Journal of Cardiovascular Disease Research
ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

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

[1]T. Nagendra Kumar *, [2]K. Nagaraju, [3] M. Eswar gupta.
Department of Pharmaceutical Analysis & Quality Assurance,
Sir c r reddy College of Pharmaceutical sciences, Eluru, Santhinagar-534007, West Godavari (DT), Andhra Pradesh, India.
***Corresponding author:**
Department of pharmaceutical analysis & Quality Assurance**,** Sir c r reddy college of pharmaceutical sciences, Andhra University, Santhinagar, Eluru, West Godavari district 534007, Andhra Pradesh, India.
E-mail address: kumarchinni94924@gmail.com

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 SHIMADZU Prominence HPLC , Zodiac - C_{18} (250 x 4.6 mm, 5 µ) column within a runtime of 10 min under isocratic elution Acetonitrile and methanol and water at a flow rate of 1.0ml/min. UV detector set at 275nm 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 form.

Keywords
Sofosbuvir, Ledipasvir, ICH, HPLC, HCV

INTRODUCTION

Hepatitis C is an infectious disease caused by the Hepatitis C Virus (HCV) that primarily affects the liver, it is a type of viral hepatitis [5]. Sofosbuvir (SBR) is a medication used to treat hepatitis c. Sofosbuvir inhibits Hepatitis C NS5B protein and sofosbuvir appears to have a high barrier to the development of resistance [6]. Ledipasvir (LDR) is also used for the treatment of Hepatitis C. It inhibits an important viral phospoprotein NS5A, which id involved in viral replication, assembly and secretion [7]. The combination of sofosbuvir (SBR) and ledipasvir (LDR) for the treatment of HCV is approved by FDA in 2014. After literature survey we found many chromatographic methods for the determination of sofosbuvir (SBR) and ledipasvir (LDR) by using RP-HPLC[9,10,11,12,13,14,15,16]. In present study we developed and validated a rapid and robust HPLC method for the simultaneous estimation of sofosbuvir (SBR) and ledipasvir (LDR).

Figure-I Structure of ledipasvir

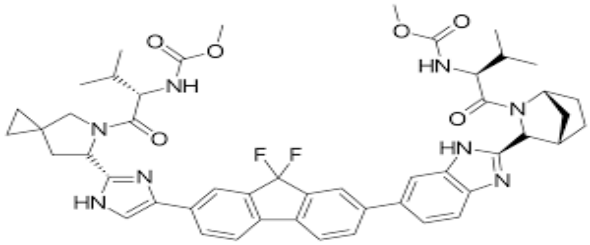
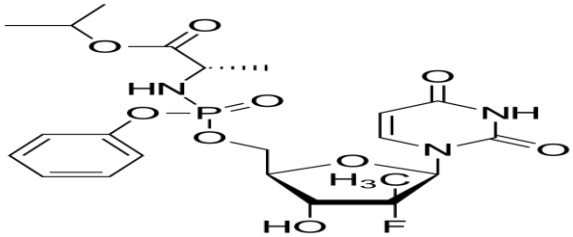


Figure 2. Structure of sofosbuvir



ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Materials and Methods

All chemicals and reagents used were of high quality, purity procured from various sources, Acetonitrile, Methanol, Water Merck (HPLC- Grade), Ledipasvir and Sofosbuvir Reputed pharmaceutical company, SHIMADZU Prominence, HPLC series with UV Detector, Zodiac -C18, ODS column, Detector wavelength 275nm, Colum Temperature is ambient The Optimized chromatographic conditions are listed in Table No 1

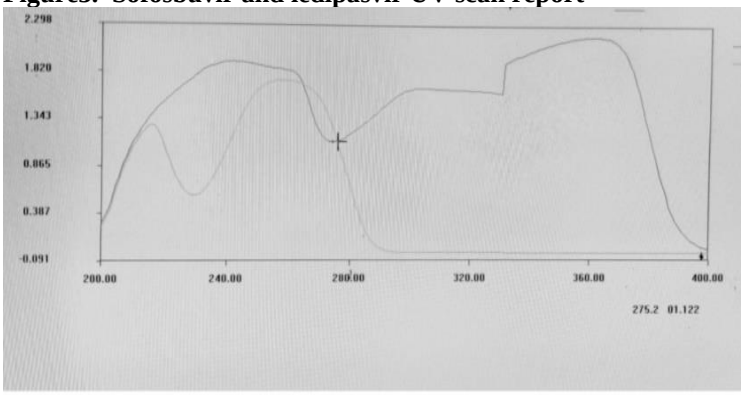
Preparation of Ledipasvir Standard Solution

Weigh accurately about 10mg of Ledipasvir is dissolved in 10ml of Methanol taken in to 10ml of volumetric flask and sonicated for 10 minutes to get 1000ppm and 0.1 ml was taken from the solution into a 10ml volumetric flask and diluted to 10 ml with methanol.

Preparation of Sofosbuvir Standard Solution

Weigh accurately about 10mg of Sofosbuvir is dissolved in 10ml of Methanol taken in to 10ml of volumetric flask and sonicated for 10 minutes to get 1000ppm and 0.1 ml was taken from the solution into a 10ml volumetric flask and diluted to 10 ml with methanol. The samples were scanned in UV spectrophotometer and the wavelength was found to be 275 nm

Figure3. Sofosbuvir and ledipasvir UV scan report



Validation of the Method

The method was validated according to ICH guidelines in terms of specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness of the sample.

Table 1. Optimized method conditions

Parameters	Method
Stationary phase (column)	Zodiac ODS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Acetonitrile : Methanol: water (60:20:20)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20 µl
Detection wavelength (nm)	275nm
Drug RT (min)	2.9 min for sofosbuvir and 6.2 min for ledipasvir

Results

Specificity

Specificity was performed by injecting blank. It was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. There is no interference of any impurities on the

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

retention time of analytical peak.

Figure 4. Blank chromatogram

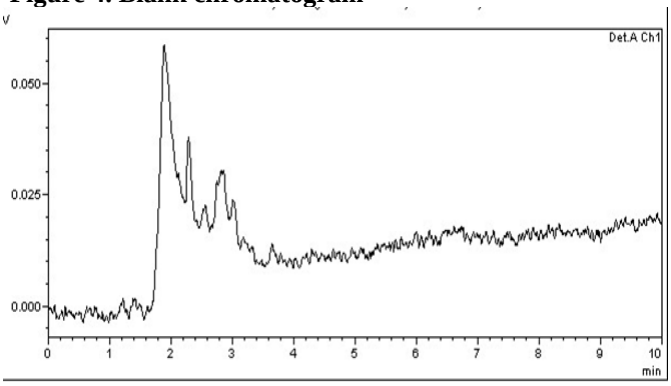
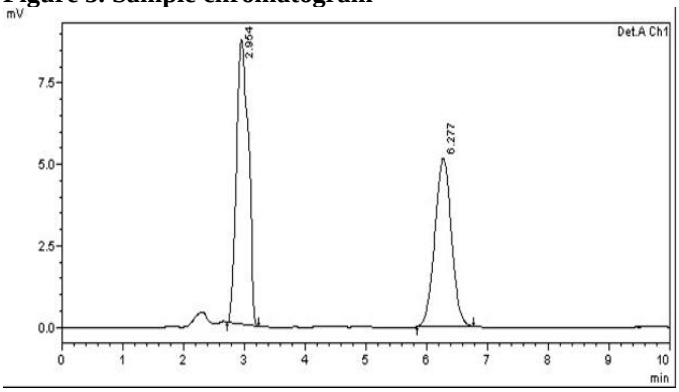


Figure 5. Sample chromatogram



Linearity

The linearity range of Ledipasvir and Sofosbuvir was evaluated by varying concentrations ranging from 10-50µg/ml of standard solutions were injected into HPLC system. The linearity graph was plotted from (Fig: 3-4). 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 = 11148x + 9269.3 for Ledipasvir .The regression line were observed to be in the form of y = 13098x – 4981 for Sofosbuvir and Linearity data for ledipasvir and Sofosbuvir are presented in Table no 2&3.

Figure 6. Ledipasvir linearity graph

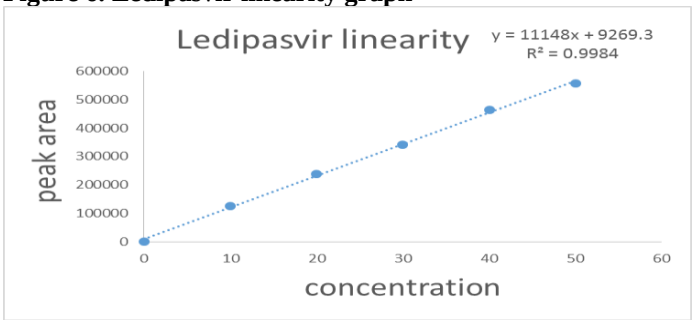


Figure 8. Sofosbuvir linearity graph

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

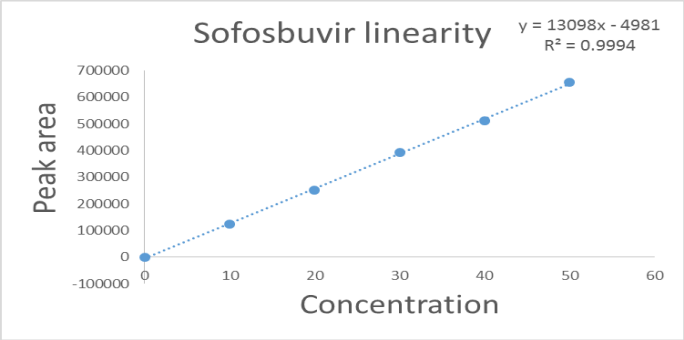


Table 2. Linearity Data for Ledipasvir

Concentration (µg)	Average Area	Statistical Analysis	
0	0	Slope	11148
10	126316		
20	239510	y-Intercept	9269
30	340191		
40	464528	Correlation Coefficient	0.998
50	557319		

Table 3. Linearity Data for Sofosbuvir

Concentration (µg)	Average Area	Statistical Analysis	
0	0	Slope	13098
10	124005		
20	251590	y-Intercept	4981
30	391440		
40	511176	Correlation Coefficient	0.999
50	656574		

Precision

Repeatability

The % Relative standard deviations of ledipasvir and sofosbuvir for repeatability was found to be 1.41 and 1.14. Hence the %RSD values indicate a good degree of precision within the specified range. The results are tabulated in Table No 4.

Table 4. Data of Repeatability for Ledipasvir and Sofosbuvir

	Injection	Ledipasvir	Sofosbuvir
		Peak area	**Peak area**
Concentration 10µg/ml	1	169221	133355
	2	165813	132100
	3	163984	130824
	4	164715	131071
	5	165055	129109
	6	162218	132609
Statistical Analysis	**Mean**	165167	131511
	SD	2330	1509
	%RSD	1.41	1.14

Intermediate precision

The % Relative standard deviations of Ledipasvir and Sofosbuvir for intermediate precession was found to be 1.04 and 1.26.Hence the %RSD values indicate a good degree of precision within the specified range. The results are

tabulated in Table No 5.

Table 5. Data of Intermediate precision for Ledipasvir and Sofosbuvir

	Injection	Ledipasvir	Sofosbuvir
		Peak area	Peak area
Concentration 10µg/ml	1	113414	141592
	2	113187	146701
	3	113417	141928
	4	113467	143088
	5	113545	143157
	6	116234	143506
Statistical Analysis	Mean	113895	143338
	SD	1195	1816
	%RSD	1.04	1.26

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 .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.

Table 6. 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	591594	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 7. 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		

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%, this indicates that the method is precise, accurate and robust; the results are tabulated in 10-11.

Table- 8 Data for Effect of variation in flow rate (Ledipasvir)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
	1239361	1.138372		1239678	1.146235		1243389	1.129133
	1243411	1.132285		1243389	1.129133		1264984	1.159150
	1237979	1.125927		1264984	1.159150		1248352	1.141469
Flow 0.8 ml	1246482	1.127428	**Flow1.0 ml**	1248352	1.141469	**Flow 1.2 ml**	1256493	1.130372
	1241537	1.123857		1248352	1.130372		1239664	1.133372
Avg	1241755	1.129573	**Avg**	1250579	1.141272	**Avg**	1249382	1.077644
SD	3358.178	0.002908	**SD**	10222.12	0.001235	**SD**	9602.688	0.005207
%RSD	0.270438	0.257442	**%RSD**	0.817390	0.108212	**%RSD**	0.768595	0.483188

Table- 9 Data for Effect of variation in flow rate (Sofosbuvir)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
	578649	1.134753		568561	1.125684		568428	1.139145
	568543	1.124158		568534	1.121983		561542	1.149125
	561524	1.143582		576984	1.149124		564857	1.142561
Flow 0.8 ml	568568	1.124587	**Flow1.0 ml**	569578	1.125685	**Flow 1.2 ml**	574596	1.123562
	566895	1.135681		559852	1.136379		568536	1.123374
Avg	566835	1.132552	**Avg**	568701	1.131771	**Avg**	567591	1.135553
SD	4126.90	0.004107	**SD**	3038.31	0.005546	**SD**	2433.21	0.005800
%RSD	0.27043	0.36263	**%RSD**	0.534254	0.49002	**%RSD**	0.42869	0.51076

Limit of detection [LOD]

Calculation for sofosbuvir
LOQ = 3.3σ / Slope
3.3×1816 / 13098= 0.45

Calculation for ledipasvir
LOQ = 3.3σ / Slope
3.3×1195 / 11148=0.30

Limit of quantification [LOQ]

Calculation for sofosbuvir
LOQ = 10 σ / Slope
10×1816 / 13098= 1.38

Calculation for ledipasvir
LOQ = 10 σ / Slope
10×1195 / 11148= 1.07

Discussion & Conclusion

The HPLC method was developed by using, Zodiac C18 (250mmX 4.6mm id particle size 5µ) reverse phase packed with Octadecyl silane chemically bonded to porous silica or ceramic micro-particle with mobile phase 60:20:20 (v/v) acetonitrile, methanol, water. Flow rate was 1 ml / min with UV detection at 270 nm and the injection volume was set at 20 µl, with 10 min runtime. The developed method was validated by using various parameters according to ICH guidelines. It was validated for specificity, stability in analytical solution, linearity, precision, accuracy studies, LOD, LOQ, robustness and ruggedness. All the validation parameters were found to be well within the acceptance criteria. The system suitability parameters also reveal that the values are within the specified limit for the proposed method. The theoretical plates for Sofosbuvir and Ledipasvir were found to be more than 2000 and the tailing factor is NMT 2.0. The precision of the System and Method were checked and found to be within limits. This indicates that the method is precise. From the linearity studies, the specified range for Sofosbuvir was found to be (10-50) µg/ml and for Ledipasvir was found to be (10-50) µg/ml. It was evaluated by the visual inspection of the plot of Peak area vs. Concentration and the correlation was found to be linear.

The accuracy studies found that the recovery value of pure drug and sample is in between 99.97 % to 100.04% which indicates that the method is accurate. The system suitability should passes as per the test method at variable conditions, hence it was concluded that the test method was Robust. There is a wide scope for the development of

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

new analytical methods for the assay of the above drugs. RP-HPLC technique has been used as a tool in the present work.

An efficient high performance liquid chromatographic method was developed and validated for the estimation of Sofosbuvir and Ledipasvir by RP-HPLC.

The proposed method was found to be simple, sensitive, precise, accurate and robust. The developed method was checked for the performance characteristics and has also been validated.

Acknowledgment

I take this opportunity to acknowledge my thanks and regards to management of sir c r reddy college of pharmaceutical sciences.

References:

1. ICH Guideline on Impurities in new drug products; Q3B (R2), 2006.
2. ICH Guideline on Validation of analytical procedures: text and methodology; Q2 (R1), 2005.
3. Sharma BK. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23th ed.Goel publishing house Meerut, 2004, P12-23.
4. P.D. Sethi. HPLC: Quantitative analysis pharmaceutical formulations, CBS Publishers and distributors, New Delhi (India), 2001, P.3-137.
5. https://en.wikipedia.org/wiki/Hepatitis_C
6. https://en.wikipedia.org/wiki/Sofosbuvir
7. https://en.wikipedia.org/wiki/Ledipasvir
8. http://www.rajaha.com/types-of-spectroscopy
9. Kranthi k k, et al, A new analytical method development and validation for the simultaneous estimation of ledipasvir and sofosbuvir using RP-HPLC. Icjpir2017, 4(1), 142-165.
10. Devilal, et al, New method development and validation for the determination of ledipasvir in bulk drug form by Using RP-HPLC. World journal of pharmacy and pharmaceutical sciences2016, 5(8), 1312-1321.
11. Bakht zaman, et al, RP-HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form its applications to invitro dissolution studies. Springer link, chromatographia2016, 79, 23-24.
12. Battulasreenivasarao, et al, Simultaneous analysis of ledipasvir and sofosbuir in bulk and tablet dosage form by stability indicating high performance liquid chromatographic method. Gjra2017, 6(4), 505-509.
13. Mohammed El-Kaseem, et al, Assay and dissolution methods development and validation for simultaneous determination of sofosbuvir and ledipasvir by RP-HPLC method. Juniper publishers2017, 1(3), 0001-0011.
14. Ravi kumar, et al, Estimation and validation of sofosbuvir in bulk and tablet dosage form by RP-HPLC. Int J pharm2016, 6(2), 121-127.
15. Yogendrachari, et al, Analytical method development and validation for simultaneous determination of ledipasvir and sofosbuvir in tablet dosage form by RP-HPLC. J Global Trends Pharm Sci2016, 7(3), 3386-3393.
16. Suryaprakash, et al, Devolopment and validation of RP-HPLC and UV spectroscopy methods for simultaneous estimation of sofosbuvir and ledipasvir in their combined tablet dosage form. Pharma Science Monitor2017, 8(2), 369-388.