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RAPID ANALYTICAL METHOD DEVELOPMENT & VALIDATION OF LEDIPASVIR AND SOFOSBUVIR IN BULK AND PHARMACEUTICAL FORMULATIONS BY RP-HPLC METHOD

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ABSTRACT

Ledipasvir and Sofosbuvir dose forms were simultaneously estimated using a straightforward, accurate, and exact procedure. The standard Discovery C8 150 x 4.6 mm, 5 m chromatogram was used. Phase of mobility with Buffer 0.1% OPA: One millilitre per minute of acetonitrile in a 60:40 ratio was pushed through the column. This procedure uses 0.1% OPA buffer as buffer. A constant 30°C was maintained. The chosen optimised wavelength was 260 nm. Ledipasvir and Sofosbuvir were shown to have retention times of 3.436 and 2.367 minutes, respectively. Ledipasvir and Sofosbuvir were found to have %RSDs of 0.5 and 0.6, respectively. %For sofosbuvir and ledipasvir, recovery rates were 99.61% and 99.80%, respectively. The regression models for sofosbuvir and ledipasvir yielded LOD and LOQ values of 0.67, 2.02 and 0.70, 2.12, respectively. The regression equations for Ledipasvir and Sofosbuvir are y = 4861.x + 2656 and y = 4266.x + 7700, respectively. Because both the retention and run durations were shortened, the approach was straightforward and affordable, making it suitable for use in industry-wide routine quality control testing.

Key Words: Sofosbuvir, Ledipasvir, RP-HPLC, Validation.

INTRODUCTION

The creation of straightforward and repeatable analytical techniques for multicomponent medication estimate is a crucial component of the quality control and social awareness initiatives that the current study has developed.

Sofosbuvir is a prodrug nucleotide analogue that is used in combination treatment to treat co-infection of HIV and HCV or hepatitis C virus (HCV) infection. Following conversion to 2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate (GS-461203) Fig-1, the triphosphate acts as a deficient substrate for the RNA-dependent RNA polymerase NS5B protein, which is necessary for the replication of viral RNAMore recently, sofosbuvir and levipasvir (marketed under the brand Harvoni) were made available as a fixed dosage medication combination therapy for the treatment of chronic Hepatitis C, an infectious liver condition brought on by HCV infection. Ledipasvir and sofosbuvir are direct-acting antiviral medications that were approved by the FDA in October 2014 and are prescribed for the treatment of HCV genotype 1 with or without cirrhosis.

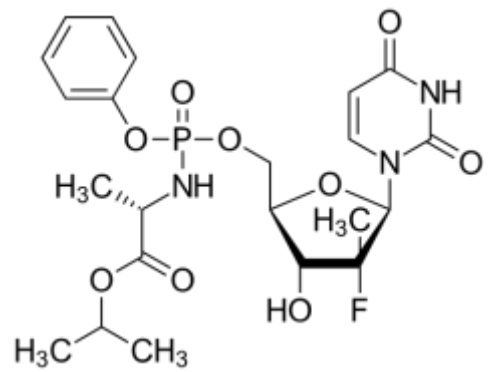

Fig. 1: Chemical structure of Sofosbuvir

Ledipasvir, formerly known as GS-5885, is an inhibitor of the NS5A protein of the Hepatitis C virus (HCV), which is necessary for the replication of viral RNA and the formation of HCV virions. Its precise mode of action is unknown, although it is thought to work by inhibiting NS5A's hyperphosphorylation, which is necessary for the creation of viruses. It has reduced action against HCV genotypes 2a and 3a but is efficient against genotypes 1a, 1b, 4a, and 5a. Ledipasvir is marketed under the brand name Harvoni and is offered as a fixed dosage medication combination product with Sofosbuvir (fig-2) for the treatment of chronic Hepatitis C, an infectious liver condition brought on by HCV infection. FDA-approved in October 2014.

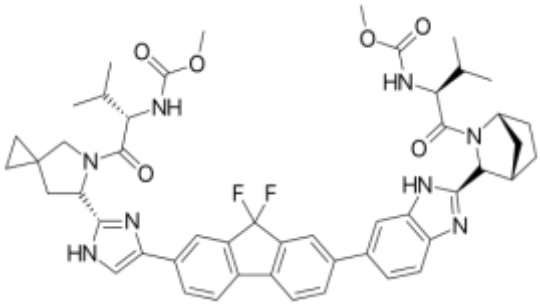

Fig. 2: chemical structure of Ledipasvir

MATERIALS AND METHODS

Materials

Potassium dihydrogen, methanol, phosphate buffer, ortho-phosphoric acid, distilled water, acetonitrile, phosphate buffer, combination sofosbuvir and ledipasvir tablets (Harvoni), and pure pharmaceuticals of sofosbuvir and ledipasvir (API). The solvents and compounds listed above are all from Rankem.

HPLC method

Instrument

UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Sofosbuvir and Ledipasvir solutions having universal loop injector of injection capacity 20 µL. The column used was Discovery C18 (4.6 x 250mm, 5µm) at ambient temperature. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously. Optimised Chromatographic conditions. The mobile phase having 60% OPA (0.1%): 40% Acetonitrile was selected because it was found that it ideally resolve the peaks with retention time (RT) 2.380 min and 3.449 min for Sofosbuvir and Ledipasvir respectively (fig-3). Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200nm to 350nm. Both the components showed reasonably good response at 260 nm.

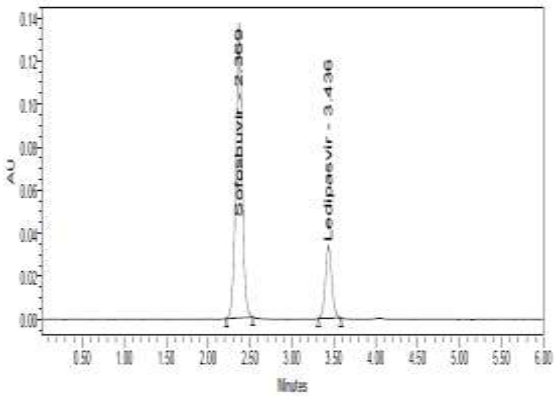

Fig. 3: Optimized chromagram of Sofosbuvir and Ledipasvir

Preparation of solutions

Diluent

Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions

Accurately weighed 40mg of Sofosbuvir, 9mg of Ledipasvir and transferred to 25ml & 25ml volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1600µg/ml of Sofosbuvir and 360µg/ml Ledipasvir).

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (160µg/ml of Sofosbuvir and 36µg/ml of Ledipasvir).

Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (800µg/ml of Sofosbuvir and 1800µg/ml of Ledipasvir).

Preparation of Sample working solutions (100% solution)

0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (160µg/ml of Sofosbuvir and 36µg/ml of Ledipasvir).

Preparation of buffer
1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Linearity
Six linear concentrations of Sofosbuvir (40-240µg/ml) and Ledipasvir (9- 54µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Sofosbuvir was y = 4266.x + 7700and of Ledipasvir wasy = 4861.x + 2656Correlation coefficient obtained was 0.999 for the two drugs and results are tabulated in table 2 and figure 4 & 5.

System suitability parameters
The system suitability parameters were determined by preparing standard solutions of Sofosbuvir (160ppm) and Ledipasvir (36ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.
The % RSD for the area of six standard injections results should not be more than 2% and results tabulated in table 3.

Specificity
Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision
Preparation of Standard stock solutions
Accurately weighed 40mg of Sofosbuvir, 9mg of Ledipasvir and transferred to 25ml & 25ml volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1600µg/ml of Sofosbuvir and 360µg/ml Ledipasvir)

Repeatability
Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.6% respectively for Sofosbuvir and Ledipasvir. As the limit of Precision was less than “2” the system precision was passed in this method.

Accuracy
Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.61% and 99.80% for Sofosbuvir and Ledipasvir respectively. The results are tabulated in table 4 & 5.

Precision
Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.6% respectively for Sofosbuvir and Ledipasvir. As the limit of Precision was less than “2” the system precision was passed in this method and results were tabulated in table 7&8.

Robustness
Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.
Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit results were tabulated in table 9.

Assay
RadhaKishan Pharmaceuticals, (Hepcvir L)bearing the label claim Sofosbuvir 400mg, Ledipasvir 90mg. Assay was performed with the above formulation. Average % Assay for Sofosbuvir and Ledipasvir obtained was 99.32 and 98.47% respectively and results were tabulated in table 10 &11 and figure 6 & 7.

Degradation studies
Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. Regarding the pH adjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time and results were tabulated in table 12 & 13.

Table 1: Trails for method development

Chromatographic conditions	Trial 1	Trial 2	Trial 3	Trial 4
Mobile phase	Water : Methanol (50:50 %)	Water : Acetonitrile (50:50%)	OPA : Acetonitrile (50:50 %)	OPA : Acetonitrile (50:50 %)
Flow rate	1 ml/min	1 ml/min	1 ml/min	1 ml/min
Column	C18 (4.6 x 250mm, 5µm)	C18 (4.6 x 250mm, 5µm)	C18 (4.6 x 250mm, 5µm)	C18 (4.6 x 250mm, 5µm)
Detector wave length	260nm	260nm	260nm	260nm
Column temperature	30°C	30°C	30°C	30°C
Injection volume	10µL	10µL	10µL	10µL
Run time	10 min	10 min	10 min	10 min
Diluent	Water and Acetonitrile 50:50	Water and Acetonitrile 50:50	Water and Acetonitrile 50:50	Water and Acetonitrile 50:50

Table 2: Linearity table for Sofosbuvir and Ledipasvir

Sofosbuvir		Ledipasvir	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
40	193238	9	48575
80	346154	18	92565
120	523238	27	135642
160	680185	36	174059
200	853929	45	219015
240	1041024	54	267583

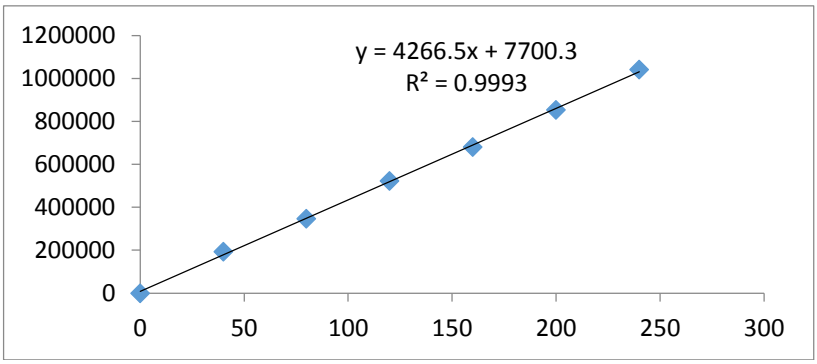

Fig. 4: Calibration curve of Sofosbuvir

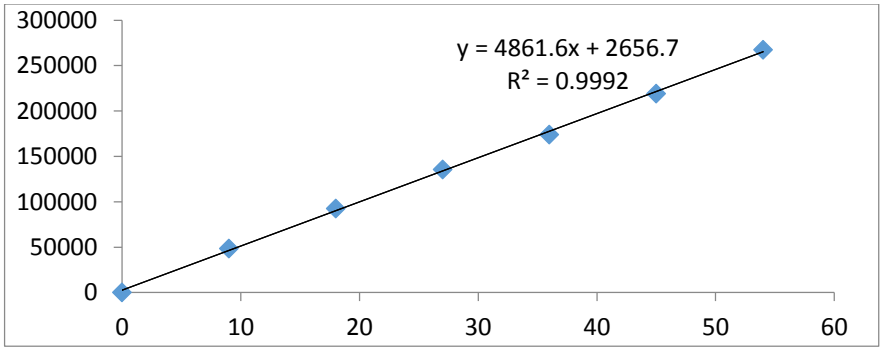

Fig. 5: Calibration curve of Ledipasvir

Table 3: System suitability parameters for Sofosbuvir and Ledipasvir

S. No.	Sofosbuvir			Ledipasvir			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.366	5341	1.08	3.434	9522	1.09	7.5
2	2.367	5497	1.09	3.436	9659	1.09	7.6
3	2.367	5685	1.08	3.436	9776	1.08	7.5
4	2.369	5082	1.04	3.436	9731	1.10	7.5
5	2.369	5104	1.03	3.438	10083	1.09	7.6
6	2.372	5095	1.03	3.447	9852	1.05	7.7

Table 4: Accuracy table of Sofosbuvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	80	79.77918	99.72	99.61%
	80	79.54383	99.43	
	80	79.30638	99.13	
100%	160	159.3926	99.62	
	160	159.7794	99.86	
	160	159.7616	99.85	
150%	240	239.079	99.62	
	240	239.0996	99.62	
	240	239.0872	99.62	

Table 5: Accuracy table of Ledipasvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	18	17.986	99.92	99.80%
	18	17.979	99.88	
	18	17.949	99.72	
100%	36	35.902	99.73	
	36	35.915	99.76	
	36	35.889	99.69	
150%	54	53.976	99.95	
	54	53.796	99.62	
	54	53.959	99.92	

Table 6: LOD & LOQ of Sofosbuvir and Ledipasvir

Molecule	LOD	LOQ
Sofosbuvir	0.67	2.02
Ledipasvir	0.70	2.12

Table 7: System precision table of Sofosbuvir and Ledipasvir

S. No.	Area of Sofosbuvir	Area of Ledipasvir
1.	698943	177127
2.	695463	176673
3.	693621	177445
4.	704923	179081
5.	698452	176591
6.	693668	177154
Mean	697512	177345
S.D	4288.8	908.6
%RSD	0.6	0.5

Table 8: Intermediate precision table of Sofosbuvir and Ledipasvir

S. No.	Area of Sofosbuvir	Area of Ledipasvir
1.	695241	176978
2.	695200	176075
3.	694259	176873
4.	694723	176902
5.	697754	176596
6.	696181	177083
Mean	695560	176751
S.D	1250.9	368.9
%RSD	0.2	0.2

Table 9: Robustness data for Sofosbuvir and Ledipasvir

S. No.	Condition	%RSD of Sofosbuvir	%RSD of Ledipasvir
1	Flow rate (-) 0.9ml/min	0.5	0.4
2	Flow rate (+) 1.1ml/min	0.5	0.7
3	Mobile phase (-) 65B:35A	0.5	0.4
4	Mobile phase (+) 55B:45A	0.5	0.5
5	Temperature (-) 25°C	0.3	0.3
6	Temperature (+) 35°C	0.1	0.1

Table 10: Assay Data of Sofosbuvir

S. No.	Standard Area	Sample area	% Assay
1	698943	695241	99.28
2	695463	695200	99.27
3	693621	694259	99.14
4	704923	694723	99.20
5	698452	697754	99.63
6	693668	696181	99.41
Avg	697512	695560	99.32
Stdev	4288.8	1250.9	0.18
%RSD	0.6	0.2	0.18

Table 11: Assay Data of Ledipasvir

S. No.	Standard Area	Sample area	% Assay
1	177127	176978	99.59
2	176673	176075	99.09
3	177445	176873	99.53
4	179081	176902	99.55
5	176591	176596	99.38
6	177154	177083	99.65
Avg	177345	176751	99.47
Stdev	908.6	368.9	0.2
%RSD	0.5	0.2	0.2

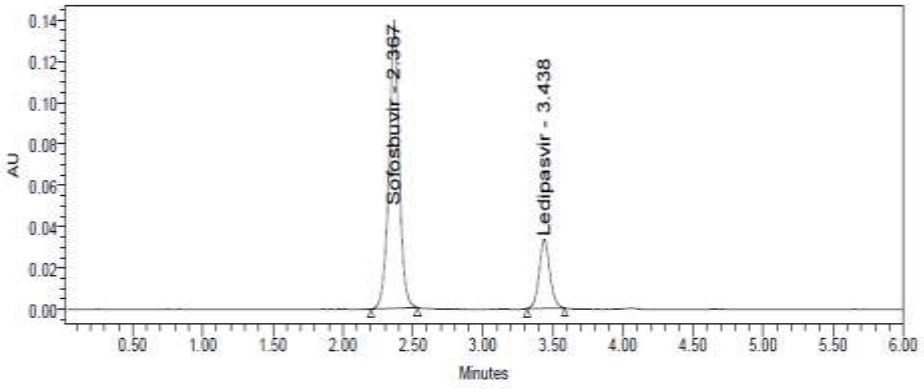

Fig. 6: Chromatogram of working standard solution

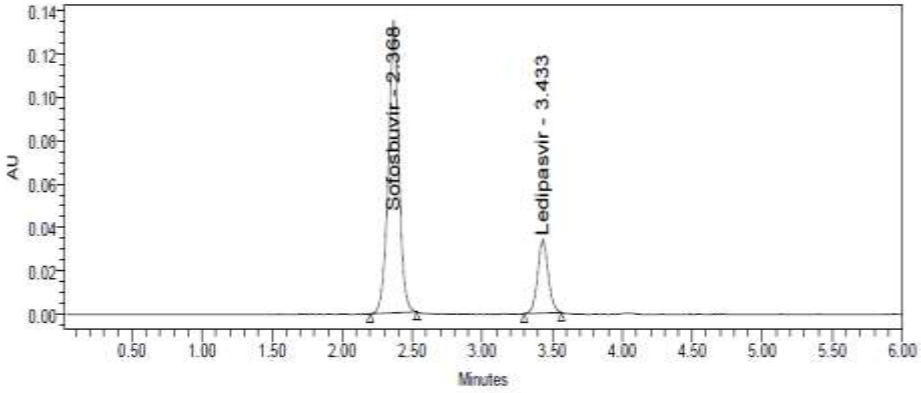

Fig. 7: Chromatogram of working sample solution

Table 12: Degradation Data of Sofosbuvir

S. No.	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.21	0.051	0.296
2	Alkali	3.96	0.124	0.252
3	Oxidation	3.89	0.159	0.304
4	Thermal	2.61	0.197	0.294
5	UV	1.48	0.133	0.280
6	Water	1.48	0.044	0.287

Table 13: Degradation Data of Bromhexine

S. No.	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.62	0.187	0.320
2	Alkali	4.22	0.162	0.587
3	Oxidation	3.78	0.171	0.328
4	Thermal	2.78	0.197	0.297
5	UV	1.22	0.130	0.296
6	Water	0.90	0.123	0.299

CONCLUSION

For the simultaneous estimate of sofosbuvir and ledipasvir in tablet dose form, a straightforward, accurate, and exact approach was established. Sofosbuvir and Ledipasvir were shown to have retention times of 2.367 and 3.436 minutes, respectively. Ledipasvir's and sofosbuvir's percentage RSD were discovered to be 0.5 and 0.6, respectively. %) Ledipasvir and Sofosbuvir showed recovery rates of 99.80% and 99.61%, respectively. The values of LOD and LOQ derived from the regression equations of Ledipasvir and Sofosbuvir were 0.70, 2.12 and 0.67, 2.02, respectively. Ledipasvir's regression equation is y = 4861.x + 2656, while Sofosbuvir's is y = 4266.x + 7700. The suggested approach was straightforward and cost-effective, suitable for routine quality control testing in industries, as both retention times and run times were reduced.

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