvir)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	1239361	1.133372	**Flow 1.0 ml**	1239678	1.146235	**Flow 1.2 ml**	1243389	1.129133
	1243411	1.164125		1243389	1.129133		1264984	1.159150
	1237979	1.123397		1264984	1.159150		1248352	1.141469
	1246482	1.125612		1248352	1.141469		1256493	1.130372
	1241537	1.123857		1248352	1.130372		1239664	1.133372
Avg	1241755	1.134073	**Avg**	1250579	1.141272	**Avg**	1249382	1..0776444
SD	3358.178	0.017274	**SD**	10222.12	0.0012354	**SD**	9602.688	0.005207937
%RSD	0.270438	1.523171	**%RSD**	0.817391	1.14272	**%RSD**	0.768595	0.4832705

Table No.11: Data for Effect of variation in flow rate (Sofosbuvir)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	547437	1.086917	**Flow 1.0 ml**	549407	1.082014	**Flow 1.2 ml**	547265	1.075439
	549117	1.074793		547265	1.075439		553482	1.074589
	546517	1.075516		553482	1.074589		551981	1.075276
	550490	1.076837		551981	1.075276		551495	1.076001
	547427	1.077863		551495	1.075276		547437	1.086917
Avg	548197.6	1.078385	**Avg**	550726	1.076664	**Avg**	550129.5	1.0776444
SD	1588.8	0.004914	**SD**	2422.819	0.003033	**SD**	2569.51246	0.012401
%RSD	0.289823	0.4557	**%RSD**	0.439932	0.281698	**%RSD**	0.46707411	1.089051

Intermediate precession:

The % Relative standard deviations of Ledipasvir and Sofosbuvir for Intermediate precession was found to be 0.85 and 0.75.Hence the %RSD values indicate a good degree of precision within the specified range. The results are tabulated in Table No 6.

System Precision Ruggedness

The standard and sample solutions prepared by analyst-1 and analyst-2 are injected in different HPLC systems, on different day, using a different column. The system suitability parameters calculated by analyst -2 can be compared with those of Analyst -1. The results were tabulated in Table 9. These results indicated that the developed method is rugged.

Linearity

The linearity range of Ledipasvir and Sofosbuvir was evaluated by varying concentrations of standard solutions were injected into HPLC system. The linearity graph was plotted from (Fig: 4-5). A calibration curve was constructed for each sample by plotting the peak area obtained the concentration. The correlation coefficient for the data was calculated as 0.999. The regression line were observed to be in the form of y = 32226x - 44792. The linearity data for Ledipasvir.The regression line were observed to be in the form of y = 14792x - 19692 and Sofosbuvir are presented in Table 7-8.

Robustness

Small changes in flow rate, composition of mobile phase and temperature, performed the robustness of method. Robustness was studied using three replicates of concentration level at 100%. The % RSD in robustness study was less than 2%, his indicates that the method is precise, accurate and robust, the results are tabulated in 10-11.

CONCLUSION

The present proposed RP-HPLC method for the assay of Ledipasvir and Sofosbuvir in tablet formulation was validated as per ICH Q2(R1) guideline and it meets to specific acceptance criteria. It is concluded that the developed method was specific, precise, linear, accurate, robust, cost effective and it proves all validation characteristics and it can be effectively applied for routine analysis in research institutions, quality control department in industries.

ACKNOWLEDGEMENT

The authors thank Sri. C. Gangi Reddy, Founder, Annamacharya Educational Trust for providing all the facilities for graduate studies and dissertation work.They also thank Reputed pharmaceutical company (P) Ltd. (India), for providing the gift sample of standard Ledipasvir and Sofosbuvir.

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