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ESTIMATION OF SOFOSBUVIR AND VELPATASVIR BY VARIOUS ADVANCED ANALYTICAL TECHNIQUES – A DETAILED REVIEW

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ABSTRACT

The present review article focuses on the estimation of Sofosbuvir and Velpatasvir and its combinations by various analytical techniques. Sofosbuvir is in a class of antivirals called nucleotide hepatitis C virus (HCV) NS5B polymerase inhibitors. Velpatasvir is in a class of antivirals called HCV NS5A replication complex inhibitors. This review discusses the estimation of sofosbuvir and velpatasvir by using various analytical tools like UV-Visible spectrophotometer, Fluorimetry, Capillary Electrophoresis, High–Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Ultra Performance Liquid Chromatography (UPLC), Liquid Chromatography (LC), Liquid Chromatography-Mass Spectroscopy (LC-MS/MS), Ultra Performance Liquid Chromatography-Mass Spectroscopy (UPLC-MS/MS). This review enlightens the various solvents and conditions suitable for the estimation of Sofosbuvir and Velpatasvir and its combinations. The techniques illustrated here may find application in the qualitative and quantitative estimation of Sofosbuvir and Velpatasvir.

Keywords: Sofosbuvir, Velpatasvir, Chromatography, Hepatitis C virus

INTRODUCTION

Antiviral agents are medications that have been approved by the FDA for the treatment or control of viral infections. The steps of the viral life cycle that are targeted are viral attachment to the host cell, uncoating, viral mRNA synthesis, translation, viral RNA and DNA replication, maturation of new viral

proteins, budding, release of freshly manufactured virus, and free virus in body fluids. At least half of the antiviral drugs on the market are used to treat human immunodeficiency virus (HIV) infections. Herpesviruses, hepatitis B virus (HBV), hepatitis C virus (HCV), and respiratory viruses are treated with the others **[1]**.

Hepatitis C virus (HCV) has three targets that suggest direct-acting antiviral (DAA) drugs attack to kill the virus. Each DAA drug targets one of these targets; combination DAA drugs target many targets. DAA drugs are categorised according to the mode of action they employ to combat HCV. There are three types of DAA medicines that are now recommended **[2]**:

1. NS3/4A protease Inhibitors
2. NS5A polymerase Inhibitors
3. NS5B polymerase Inhibitors

Sofosbuvir (SOF)is an oral nucleoside analogue and powerful inhibitor of hepatitis C virus (HCV) RNA polymerase that is used to treat chronic hepatitis C in combination with other antiviral drugs **[3]**. Sofosbuvir is the first DAA, was released in India in March 2015 at a low price **[4]**. IUPAC name (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl] methoxy-phenoxy-phosphoryl] amino] propanoate **(Figure 1) [3]**.

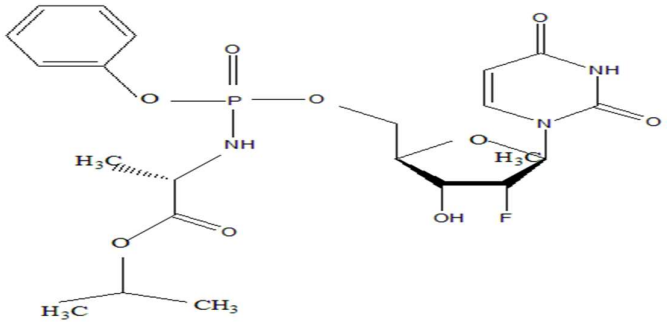

Figure 1: Structure of Sofosbuvir

Velpatasvir (VEL) is a complex organic hetero pentacyclic molecule that inhibits the non-structural protein 5A of the hepatitis C virus. It is used in combination with sofosbuvir (marketed as Epclusa) to treat patients with chronic hepatitis C of all six genotypes. It works as an antiviral and a non-structural protein 5A inhibitor for the hepatitis C virus **[5]**.

IUPAC name for VEL is 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 – dihydro isochromeno [4', 3': 6,7] naphtha [1,2-d] imidazol-2-yl) - 5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} Carbamate **(Figure 2) [5]**.

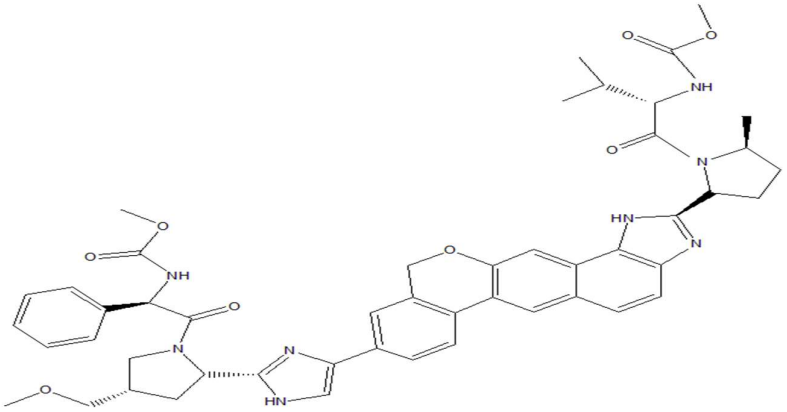

Figure 2: Structure of Velpatasvir

METHODS FOR ESTIMATION

Spectrophotometric methods

Various Spectrophotometric methods for Sofosbuvir and Velpetasvir in single and combination are developed and are listed in **Table 1, 2**.

Electrophoretic methods:

Estimation of Sofosbuvir and Velpetasvir by Capillary Electrophoresis are listed in **Table 3**.

Chromatographic methods:

Various chromatographic methods like HPLC, HPTLC, UPLC are listed in **Table 4, 5, 6**

Hyphenation techniques:

Hyphenation techniques was developed for estimation of Sofosbuvir and Velpatasvir are listed in **Table 7, 8**.

Table 1: Method for estimation of Sofosbuvir and velpatasvir by UV-Visible Spectroscopy

S.No	Drugs	Description	Reference
1.	SOF+VEL in their pure forms and pharmaceutical formulation	Detection Wavelength- VEL- 302.5 and 337.0 nm. (Zero-order spectrophotometric method (D°)), SOF- 239.6 and 260.0 nm. Solvent- Methanol Linearity Range- SOF- 5.0-90.0 µg/mL, VEL- 2.0-30.0 µg/mL	[6]

Table 2: Method for estimation of Sofosbuvir and velpatasvir by Fluorimetric method.

S.No	Drugs	Description	Reference
2.	VEL in human plasma in the presence of sofosbuvir.	Wavelength- 383 nm with 339 nm for excitation wavelength. Solvent- Methanol Concentration range- 5 to 5 × 103 ng /mL LOD- 0.23ng/mL LOQ- 0.70ng/mL	[7]
3.	VEL+SOF in its bulk and in combined tablet.	Wavelength- 385 nm and 400 nm after excitation at 295 nm Solvent- Methanol Concentration range- 2.0–20.0 ng /mL LOD- 0.146 ng /mL and 0.378 ng /mL LOQ- 0.444 ng /mL and 1.147 ng/ mL	[8]

Table 3: Method for estimation of Sofosbuvir and velpatasvir by Capillary electrophoresis

S.No	Drugs	Description	Reference
4.	SOF+VEL in their new FDA-approved pharmaceutical dosage form.	Detection Wavelength: 225 nm Temperature: 25°C Electrolyte Solution: borate buffer (40 mM, pH10). Capillary: fused silica capillary (50 cm × 75 mm internal diameter)	[9]

Table 4: Method for estimation of Sofosbuvir and velpatasvir by RP-HPLC

S.No	Drugs	Description	Reference
5.	SOF+VEL in bulk drug and pharmaceutical dosage form.	Detection Wavelength- 260nm Mobile Phase- Buffer: Acetonitrile (45:55 v/v) Column- Kromasil C18 column (250mm×4.6mm, 5µm particle size) Flow Rate- 1.0 ml/min Linearity Range- SOF-100 to 600 ppm, VEL- 25 to 150 ppm Retention Time- SOF-2.124 min, VEL-3.334 min.	[10]
6.	SOF+VEL+VOX (Voxilaprevir) in bulk and tablet dosage forms.	Detection Wavelength- 260 nm Mobile Phase- Solution-A was 0.1% OPA (pH 1.8) in water. Solution -B was Acetonitrile (mobile phase: solution A: solution B, (50:50 v/v). Column- C18 (250×4.6mm,5µm) Flow Rate- 1.0 ml/min Linearity Range- SOF-100 to 600 ppm, VEL&VOX- 25 to 150 ppm Retention Time- SOF-2.458 min, VEL- 3.282 min and VOX- 4.003 min.	[11]
7.	SOF+VEL in raw and tablet forms.	Detection Wavelength- 240nm Mobile Phase- potassium dihydrogen phosphate (0.1 M) and methanol (pH 4.5, 60:40 v/v) Column- Spursil C18 (250 × 4.5 m5.m and i.d., 5 µm particle size) Flow Rate- 1.0 mL/min Linearity Range- VEL- 10–30 µg/mL and SOF-40–120 µg/mL Retention time- VEL- 3.078 and SOF- 4.054	[12]
8.	SOF+VEL in fixed dose combination tablets and plasma.	Detection Wavelength- 270 nm Mobile Phase- ammonium acetate buffer (pH 7.0), acetonitrile and methanol (20:40:40, v/v/v) Column- Promosil C18 (250 mm × 4.6mm ID, 5µm) Flow Rate- 1.0ml/min Linearity Range- SOF-10 -60 µg/mL and VEL 1-6µg/mL. Retention Time- SOF- 3.72 min and VEL -7.09 min.	[13]
9.	SOF+ VEL in bulk and pharmaceutical dosage form	Detection Wavelength- 260nm Mobile Phase- buffer (60 %; 0.01 N KH2PO4: 40 % acetonitrile, 1.0 ml/min Column- C18 (4.6x250 mm, 5 µm) Flow Rate- 1.0 ml/min Linearity Range- SOF-100-600 µg/mL and VEL- 25-150 ppm Retention Time- SOF- 2.373 and VEL- 2.967 min.	[14]
10.	SOF+ VEL in solid pharmaceutical dosage form	Detection Wavelength- 262nm Mobile Phase- acetonitrile, phosphate buffer and methanol (60:30:10 v/v, pH 3.0) Column- Purospher Star C18 column (5 µm, 4.6 × 250 mm) Flow Rate- 1.0 mL/min Linearity Range- SOF- 10.0-70 µg/mL and VEL- 5.0-35.0 µg/mL Retention Time- SOF-3.251 and VEL- 4.512 min.	[15]
11.	SOF+VEL+VOX in Bulk and Pharmaceutical dosage form	Detection Wavelength- 220nm Mobile Phase- buffer 0.01N Na2HPO4 and acetonitrile (60:40 v/v). Column- Agilent C18 (150 x 4.6mm, 5m). Flow Rate- 1.0 ml/min Retention Time- SOF- 2.229 min, VEL- 2.957 min, VOX- 3.568 min	[16]
12.	SOF+VEL in their combined tablet dosage form	Detection Wavelength- 254nm Mobile Phase- Methanol and phosphate buffer (60: 40 v/v). Column- Inertsil C18 (250 × 4.6mm, 5 µ) Flow Rate- 1.0 ml/min Linearity Range- SOF-2- 8 µg/ml, VEL- 0.5 - 2µg/ml Retention Time- SOF- 3.049 min and VEL- 4.316 min.	[17]
13.	SOF+ VEL in bulk and tablet dosage form.	Detection Wavelength- 255 nm Mobile Phase- acetonitrile and 0.025M KH2PO4 adjusted to pH3.0 with orthophosphoric acid (50:50 v/v). Column- YMC column (4.6×15mm,5µ) Flow Rate- 1.0 ml/min Linearity Range- SOF- 200-1000µg/ml, VEL- 50-250 µg/ml. Retention Time- SOF-5.463 and VEL- 3.473	[18]
14.	SOF+VEL in tablet dosage forms	Detection Wavelength- 240 nm Mobile Phase- buffer 0.1% OPA: acetonitrile (50:50 v/v). Column- C18 (250 x 4.6 mm, 5µm) Flow Rate- 1.0 ml/min Linearity Range- SOF-100-600 µg/mL and VEL- 25-150µg/mL. Retention Time- SOF- 2.473 min and VEL- 3.316 min.	[19]

Table 5: Method for estimation of Sofosbuvir and velpatasvir by HPTLC

S.No	Drugs	Description	Reference
15.	SOF+VEL in pharmaceutical formulation and human plasma using ledipasvir (LED) as an internal standard.	Mobile Phase- ethyl acetate-isopropanol (90:10, v/v) Stationary phase- silica gel 60 F254 aluminum plates Densiometric scanning- SOF- 260nm and VEL- 302 nm Retardation factor- SOF- 0.76, VEL- 0.33 and LED- 0.22. Linearity Range: SOF- 400-10000ng/mL and VEL- 200 – 10000 ng/ml.	[20]
16.	SOF+VEL in Their Pure Forms and Tablet Dosage Form.	Detection Wavelength- SOF- 275nm and VEL- 378nm. Mobile Phase- methylene chloride–methanol–ethyl acetate–ammonia (25%) at a ratio of 5:1:3:1 (v/v) Stationary phase- silica gel 60 F254 aluminum plates Linearity Range: 100–2000 ng per spot for both SFS and VLP.	[21]

Table 6: Method for estimation of Sofosbuvir and velpatasvir by UPLC.

S.No	Drugs	Description	Reference
17.	SOF+VEL in bulk powder and in their pharmaceutical dosage form.	Detection Wavelength- SOF- 245.8 nm, VEL- 287.4nm Mobile Phase- diammonium phosphate buffer pH 6±0.02: acetonitrlie (40:60, v/v) Column- Waters Acquity C18 (150 x 2.1mm, 1.7µm) column Flow Rate- 0.1 mL/min Linearity Range- SOF- d 5- 240 µg/mL and VEL- 5-90 µg/mL	[22]
18.	SOF+VEL in Co-formulated tablet,	Detection Wavelength- t 405 nm after excitation at 340 nm (Method 1), 260 nm (Method 2) Mobile Phase- NaH2PO4, pH 2.5 (with phosphoric acid) and acetonitrile (60:40 v/v) Column- Acclaim RSLC 120 C18, 5.0 µm, 4.6×150 mm (column A) and Acclaim RSLC 120 C18, 2.2 µm, 2.1×100 mm (Column B) Flow Rate- 1.0 mL/min column, A, 0.5 mL/min column B Linearity Range- SOF- 120–600 ng/mL and VEL- 30–150 ng/mL	[23]

Table 7: Method for estimation of Sofosbuvir and velpatasvir by LC-MS/MS and UPLC-MS/MS.

S.No	Drugs	Description	Reference
19.	SOF+VEL in human plasma using ledipasvir as an internal standard.	Mobile Phase- 0.1% formic acid in water: acetonitrile: methanol (30:60:10, v/v/v) Column- C18 Zorbox eclipse plus (100 x 4.6 mm, 5µm) Column temp- 40°C Flow Rate- 0.55 ml/ min Linearity Range- SOF- 5-5000 ng/mL and VEL- 10-1500 ng/mL Retention time- SOF- 2.04 min, VEL-1.8 min and LED- 2.02 min.	[24]
20.	SOF+VEL in spiked human plasma using ledipasvir as an internal standard.	Mobile Phase- acetonitrile: 1% formic acid (50:50v/v) Column- Zorbax C18 Stable Bond (SB), C18 (4.6mm id x 50 mm) Flow Rate- 600µl/min Ion spray voltage- 5400V Source temperature- 140°C Desolvation temp- 800°C Linearity Range- SOF-0.5-5000 ng/mL, VEL-1.5-2000 ng/mL Retention Time- SOF- 1.13± 0.3 min, VEL- 1.32 ± 0.3 min and LED- 12.5± 0.3 min.	[25]

Table 8: Method for estimation of Sofosbuvir and velpatasvir by UPLC-MS/MS

21.	SOF+VEL in human plasma using ledipasvir as an internal standard (IS)	Mobile Phase- 0.1% formic acid and acetonitrile (50: 50, v/v) Column- BEH C_{18} column (50 x 2.1 mm, 1.7 µm) Colunm temp- 35°C Source temp- 120°C Desolvation temp- 350°C Flow Rate- 0.3 ml/min Run Time- 1.5 min. Linearity Range- SOF- 0.25–3500 ng/mL, VEL- 1–1000 ng/mL	[26]

CONCLUSION

This review discussed the reported spectroscopic techniques such as UV Spectroscopy and chromatographic techniques such as HPLC, HPTLC, UPLC, UPLC/MS, LC-MS/MS, Fluorimetry, and Capillary electrophoresis methods developed and validated for the estimation of Sofosbuvir and Velpatasvir in single and combined formulations. Out of all of these approaches, HPLC with UV detection was used extensively using the best solvents for improved resolution, such as acetonitrile, methanol, potassium dihydrogen orthophosphate, and phosphate buffer. A flowrate of 1.0ml/min was found to be the most effective for detecting chemicals. As a result, all of these procedures were simple, precise, and accurate, and they provided repeatability and reliability at a reasonable cost when compared to modern technologies.

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