



## **Development and Validation of UV Spectrophotometric method for the estimation of Sofosbuvir and Ledipasvir in combined Pharmaceutical dosage forms**

**M. Siva Kishore<sup>1\*</sup> and C. Rambabu<sup>2</sup>**

<sup>1</sup>Department of Chemistry, K.B.N. P.G. College, Vijayawada, India

<sup>2</sup>Department of chemistry, Acharya Nagarjuna University, Guntur, India

**Abstract** : Two simple spectrophotometric methods have been developed for simultaneous estimation of Sofosbuvir and Ledipasvir in combined dosage forms has been developed. Method-I simultaneous equation method involves the measurement of absorbances at two wavelengths 237 nm ( $\lambda_{\text{max}}$  of Sofosbuvir) and 247 nm ( $\lambda_{\text{max}}$  of Ledipasvir) in diluents of water and methanol in the ratio of 8:2 (v/v). Method-II involves derivative spectrophotometry method for simultaneous estimation of Sofosbuvir and Ledipasvir. In this method, the absorbance was measured at 237 nm for Sofosbuvir and 247 nm for Ledipasvir. Linearity was observed in range of 20-120  $\mu\text{g/ml}$  and 4.5-27  $\mu\text{g/ml}$  for Sofosbuvir and Ledipasvir respectively. Accuracy of method was found between 98 to 102%. The precision (intra-day, inter-day) of method was found within limits. Both method were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of Sofosbuvir and Ledipasvir in combined dosage form.

**Keywords** : Sofosbuvir and Ledipasvir, method development, Validation, UV spectrophotometer.

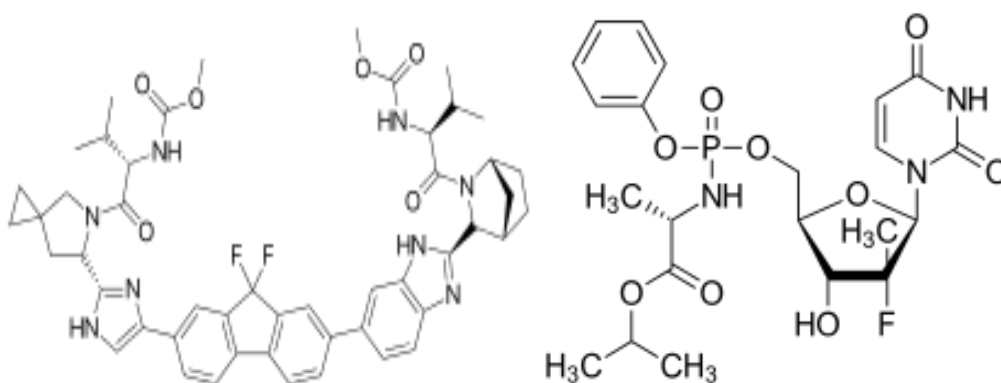
### **Introduction:**

Ledipasvir, is an inhibitor of the Hepatitis C Virus (HCV), most commonly used in combination with sofosbuvir for treatment in chronic hepatitis C genotype 1 patients. The drug has approved in October 2014 by the FDA of ledipasvir/sofosbuvir fixed-dose combination tablet for genotype 1 hepatitis C [1-3]. The ledipasvir/sofosbuvir combination is a direct-acting antiviral agent that interferes with HCV replication and can be used to treat patients with genotypes 1a or 1b without PEG-interferon or ribavirin. The drug works by inhibiting the Hepatitis C Virus (HCV) NS5A protein required for viral RNA replication and assembly of HCV virions [4-8]. Ledipasvir is available as a fixed dose drug combination product with sofosbuvir (trade name Harvoni) used for the treatment of chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). This drug also has been tested and shown efficacy in treatment-naive and treatment experienced patients [4].

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Sofosbuvir is a prodrug nucleotide analog used for the treatment of chronic hepatitis C, genotypes 1, 2, 3, 4, 5, and 6, usually in combination with other medications depending on the specific genotype. Sofosbuvir, is a medication used in combination with other medications for the treatment of hepatitis C [9-11]. The drug has approved in December 2013 [9] and also it is listed on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system [12-15]. Sofosbuvir is nucleotide analog inhibitor, which specifically inhibits HCV NS5B polymerase. For the treatment of genotypes 1, 4, 5, and 6 hepatitis C infections, sofosbuvir is used in combination with the viral NS5A inhibitor ledipasvir. Fixed dose combination drug sofosbuvir/ledipasvir, was approved and has been granted breakthrough status in 2014 [16,17]. Review of literature survey reveals that there is two spectrophotometry method [18,19], two RP-HPLC methods [20-21] and one UPLC methods [22] has been described for sofosbuvir along with daclatasvir for formulation analysis. Hence this present study aimed to develop and validate the ultraviolet and derivate spectroscopic methods for simultaneous estimation of sofosbuvir and ledipasvir in combined dosage forms.



**Fig. 1: Chemical structure of Ledipasvir and Sofosbuvir**

## Material and methods:

### Chemicals and Materials

Pure standard drugs sofosbuvir and ledipasvir were obtained as gift sample from reputed Pharmaceutical Company. Methanol, water (Merck, Mumbai, India) was of analytical grade (Merck Specialties Private Limited, Mumbai, India). Formulations of HEPcVIR-L tablet pharmaceutical dosage form of sofosbuvir and ledipasvir containing labeled amount of sofosbuvir- 400mg, ledipasvir -90mg were procured from local market.

### Apparatus and Instrumentation

A double beam UV/Visible spectrophotometer model Teccomp UV-2301 was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234).

### Preparation of Standard and Sample solutions

Individual standard stock solutions of Sofosbuvir and Ledipasvir were prepared by 10mg of drug was accurately weighed individually and dissolved in 5ml diluent water and methanol in the ratio of 8:2 (v/v) transferred to a 10ml volumetric flask sonicate it for 5min, finally volume was made up to the mark with same solvent to prepare 1000 $\mu$ g/ml stock solution. Working standard solutions were prepared by 1ml of stock solutions was again diluted to 10ml to get a concentration of 100 $\mu$ g/ml solution of Ledipasvir and Sofosbuvir were obtained individually. Further concentration solutions were prepared by dilution with diluent.

Ten formulation tablets from 2 different strips of Sofosbuvir and Ledipasvir (HEPCVIR-L; Sofosbuvir -400mg and Ledipasvir - 90mg) were powdered and 10mg tablet powder was weighed accurately and was dissolved in 5ml diluent. Solution was sonicated for 10-15min to dissolve the drugs complete. Then it was filtered and makes up to 10ml with same diluents to make 1000 $\mu$ g/ml stock solution. As per the label claim of

the two drugs a Ledipasvir concentration of 18 $\mu$ g/ml was obtained by subsequent dilution. The resultant solution was used for the simultaneous estimation of Sofosbuvir and Ledipasvir in combined dosage forms.

### Simultaneous equation method (Method-1)

$\lambda$  maximum of individual drugs were at 237 nm for Sofosbuvir and 247 nm for Ledipasvir. Different aliquots of the standard solution of Sofosbuvir and Ledipasvir, was transferred into volumetric flask. The solutions were then completed to the volume with diluents, so the final concentration for Sofosbuvir was in range of 20-120 $\mu$ g/mL and for Ledipasvir were in range of 4.5-90 $\mu$ g/ml. At the absorbance of these standard solutions calibration curves were plotted at these wavelengths. Two simultaneous were formed using these Absorptivity coefficient values.

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}}$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}}$$

where  $a_{x1}$  = Absorptivity of Sofosbuvir at 237nm

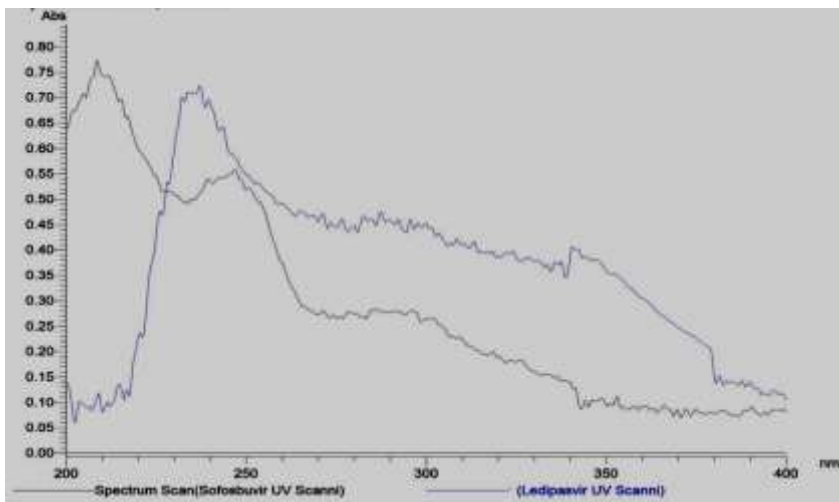
$a_{x2}$  = Absorptivity of Sofosbuvir at 247nm

$a_{y1}$  = Absorptivity of Ledipasvir at 247nm

$a_{y2}$  = Absorptivity of Ledipasvir at 237nm

$A_1$  and  $A_2$  are the absorbance of the diluted sample at 237nm and 247nm respectively.

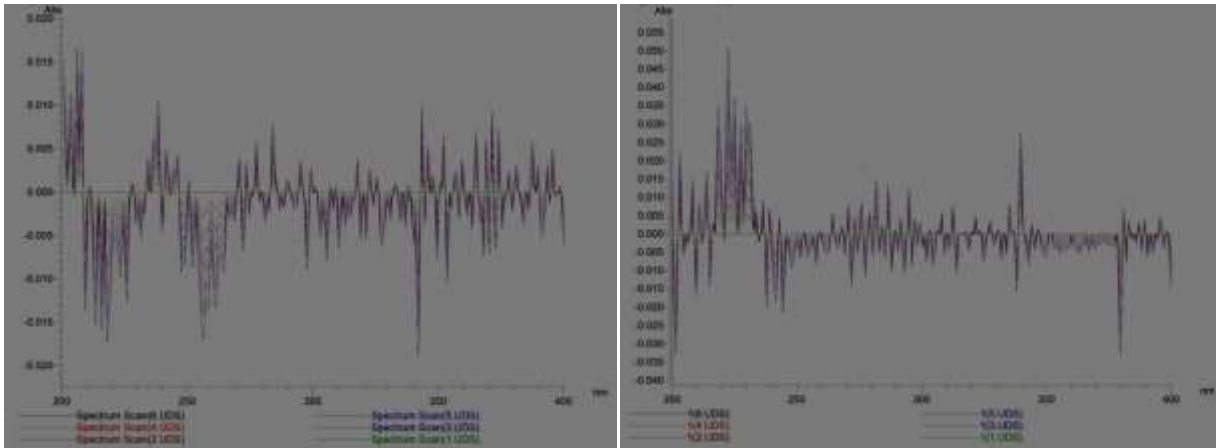
Aliquots of this tablet solution were diluted to get the concentrations of 80 $\mu$ g/ml Sofosbuvir and 18 $\mu$ g/ml Ledipasvir absorbance of these solutions were measured at 237 nm and 247 nm and from the absorbance values, the concentration of drugs in the sample solution was determined by using the simultaneous equations.



**Fig. 2: Overlay spectra of Ledipasvir and Sofosbuvir for Simultaneous equation method**

### First Order Derivative Spectrophotometry (Method -2):

In this method solution of Sofosbuvir and Ledipasvir standard stock solutions was prepared and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra obtained were derivatized from first fourth order. First order derivative spectra were selected for analysis of drug. From spectra of drug the absorbance was measured at 237 nm for Sofosbuvir and 247 nm for Ledipasvir and zero cross=217.0nm, amplitude difference ( $dA$ ) with respect to wavelength difference ( $d\lambda$ ) was measured for the respective concentration of standard and was plotted against concentrations and regression equation was calculated. All the validation tests were conducted in above prepared range.

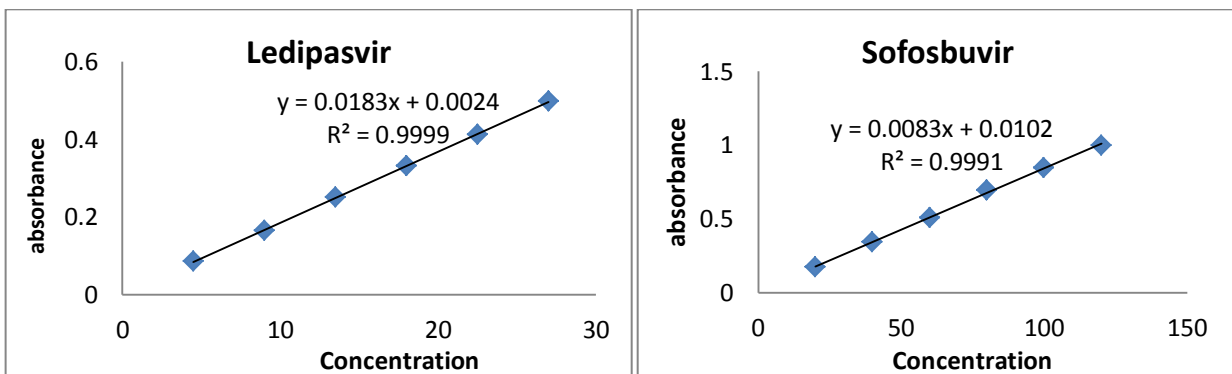


**Fig. 3: Overlay spectra of Ledipasvir and Sofosbuvir for First Order Derivative Spectrophotometry methods**

## Result and discussion:

### Simultaneous equation method (Method-1)

The proposed methods are based on spectrophotometric absorption for the simultaneous estimation of Ledipasvir and Sofosbuvir in UV region using water and methanol in the ratio of 8:2 (v/v) as solvent. The overlain spectra of Sofosbuvir and Ledipasvir are shown in Figure-B. Beer's law obeyed in the concentration range of 20-120  $\mu\text{g/ml}$  and 4.5-27  $\mu\text{g/ml}$  for sofosbuvir and ledipasvir respectively. The correlation coefficient ( $r^2$ ) values were found 0.999 for both drugs, which shows that absorbance of both the drugs, was linear with concentration. Results are presented in the figure D and table 1. For intraday precision method was repeated six times in a day and the average % RSD was found to be 0.739 for Ledipasvir and 0.695 for Sofosbuvir. Similarly, the method was repeated on five different days and average % RSD was found to be 0.334 for Ledipasvir and 0.423 for Sofosbuvir. These values confirm the intra and interday precision of the method. Accuracy of the method was confirmed by recovery studies on preanalyzed formulations. Recovery greater than 98% with the low standard deviation justifies the accuracy of the method. Commercial formulation (HEPCVIR-L) containing Ledipasvir and Sofosbuvir were analyzed by the proposed method at 80  $\mu\text{g/ml}$  Sofosbuvir and 18  $\mu\text{g/ml}$  Ledipasvir concentration. Percentage of assay has been found 99.75% and 99.01% for Ledipasvir and Sofosbuvir respectively. The results are in good agreement with the label claim. With proposed method Limit of detection (LOD) and Limit of Quantitation (LOQ) has been found 1.0  $\mu\text{g/ml}$  and 3.5  $\mu\text{g/ml}$  for Ledipasvir and 0.5  $\mu\text{g/ml}$  and 2.0  $\mu\text{g/ml}$  for Sofosbuvir respectively. Summary of validation results are presented in table-2. Hence it is found that the proposed method is found to be simple, accurate and sensitive and therefore, can be used for the simultaneous estimation of both drugs from their combined dosage form.



**Fig. 4: Linearity Graph of Ledipasvir and Sofosbuvir for Simultaneous equation method**

**Table-1: Result of Linearity for Simultaneous equation method:**

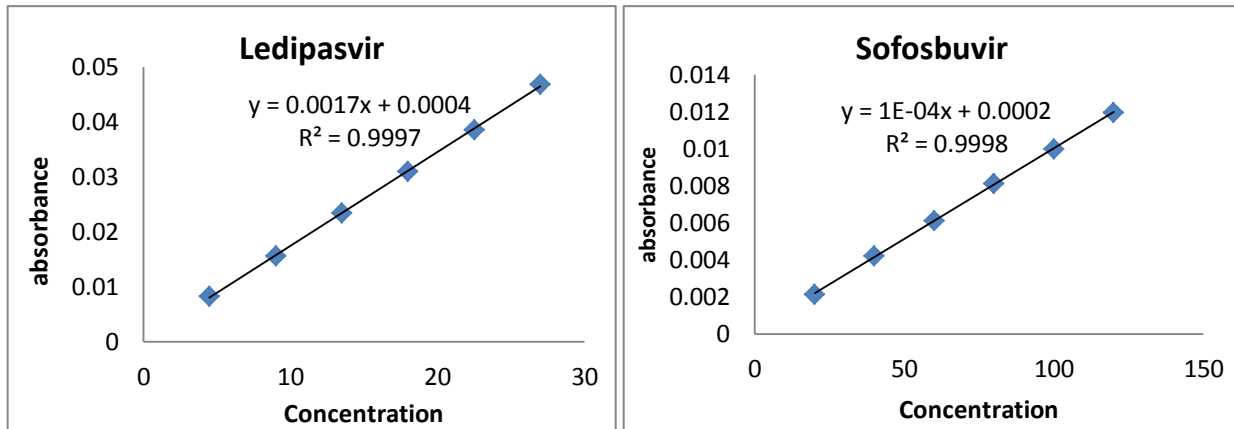
S.NO	Ledipasvir		Sofosbuvir	
	Concentration	Absorbance	Concentration	Absorbance
1	4.5	0.086	20	0.174
2	9	0.165	40	0.341
3	13.5	0.251	60	0.505
4	18	0.331	80	0.693
5	22.5	0.412	100	0.846
6	27	0.498	120	0.999

**Table-2: summary of validation for Ledipasvir and Sofosbuvir for Simultaneous equation method**

Parameter with validation limit	Ledipasvir	Sofosbuvir
Intraday Precision (% RSD)	0.739	0.695
Interday Precision (% RSD)	0.334	0.423
Ruggedness (% RSD)	0.553	0.310
Recovery (98-102%)	99.6-100.9	100.1-100.8
LOD	1.0µg/ml	0.5µg/ml
LOQ	3.5µg/ml	2.0µg/ml
Formulation assay (98-102%)	99.751	99.008

**First Order Derivative Spectrophotometry (Method -2):**

The zero order absorption spectra of Ledipasvir and Sofosbuvir are represented in the figure-C. The close overlap of the absorption spectra of Ledipasvir and Sofosbuvir prevents the correct use of zero-order absorption measurements for their simultaneous determination in binary mixtures. On the other hand, the first-order spectrum did not suffer any interference at the determination wavelength of Ledipasvir (247.0 nm), Sofosbuvir (237.0 nm) as expected. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, LOD and LOQ. The calibration curve (figure-E) was obtained with six concentrations of the standard solution. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in Table 3. Linearity for Ledipasvir and Sofosbuvir was observed in the concentration range of 4.5-27µg/ml for Ledipasvir and 20-120µg/ml for Sofosbuvir. The method and correlation coefficient was found to be 0.999 for both the drugs. The results of intra-day, inter-day and ruggedness precisions of the proposed method were conducted with six repetitive analyses and the percentage relative standard deviation has been found below 2 percent for both drugs. The recovery was performed at three levels, 50, 100 and 150% of AMB and OLM standard concentration. Results are found to be 99.6-100.05% for Ledipasvir and 98.9 – 100.45% for Sofosbuvir. The LOD were 1.0µg/ml, 0.5µg/ml and the value for LOQ were 3.5µg/ml, 2.0µg/ml for Ledipasvir and Sofosbuvir, respectively. Validation results with the proposed method are presented in the table-4. These low values indicated the good sensitivity of the methods proposed. The applicability of the proposed methods for the determination of Ledipasvir and Sofosbuvir commercial dosage forms was examined by analyzing marketed products. It is evident that there is good agreement between the amount estimated and those claimed by the manufacturers. Percent label claims are very close to 100, with low value of standard deviation. The proposed method is more sensitive and the methods depends on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets [23].



**Fig. 5: Linearity Graph of Ledipasvir and Sofosbuvir for First Order Derivative method**

**Table-2: Result of Linearity for First Order Derivative Spectrophotometry method**

S.NO	Ledipasvir		Sofosbuvir	
	Concentration	Absorbance	Concentration	Absorbance
1	4.5	0.00835	20	0.00213
2	9	0.01568	40	0.00421
3	13.5	0.02345	60	0.00613
4	18	0.03105	80	0.00814
5	22.5	0.03865	100	0.00998
6	27	0.046916	120	0.011967

**Table-4: summary of validation for Ledipasvir and Sofosbuvir for First Order Derivative Spectrophotometry method:**

Parameter with validation limit	Ledipasvir	Sofosbuvir
Intraday Precision (% RSD)	0.031	0.298
Interday Precision (% RSD)	1.454	0.351
Ruggedness (% RSD)	1.027	0.460
Recovery (98-102%)	99.91-100.05	98.89-100.48
LOD	1.0 $\mu$ g/ml	0.5 $\mu$ g/ml
LOQ	3.5 $\mu$ g/ml	2.0 $\mu$ g/ml
Formulation assay (98-102%)	99.751	99.008

### Conclusion:

Percentage of RSD for intra-day and inter-day precision studies for both drugs was well within the acceptable range (< 2 %) indicating that both the methods have excellent repeatability and reproducibility. The percentage relative standard deviation for precision and accuracy was found to be low, which indicates that the methods have considerable accuracy and precision. Percent recovery for Ledipasvir and Sofosbuvir, by both methods, was found in the range of 98.99 % to 100.4 % with standard deviation well below 2 indicating accuracy of the methods. Recovery greater than 98% with the low standard deviation justifies the accuracy of the method. Intra-day and Inter-day precision studies were carried out by analyzing tablet formulation, by both methods. The results are in good agreement with the label claim. The proposed method is found to be simple, precise, accurate and sensitive and therefore, can be used as a quality control tool for the simultaneous estimation of both drug from their combined dosage form in quality control laboratory.

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