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DEVELOPMENT AND APPLICATION OF LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF SOFOSBUVIR AND VELPATASVIR IN FIXED DOSAGE FORM

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ABSTRACT

A new method was established for simultaneous estimation of Sofosbuvir and Velpatasvir by RP – HPLC. The chromatographic conditions were successfully developed for the separation of Sofosbuvir and Velpatasvir by using Eclipse XDB -C18 (150x4.6mm, 5µm particle size) flow rate was 1.0 ml/min, mobile phase ratio was Acetonitrile and Water in the ratio of 50:50 (v/v), 0.025M Potassium dihydrogen orthophosphate in 1000 ml of water adjust pH 2.5 with dilute *Ortho*-phosphoric acid, detection wavelength was 240 nm.The retention times were found to be 7.668 mins and 3.872 mins.The percentage purity of Sofosbuvir and Velpatasvir was found to be 99.89% and 99.80% respectively.the analytical method was validated according to ICH guidelines .the precision study was ,robustness and repeatability.LOD value was 0.3 and 0.15 and LOQ value was 0.9 and 0.45 respectively.

INTRODUCTION:

Epclusa is a fixed-dose combination of sofosbuvir, a hepatitis C virus (HCV) nucleotide analog NS5B polymerase inhibitor [1,2], and velpatasvir, an HCV NS5A inhibitor, and is indicated for the treatment of adult patients with chronic HCV genotype 1, 2, 3, 4, 5, or 6 infection. The tablets include the following inactive ingredients: copovidone, croscarmellose sodium, magnesium stearate, and microcrystalline cellulose. The tablets are film-coated with a coating material containing the following inactive ingredients: iron oxide red, polyethylene glycol, polyvinyl alcohol, talc, and titanium dioxide [3]. Sofosbuvir is an inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is required for viral replication [4]. Velpatasvir is an inhibitor of the HCV NS5A protein, which is required for viral replication. Resistance selection in cell culture and cross-resistance studies indicate velpatasvir targets NS5A as its mode of action [5].Epclusa (sofosbuvir 400mg/velpatasvir 100mg) is a first all oral, pan genotypic treatment for Hepatitis C.

Literature Survey: Zaman et al [6] developed a reversed-phase high-performance liquid chromatographic method developed for the simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form. The analysis was performed on Luna analytical column 250 × 4.6 mm, 5 µm, octyl silica packing (Si–[CH2]7–CH3) C8, using ammonium acetate buffer solution pH 7.0 and acetonitrile 35:65 % v/v as mobile phase at flow rate of 0.7 mL min−1 for isocratic elution. Detection of sofosbuvir and ledipasvir was performed on a UV detector at 245 nm. The retention times of sofosbuvir and ledipasvir were 4.468 ± 0.013 min and 8.242 ± 0.012 min, respectively, and the total run time was 20 min. Maria et al [7] developed a new method based on reversed phase (RP)-ultra-high performance liquid chromatography (UHPLC) coupled to diode array detection (DAD) and mass spectrometry (MS) was devel oped to facilitate the qualitative and quantitative analysis of sofosbuvir in film coated tablets.

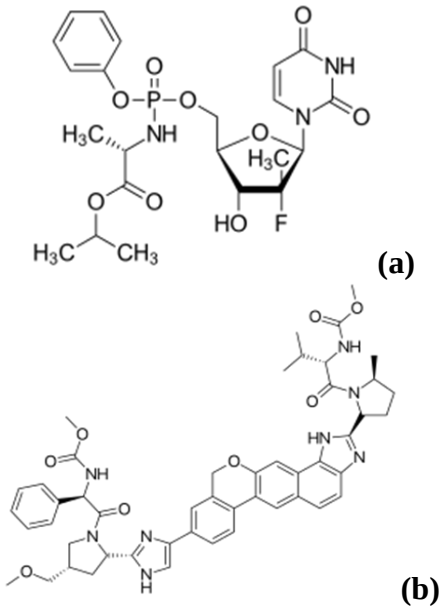

Figure 1: Chemical structure of Velpatasvir and Sofosbuvir (a and b)

MATERIALS AND METHODS

Materials and reagents: Glass-distilled and deionized water (Nanopure, Barnstead, USA), HPLC-grade methanol, sodium hydroxide, hydrochloric acid, hydrogen peroxide and *ortho*-phosphoric acid (S.D.Fine chem, Mumbai, India) were used. Samples of active pharmaceutical ingredients as reference standards the active pharmaceutical ingredients were a kind of gift from M/s Mylan Laboratories India Pvt. Ltd, Hyderabad.

Apparatus/Instruments: Liquid chromatograph: HPLC, Waters Alliance 2695 separation module equipped with 2489 UV/visible detector or 2998 PDA detector with Empower 2 software. pH meter

Analytical procedures: Preparation of mobile phase: 0.025M Potassium dihydrogen orthophosphate in 1000 ml of water adjust pH 2.5 with dilute *Ortho*-phosphoric acid as solvent-A and Acetonitrile as Solvent-B was used in gradient mode of separation. The resultant solution was thoroughly mixed and filtered through a poly-tetra-fluoro ethanol (PTFE) filter of 0.45 µm pore size using vacuum pump and degassed by sonication to expel the dissolved gases in solvent system.

Preparation of Individual Velpatasvir standarad solution: Transfer 100 mg of Velpatasvir working standards into a 100 ml volumetric flask, dissolved and dilute with acetonitrile and water in the ratio of 50:50 (v/v) as diluent. 5 ml of the resulting solution is further diluted up to 50 ml in volumetric flask with diluents. The resulting solution contains 100 µg/mL of Velpatasvir as working standard solutions. The prepared stock solutions were stored at 4 ^{0}C and protected from light.

Preparation of Individual Sofosbuvir standarad solution: Transfer 400 mg of Sofosbuvir working standards into a 100 ml volumetric flask, dissolved and dilute with acetonitrile and water in the ratio of 50:50

(v/v) as diluent. 5 ml of the resulting solution is further diluted up to 50 ml in volumetric flask with diluents. The resulting solution contains 400 µg/mL of Sofosbuvir as working standard solutions. The prepared stock solutions were stored at 4 ^{0}C and protected from light.

Assay of Tablet Formulation:

Twenty tablets of eEpclusa®-containing 100 mg of Velpatasvir and 50 mg of Sofosbuvir were triturated in mortar and pestle to get uniform blend and free flowing powder. The contents were mixed properly to get a homogeneous powder. The resulting sample contents are measured a quantity equivalent to 100 mg of Velpatasvir, and 400 mg of Sofosbuvir working standards into a 100 ml volumetric flask, dissolve and dilute with acetonitrile and water in the ratio of 50 : 50 (v/v) as diluents and transferred in to a 100-mL volumetric flask, extracted in diluent by sonication, and filtered through Whatman no. 41 filter paper. The filtrate (5 mL) was quantitatively transferred to a 50-mL volumetric flask, and solution was diluted to volume with the diluents. The resulting solution contains 100 µg/mL of Velpatasvir, and 400 µg/mL of Sofosbuvir as working test or sample solutions. The prepared stock solutions were stored at 4 ^{0}C and protected from light.

RESULTS AND DISCUSSION

The present study was aimed at developing a chromatographic method for separation and quantitative determination of Velpatasvir, and Sofosbuvir in fixed dosage form.

Specificity:

Specificity is the ability of the method to measure the analyte response in presence of all the potential impurities and excipients. The terms selectivity and specificity are often used interchangeably. The specificity of the developed LC method for quantification of all the three drugs was determined in the presence of excipients present in pharmaceutical products. In specificity study, interference between drugs and tablet excipients were evaluated from the comparison of spectral purity obtained from the analysis for the standard solutions and sample solutions. The specificity of method will be demonstrated by the ability to analyze, Velpatasvir and Sofosbuvir as fixed dosage form in finished product sample matrix. The separate solution of blank and standard samples of three analytes was evaluated along with excipient solutions.

Precision:

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. It is the ability to detect small changes in the concentration of the analytes in the sample. The intra-day repeatability was investigated using six separate sample solutions prepared, as reported above; from the freshly reconstructed tablet formulations at 100% of the target level contains 100 µg/mL of Velpatasvir and 400 µg/mL of the Sofosbuvir. Each solution was injected in six replicates and the peak areas obtained were used to calculate means and RSD% values.

Linearity and Range:

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The peak areas of the drugs to drugs concentration were used for plotting the linearity graph. The linearity data is reported in Table-3.

Accuracy/Recovery:

The accuracy of the method was determined by measuring the recovery of the drugs by the method of standard additions.

Figure-2 -Calibration Graphs of Velpatasvir and Sofosbuvir:

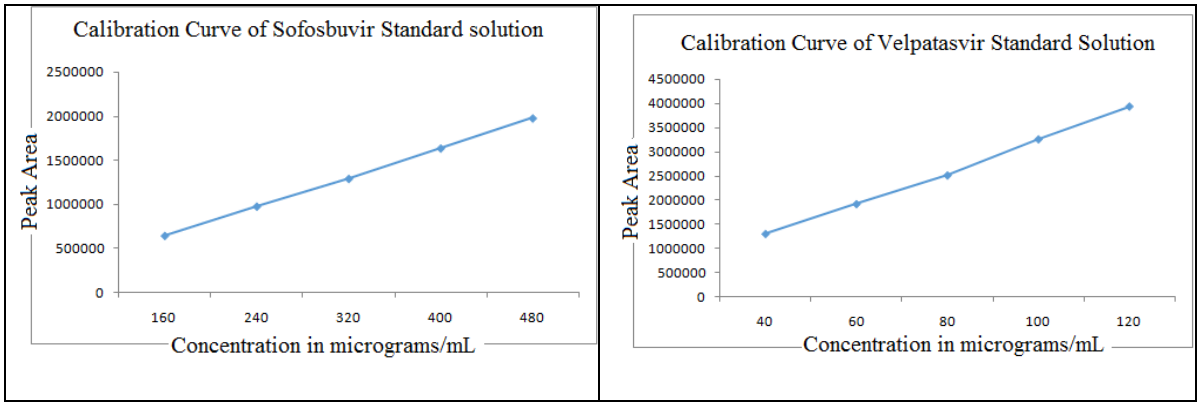

Table:1 System suitability data of Velpatasvir and Sofosbuvir

Parameter	Velpatasvir	Sofosbuvir
Retention time (min)	3.872	7.668
Theoretical plates	18991.77	36057.29
Tailing Factor	1.60	1.51
HETP	$1.3163x10^{-5}$	$6.933x10^{-6}$
USP plates/meter	75967.08	144229.16
Resolution	---	26.61
Peak area	3198382	1611771
% Peak Area	66.49	33.51

Figure-3: Typical system suitability chromatogram of Velpatasvir and Sofosbuvir:

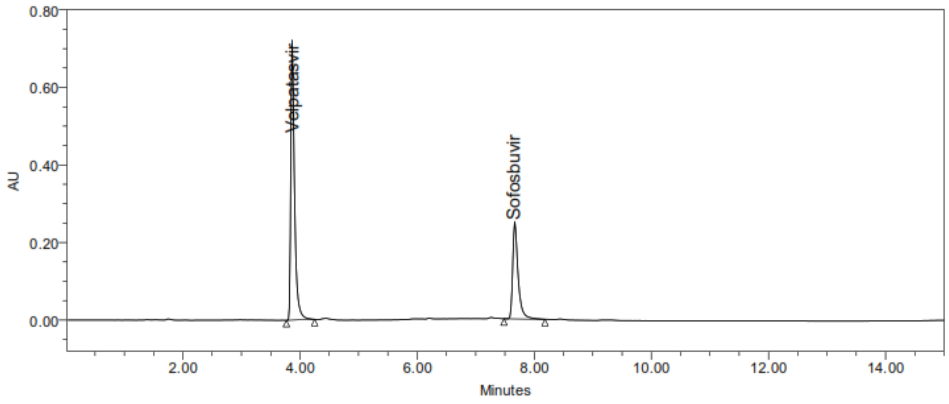

Figure-4: Specificity chromatograms of Velpatasvir and Sofosbuvir with Blank:

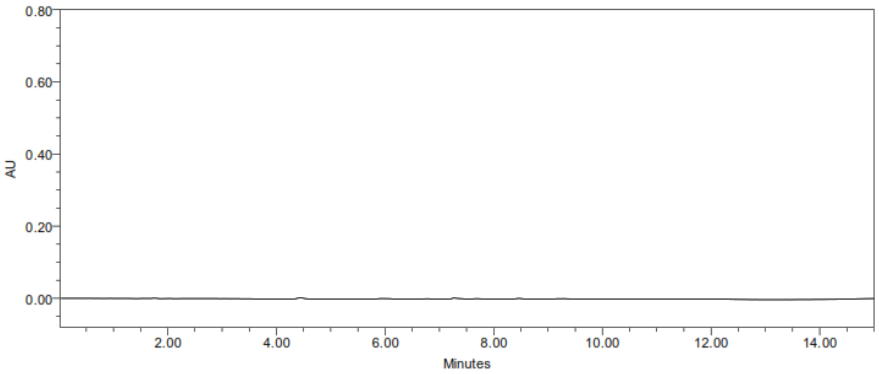

Table-2: Precision study of Velpatasvir and Sofosbuvir:

Intra-Day Precision study of Epclusa®		
Injection No:	Velpatasvir	Sofosbuvir
1	3205467	1604428
2	3217473	1605018
3	3231807	1614281
4	3244236	1611623
5	3277349	1615173
6	3225338	1616626
Mean	**3233611.7**	**1611191.5**
Std. Dev	25100.3	5272.1
% RSD	**0.8**	**0.3**
Inter-Day Precision study of Epclusa®		
Injection No	Velpatasvir	Sofosbuvir
1	3230554	1614140
2	3223084	1612085
3	3294626	1619833
4	3249390	1630478
5	3246035	1625701
6	3244289	1625489
Mean	**3247996.3**	**1621287.7**
Std. Dev	24974.9	7204.0
RSD	**0.8**	**0.4**

Figure 5: Precision Chromatograms (Intraday & Interday) of Velpatasvir and Sofosbuvir:

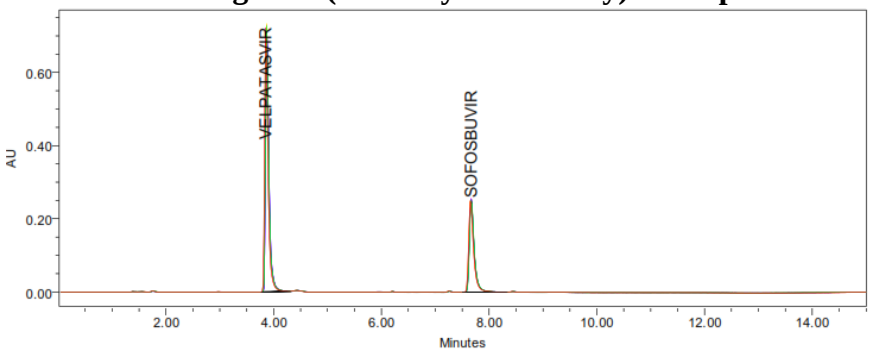

Table 3: Linearity data of Velpatasvir and Sofosbuvir:

Target Level	Velpatasvir		Sofosbuvir	
	Conc. µg/mL	Peak Area	Conc.µg/mL	Peak Area
40 %	40	1296784	160	649690
60 %	60	1963294	240	981257
80 %	80	2496320	320	1293947
100 %	100	3253365	400	1637940
120 %	120	3930953	480	1978211
Regression Equation	Y= 32792x-35220.4		Y= 4134.656x-21681	
Corr.Coeff.	**0.998**		**0.988**	

Table 4: Assay results of Velpatasvir and Sofosbuvir in Epclusa® Tablets.

Formulation	Label Claim (mg/tablet)		Amount found in (mg)	
Epclusa® is a fixed-dose tablet	Velpatasvir	Sofosbuvir	Velpatasvir	Sofosbuvir
	100	400	99.52	399.82

Known amounts of each drug (10% standard drug solution composed of Velpatasvir 10 µg/mL and Sofosbuvir 40 µg/mL) corresponding to 80% dilution (composed of Velpatasvir 80 µg/mL and Sofosbuvir 3200 µg/mL), 100% dilution (composed of Velpatasvir 100 µg/mL and Sofosbuvir 400 µg/mL), and 120% dilution composed of Velpatasvir 120 µg/mL and Sofosbuvir 480 µg/mL).

CONCLUSION:

In this study, a validated simple and reliable RP-HPLC procedure was described for the assay of a complex multi drug combination consisting of Epclusa® Tablet composed of 400 mg of Sofosbuvir and 100 mg of Velpatasvir which is indicated is indicated for the treatment of chronic hepatitis C virus (HCV) genotype 1 or 4 infection in adults.. **All the three active ingredients were successfully resolved and quantified using** Eclipse XDB -C18 150x4.6mm, 5 micron **in a relatively short run time of 15 minutes in isocratic mode of chromatographic method.** The proposed method provides a good resolution between active ingredients. The developed method reported herein was validated by parameters as described in ICH-Q2B guideline. System suitability, specificity, linearity, LOD, LOQ values, within- and between-day precision and accuracy of the proposed technique were obtained during the validation studies. The proposed method has the advantages of simplicity, repeatability, sensitivity and requires less expensive reagents.

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