Research Article

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International Journal of Pharmacy and Industrial Research

ISSN		
	Print	2231 – 3648
	Online	2231 – 3656

New analytical method development and validation for the simultaneous estimation of velpatasvir and sofosbuvir in pharmaceutical dosage forms

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ABSTRACT

A selective and sensitive reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the separation and quantification of Velpatasvir and Sofosbuvir in tablet dosage form and validated .The determination was carried out using Thermosil C18 column (250 mm ×4.6 mm id) as a stationary phase and mobile phase comprised of Methanol: 0.01M Potassium dihrogen orthophosphate buffer in proportion of 55:45(v/v) with pH adjusted to 7±0.5 by using triethyl amine. The flow rate was 1.0ml/min and the eluent was monitored at 256nm.The retention time of and Velpatasvir and Sofosbuvir were 2.089 ±0.018 min and 5.327±0.024 min respectively. The Coefficient of correlation and percentage recoveries of Velpatasvir and Sofosbuvir were 0.9986 and 100.0l % and 0.9994 and 99.98% respectively. The method is validated for accuracy, Precision, ruggedness and Robustness. The proposed method is successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies.

Keywords: Sofosbuvir, Velpatasvir, Simultaneous estimation, RP-HPLC method.

INTRODUCTION

Velpatasvir (Figure.1) is chemically Methyl {(2S) - 1 -[(2S, 5S) – 2 - (9 - {2 -[(2S ,4S) – 1 - {(2R) - 2 -[(methoxycarbonyl) amino] -2 - phenylacetyl} - 4- (methoxymethyl) -2 - pyrrolidinyl] -1 H - imidazol-4-yl} -1, 11 – dihydroisochromeno [4', 3': 6,7] naphtha [1,2-d] imidazol-2-yl) -5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate. It is white to tan or yellow hygroscopic solid, soluble in water,

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methanol and acetonitrile with pKa values of 3.72 and 5.98. It is an antiviral drug used to treat chronic hepatitis C [1-2].

Sofosbuvir (Figure.1) chemically is Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3hydroxy-4-methyl-tetrahydrofuran-2-yl]methoxy-phenoxy phosphoryl] amino] propanoate. It is white to off-white crystalline solid, slightly soluble in water with a pKa value of 9.38. It is an antiviral drug used in the treatment of Hepatitis C [3-4]. Velpatasvir is an NS5A inhibitor which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.

New Tablet formulation Epclusa (velpatasvir 100 mg and sofosbuvir 400 mg) is a combination product containing and velpatasvir and sofosbuvir. Epclusa is a prescription medicine used to treat adults with chronic hepatitis C infection. Sofosbuvir is a nucleotide hepatitis C virus (HCV) Nonstructural protein (NS) 5B polymerase inhibitors [5-7]. Velpatasvir is HCV NS5A replication complex inhibitors. Literature survey revealed that few analytical methods have been reported for individual estimation of velpatasvir and sofosbuvir and combined with other dosage forms [8-20]. The present study describes a precise, accurate, specific and sensitive RP-HPLC method as per ICH guidelines for the simultaneous estimation of velpatasvir and sofosbuvir in tablets as well as for application to dissolution testing of tablet formulations [21].

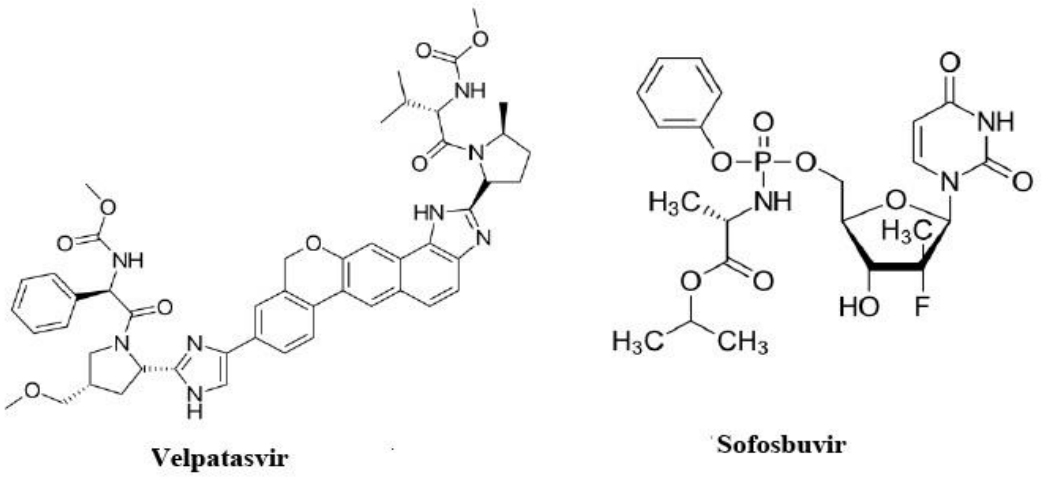

Figure1.Structure of Velpatasvir and Sofosbuvir.

EXPERIMENTATION

Equipment

Chromatographic separation was performed on Waters HPLC system consist of model 2695 having PDA detector and Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing.

Reagents and chemicals

Methanol and water of HPLC grade were procured from Rankem lab ltd. Sofosbuvir standard drug and Velpatasvir standard drug were supplied as gift samples by Spectrum labs, Hyderabad. Potassium di hydrogen ortho phosphate and Tri ethyl amine were A.R grade from Merck chemicals Mumbai, India. Tablets Epclusa were purchased from Indian market, containing a 100mg of velpatasvir and 400 mg of sofosbuvir per tablet.

Preparation of buffer

Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH adjusted to 7±0.5 by using triethyl amine solution.

Optimized chromatographic Condition

A Thermosil C18 column (250mm×4.6mm, 5µ) column was used as the stationary phase. A mixture of Methanol: 0.01M Potassium dihrogen

orthophosphate buffer in proportion of 55:45(v/v) was used as a mobile phase and pH adjusted to 7±0.5 by using triethyl amine. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1 ml/min. The eluents were monitored at 256nm.The injection volumes of samples and standard were 20µl.

STANDARD PREPARATION

Preparation of standard solution

10 mg of Velpatasvir and 10mg Sofosbuvir of were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution) . The working standard solutions were prepared and further diluted in mobile phase to and Velpatasvir and Sofosbuvir contain a mixture of in over the linearity ranges from 10-60 µg/ml and 40-240 µg/ml.

Sample preparation

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 10 mg of Velpatasvir and 40mg of Sofosbuvir was weighed and transferred to a 10ml volumetric standard flask and added 5 ml of mobile phase. The sample was kept in an ultrasonic bath for 20 min and further diluted to 10 ml by using mobile phase to get 1000 µg/ml of Velpatasvir and 4000µg/ml of Sofosbuvir. Then it is filtered through 0.22µ membrane filter paper. Then this primary sample solution were further diluted to get the concentration of 30 µg/ml Velpatasvir 120µg/ml of Sofosbuvir sample solutions.20µl of this solution was injected in to HPLC system and chromatograms were recorded. Concentrations of Velpatasvir and Sofosbuvir in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table1.

Table1: Table for Assay

Drug Name	Amount present mg	Amount found* (mg/tab)	% label claim*
Velpatasvir	100	99.98	99.98
Sofosbuvir	400	399.97	99.99

.*Each value is average of six determinations.

RESULTS AND DISCUSSION

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in Pharmaceutical industries. The chromatograms of sample and standard solution of Velpatasvir and Sofosbuvir were shown in (Figure.2) and (Figure.3). There was clear resolution between Velpatasvir and Sofosbuvir with retention time of 2.087and 5.328 minutes respectively.

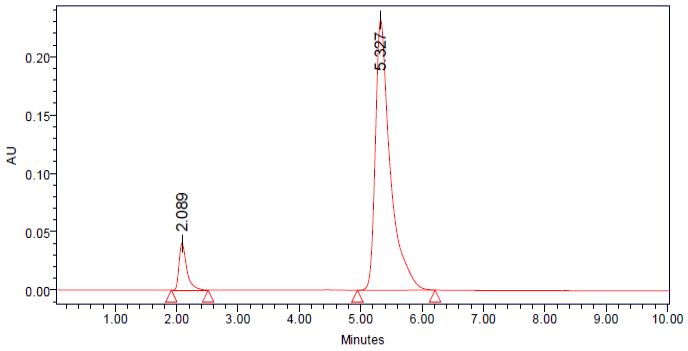

Figure 2: Typical Chromatogram of standard solution of Velpatasvir and Sofosbuvir

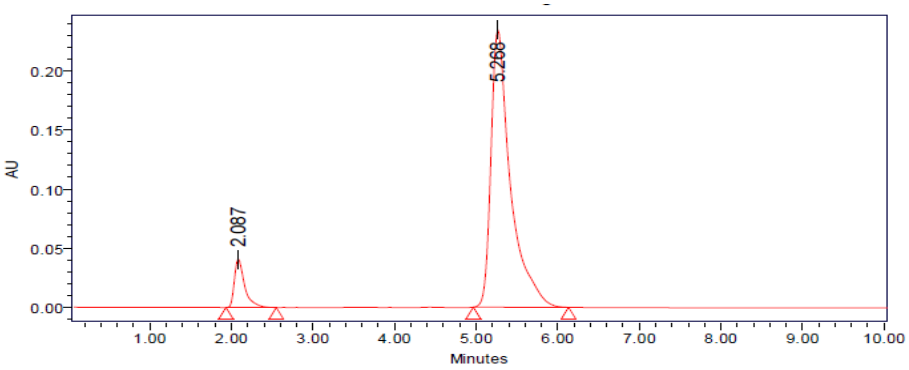

Figure 3: Typical Chromatogram of sample solution of Velpatasvir and Sofosbuvir

VALIDATION OF THE METHOD

System suitability

The system suitability test was carried out on freshly prepared stock solution of Velpatasvir and Sofosbuvir to check various parameters such as column efficiency, tailing factor and number of theoretical and presented in table 2. The values obtained were demonstrated the suitability of the system for the analysis of the drug. Here tailing factor for peaks of Velpatasvir and Sofosbuvir was less than 2% and resolution was satisfactory System suitability parameter may fall within ± 3% standard deviation range during routine performance of the method. The peaks obtained for Velpatasvir and Sofosbuvir were sharp and have clear base line separation.

Table No2: System Suitability

S.No	Parameters	Velpatasvir	Sofosbuvir
1	Capacity factor	1	1
2	Theoretical plate	6658	8456
3	Asymmetry of the peak	1.1	0.41
4	Retention time (min)	2.058	5.237
5	Resolution		8.2

Linearity

The response for the detector was determined to be linear over the range of 10-60µg/ml (10,20,30,40,50,60) of Velpatasvir and 40-240µg/ml (40,80,120,160,200,240) for Sofosbuvir. Each of this concentration was injected in six times to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. The results show that an excellent correlation exits between response factor and concentration of drugs within the concentration range indicated above. (Table 3)

Table3: Summary of analytical method validation

S.No	**Parameters**	**Acceptance criteria**	**Velpatasvir**	**Sofosbuvir.**
1	Linearity	r^2=0.995 to1.0	0.9986	0.9994
2	Specificity	No interference with placebo	specific	specific
3	Accuracy(Recovery studies)	Recovery99.0-102.0%	99.97%	101.01%
4	**Precision**			
	Intraday	RSD NMT 2.0%	0.3241	0.4611
	Interday	RSD NMT 2.0%	0.2510	0.2463

5	**Robustness**			
	Change inflow rate	NMT±1%	0.2%	0.4%
	Change in mobile phase ratio	NMT±1%	0.3%	0.4
	Change in p^H	NMT±1%	0.2%	0.4%
6	Limit of detection µg/ml	----------	0.5µg/ml	2µg/ml
	Limit of Quantification µg/ml	----------	1.5µg/ml	6µg/ml

Precision and Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard Velpatasvir and Sofosbuvir corresponding to 80%, 100%, and 120% of the label claim was added to pre analyze sample of tablet dosage form separately. The recovery studies were carried out six times at each level of recovery. From the data obtained, recoveries of standard drugs were found to be accurate (Table.3.). The %RSD of interday and intraday precision obtained was less than 2% for both the drugs. The intraday and interday precision of Velpatasvir was 0.3241 and 0.2510 and Sofosbuvir was 0.4611 and 0.2463 respectively. From the data obtained, the developed HPLC method was found to be precise and accurate.

Specificity of the method

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/ml was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Velpatasvir and Sofosbuvir from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 ∂/S and 10 ∂/S, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The LOD for Velpatasvir and Sofosbuvir was found to be 0.51µg/ml and 2.0µg/ml, respectively. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 1.52µg/ml and 6µg/ml for Velpatasvir and Sofosbuvir respectively. (Table.3)

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instrument like Waters HPLC and Agilent HPLC by different operators using different columns of similar type like HypersilC18, ZorbaxC18 column. Robustness of the method was determined by making slight changes in the experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase and the chromatographic characteristics were evaluated. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of Velpatasvir and Sofosbuvir in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolutions studies.

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