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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND LEDIPASVIR IN TABLET DOSAGE FORM

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Abstract:

Simple, specific, accurate and precise reversed phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Sofosbuvir and Ledipasvir in tablet dosage form by reversed phase C_{18} column (Kromasil C_{18} 5µ, 250 mm x 4.6 mm). The sample was analyzed using 0.1% OPA: Acetonitrile in the ratio of 55:45 as a mobile phase at a flow rate of 1.0 ml/min and detection at 230 nm. Calibration curves were linear with correlation coefficient (r^2) 0.999 over a concentration range of 100-600 µg/mL for Sofosbuvir and 0.999 over a concentration range of 22.5-135 µg/mL for Ledipasvir. The retention time was found to be 6 min. The mean recoveries were found to be 99.10% and 99.30% for Sofosbuvir and Ledipasvir respectively. The relative standard deviation (RSD) was found to be < 2.0% for both drugs. The proposed method was validated and successfully applied to the estimation of Sofosbuvir and Ledipasvir in tablet dosage form.

Keywords: *Sofosbuvir, Ledipasvir, RP-HPLC, Validation, Stability.*

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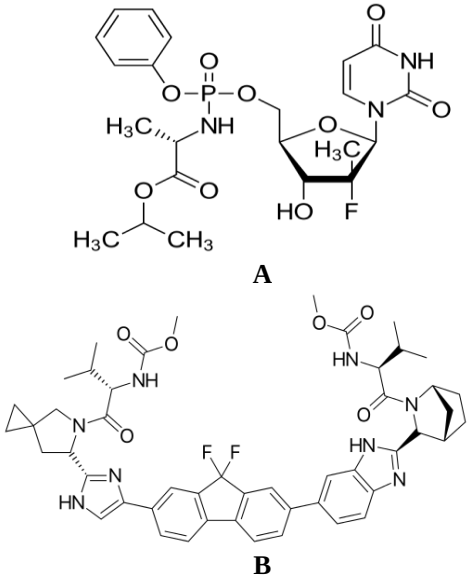
INTRODUCTION:

Reverse phase is the choice for the majority of samples, but if acidic or basic analytes [1-3] are present then reverse phase ion suppression (for weak acids or bases) or reverse phase ion pairing should be used. The stationary phase should be C18 bonded[4,5]. Sofosbuvir is a prodrug nucleotide analog used as part of combination therapy to treat hepatitis C virus (HCV) infection or to treat co-infection of HIV and HCV.

Sofosbuvir and other nucleotide inhibitors of the HCV RNA [6] polymerase exhibit a very high barrier to resistance development. Sofosbuvir has become available as a fixed dose drug combination product with ledipasvir (tradename Harvoni) used for the treatment of chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). Ledipasvir is previously known as GS-5885, is an inhibitor of the Hepatitis C Virus (HCV) NS5A protein required for viral RNA replication and assembly of HCV virions.

Ledipasvir is available as a fixed dose drug combination product [7,8] with sofosbuvir (tradename Harvoni) used for the treatment of chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). Approved in October 2014 by the FDA, ledipasvir and sofosbuvir are direct-acting antiviral agents indicated for the treatment of HCV genotype 1 with or without cirrhosis. Combination of these two drugs (Sofosbuvir 400mg and Ledipasvir 90mg is available in pharmacy under the brand name of Myhep Lvir, Ledifos tablet, Heterosofir plus and Hepcinat lp tablet. The chemical structures of both drugs were shown in Fig.1.



A

B

Fig.1: Chemical Structures of Sofosbuvir [A] and Ledipasvir [B]

Literature survey revealed that there are only three HPLC methods [9-11] have been published regarding this research work. The present study aimed to develop a simple, sensitive, less retention time and accurate RP-HPLC method for the simultaneous estimation of sofosbuvir and ledipasvir in bulk and tablet dosage forms with high sensitivity, selectivity and stability that can be used for the routine analysis.

MATERIALS AND METHODS:

Chemicals and Reagents

Pure standard samples of sofosbuvir and ledipasvir were obtained as gifted samples from Rhodes Pharmaceuticals Ltd. and its marketed formulations in the brand name of Harvoni [Label claim containing sofosbuvir 75mg and ledipasvir 325 mg] were procured from local pharmacy. Water (HPLC-Grade), Acetonitrile (HPLC-Grade; Rankem) and Methanol (HPLC-Grade Rankem), ortho phosphate buffer, Ortho-phosphoric acid (Rankem). All dilutions were performed in standard class-A, volumetric glassware.

Instrumentation

The present assay was carried out on a Waters HPLC system [Model: 2695] equipped with 2487 photodiode array detector, automated sample injector and a column Kromasil C_{18} (250mmx4.6mm I.D; particle size 5µm) respectively. Electronic Balance [Denver] and Ultra-Sonicator [SE60US; BVK enterprises] were also used in the present assay. The output of signal was monitored and integrated using waters Empower 2 software.

Buffer preparation

0.1%OPA Buffer:1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Mobile phase preparation

Prepare a filtered and degassed mixture of Buffer (pH - 2.0), 0.1% OPA: Acetonitrile (55:45%).

Diluent preparation

Mobile phase is used as diluent.

Standard preparation

Accurately weighed 40mg of Sofosbuvir, 9mg of Ledipasvir and transferred to 10ml flasks and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (40µg/ml of Sofosbuvir and 2500µg/ml Ledipasvir).

Sample preparation

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml 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 (40µg/ml of Sofosbuvir and 2500µg/ml of Ledipasvir).

RESULTS AND DISCUSSION:

Method development

Initial trials were carried by the author in developing the proposed RP-HPLC method. The mobile phase was chosen after several trials with methanol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of 0.1% OPA buffer (pH -2.0) and acetonitrile (55:45 v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5/min were studied. A flow rate of 1.0 ml/min at ambient temperature gave an optimal signal to noise ratio with a reasonable separation time. Using a Kromasil C_{18} column, the run time of analysis was 6 min. Detection wavelength of 230 nm was chosen for the analysis. A typical chromatogram for simultaneous estimation of sofosbuvir and ledipasvir obtained by using a mobile phase was shown in Fig.2**.**

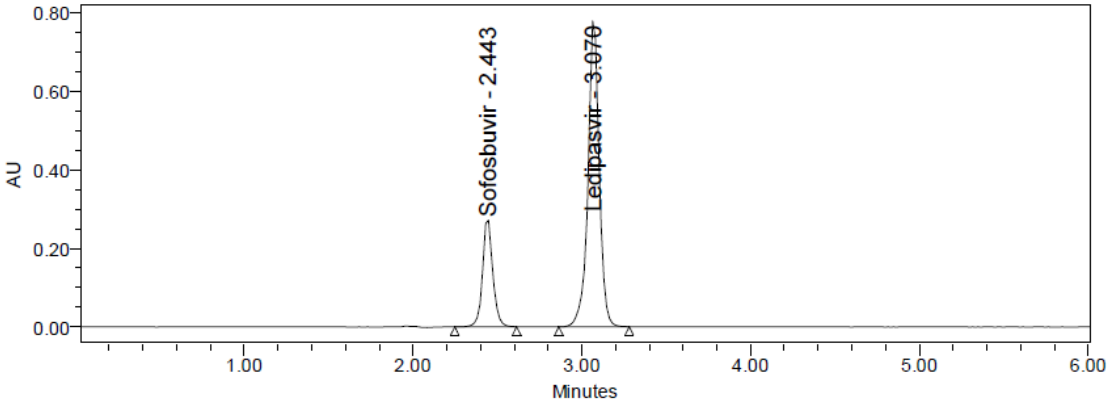


Fig.2: Typical chromatogram of standard solution (sofosbuvir and ledipasvir)

Chromatographic conditions

The isocratic mobile phase consisted of 0.1% OPA buffer (pH -2.0) and Acetonitrile (55:45 v/v), flowing through the Kromosil C_{18} column (make: 250 mmx4.6 mm i.d; particle size 5µm) at a constant flowrate of 1.0 ml/min at ambient column temperature. The mobile phase was pumped through the column at a flowrate of 1.0ml/min with a sample injection volume of 10µl. Detection of the analytes (sofosbuvir and ledipasvir) was carried out at a wavelength of 230 nm.

Method validation

The proposed RP-HPLC method was validated, in accordance with USP guidelines for system.

System suitability

RP-HPLC method System performance parameters were determined by analyzing standard working solutions of sofosbuvir and ledipasvir. The chromatographic parameters, such as number of theoretical plates (n), resolution (Rs), USP plate count and USP tailing were determined. The results are shown in Table.1, indicating the good performance of the system.

Table1: System suitability data of sofosbuvir and ledipasvir

S.No	Sample name	RT	USP Plate count	USP tailing
1.	Injection 1 (sofosbuvir)	2.442	7792	1.08
2.	Injection 1 (Ledipasvir)	3.072	11272	0.94

Specificity

Blank and placebo interference

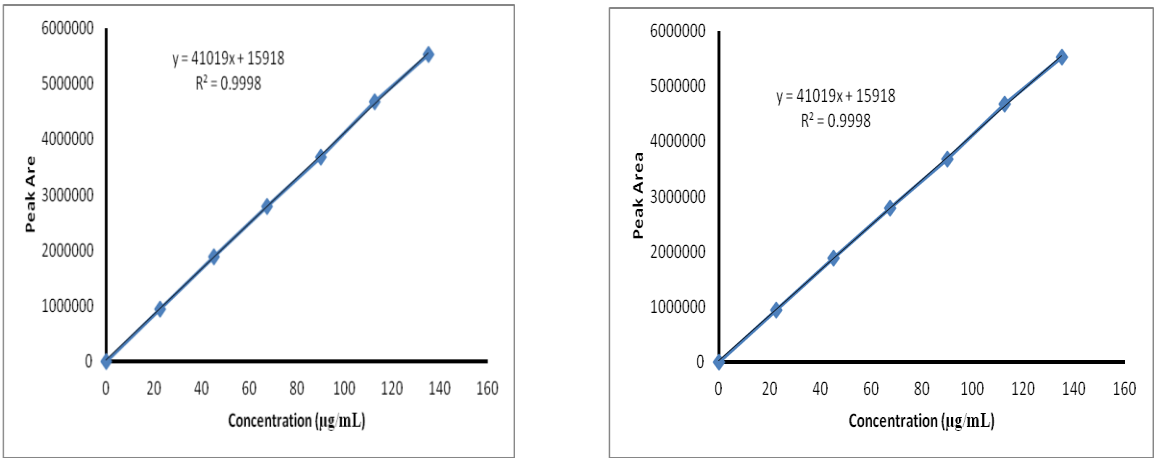
The interference of blank and placebo with the elution of the present cited drugs solutions of diluents and placebo were injected into the chromatographic system with the mentioned chromatographic conditions and their respective chromatograms were recorded. From the reported chromatograms it was observed that the placebo and blank showed no peaks at the retention time of sofosbuvir and ledipasvir peak indicating that the diluent and placebo solutions used in standard and sample preparations did not interfered in assay of sofosbuvir and ledipasvir respectively.

Linearity & Detector response

The linearity of the proposed method was accessed by calculating slope, intercept and correlation coefficient [r^2] of standard curve. Sofosbuvir and ledipasvir showed a linearity range in between 100-600 and 22.5-135µg/ml respectively and the slope and intercept of the calibration plot of sofosbuvir and ledipasvir were 2966x+2193 and 41019x + 15917 with correlation coefficients obtained was greater than 0.999 respectively. The linearity results of both the drugs were shown in Tab.2 and Linearity curves of sofosbuvir and ledipasvir were depicted in Fig.3.

Table 2: Linearity results for Sofosbuvir and Ledipasvir

Sofosbuvir		Ledipasvir	
Conc. (µg/ml)	**Peak area**	**Conc.(µg/ml)**	**Peak area**
0	0	0	0
100	293334	22.5	937588
200	597524	45	1888960
300	892905	67.5	2781827
400	1192639	90	3686223
500	1507994	112.5	4674738
600	1760574	135	5523393



A **B**

Fig.3: Linear calibration plot for Sofosbuvir (A) and Ledipasvir (B)

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were established at signal-to noise ratio of 3:1 and 10:1 respectively. The LOD of sofosbuvir and ledipasvir was found to be 0.02µg /ml & 0.28µg /ml respectively. The LOQ of sofosbuvir and ledipasvir was found to be 0.07 µg /ml & 0.85µg / ml respectively.

Precision

The precision of sofosbuvir and ledipasvir by proposed RP-HPLC method was ascertained by replicate analysis of homogeneous samples of Tablet powder. Intermediate precision of the present RP-HPLC method was studied by intra-day variation of the method was carried out. The results were given in Tab.3 and the low % RSD values of within a day for sofosbuvir and ledipasvir revealed that the proposed method is highly precise.

Table 3: System precision table of Sofosbuvir and Ledipasvir

S.No	Area of Sofosbuvir	Area of Ledipasvir
1.	1145677	3578272
2.	1152265	3587779
3.	1154492	3608922
4.	1148949	3624217
5.	1156499	3600959
6.	1160648	3601532
Mean	1153088	3600280
S.D	5357.08	16064.4
%RSD	0.5	0.4

Accuracy

The accuracy of the proposed method for sofosbuvir and ledipasvir was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The recovery studies were carried out in triplicate by adding Known amount of standard solution of sofosbuvir and ledipasvir to preanalysed tablet solutions. The resulting solutions were then reanalysed by proposed method and the results are represented in Tab.4. The percentage recoveries were found in the range of 98.82 to 100.9% for sofosbuvir and 98.50 to 100.9% for ledipasvir respectively revealing that the developed RP-HPLC method was found to be accurate.

Table 4: Results of Accuracy for Sofosbuvir and Ledipasvir

S.No	Accuracy level	Injection	Sofosbuvir	Ledipasvir
			% Recovery	% Recovery
1.	50%	1	98.82	98.50
		2	100.48	98.18
		3	98.02	98.51
2.	100%	1	98.63	100.44
		2	99.25	101.29
		3	100.09	100.65
3.	150%	1	100.34	98.19
		2	99.68	98.82
		3	99.01	99.08

Table 5: Robustness data for Sofosbuvir and Ledipasvir

S.No	Condition	% RSD of Sofosbuvir	% RSD of Ledipasvir
1	Flow rate (-) 1.1ml/min	0.5	0.4
2	Flow rate (+) 1.3ml/min	0.5	0.7
3	Mobile phase (-) 35B:65A	0.5	0.4
4	Mobile phase (+) 45B:55A	0.5	0.5
5	Temperature (-) 25°C	0.3	0.3
6	Temperature (+) 35°C	0.1	0.1

Table 6: Assay of Sofosbuvir and ledipasvir in Formulation

Drug name (Harvoni tablets)	Quantity label claim(mg)	Quantity found± SD	%Assay ± SD
Sofosbuvir	75 mg	1143799 ±0.50	99.10 ± 0.46
Ledipasvir	325 mg	3567195 ±0.30	98.98 ± 0.46

Robustness

The robustness of the developed method was evaluated by altering few experimental conditions and evaluating the resolution between two adjacent peaks of sofosbuvir and ledipasvir. The data results were shown in Tab.5.

Formulation assay

The validated method was applied on commercially available Harvoni tablets. The results of the assay undertaken yielded 99.10% and 99.30% of the label claim for sofosbuvir and ledipasvir. Results of the assay indicated that the method is quite selective for the analysis of Harvoni without interference from the excipients used to formulate and produce these tablets. The results were displayed in Tab.6 respectively.

CONCLUSION:

It was concluded that the proposed new RP-HPLC method developed for the quantitative determination of Sofosbuvir and ledipasvir in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phase was simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine estimation of Sofosbuvir and ledipasvir depending upon the availability of chemicals and nature of other ingredients present in the sample. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. Application of this method for estimation of Sofosbuvir and ledipasvir from tablet dosage form and stressed samples showed that neither the degradation products nor the excipients interfered in the estimation of drug. Hence, this method was specific, stability-indicating and can be successfully used for the estimation of drug in bulk and pharmaceutical dosage form.

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