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Overview on Hepatitis C, Treatment Strategy, Instrumental Analysis of Anti-HCV drugs

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Abstract

This literature review is dealing with Hepatitis C, treatment strategy, and different analytical techniques for determination of recently used anti-HCV drugs specifically Sofosbuvir, Simeprevir, Ledipasvir, Daclatasvir and Velpatasvir in different pharmaceutical and biological samples.

Keywords: hepatitis c; analytical techniques; sofosbuvir; simeprevir; ledipasvir; daclatasvir; velpatasvir

Hepatitis C

Liver is a very vital organ responsible for many important processes inside the body. Such processes include digestion of food, metabolism of carbohydrates, fats and proteins, storage of glucose, iron and vitamins (A, D, E, K and B12), production of some plasma proteins, regulating blood clotting, detoxification of blood from harmful substances (eg. Alcohol and drugs), immunity by producing immune factors and removing bacteria from blood stream……..etc [1, 2]. Hepatitis is defined as the inflammation of liver. Toxins, heavy alcohol consumption and even some medications can cause hepatitis. The most common cause for hepatitis is viral infection with viral Hepatitis A, Hepatitis B and Hepatitis C viruses. Hepatitis A is spread via foecal-oral route, while Hepatitis B and C are spread via contaminated blood and blood products. Viral Hepatitis A and B can be prevented by immunization. Acute hepatitis A and B infections resolve without treatment in the majority of adults. Medications are mostly given to relieve their symptoms [3].

Hepatitis C virus (HCV) is the most dangerous type of this group of viruses. HCV was first discovered in 1989 and is now affecting more than 170 million people worldwide [4]. Since then a lot of research had been made on HCV which led to decrease in its transmission. The highest prevalence is found in North Africa, Middle East and central Asia with mortality rate around 350 000 deaths per year [5].

Hepatitis C is called a silent disease because people can be infected without knowing it. Also symptoms from acute infections can include fever, gastric upset and feeling fatigue which are common with many other diseases. Only 15-25% of acute infections can be self limiting, while more than 80% develop chronic HCV infections which can take decades to develop its symptoms. But when symptoms appear, they are often indicating advanced liver disease. Chronic HCV infections are serious and can lead to liver damage, cirrhosis, liver failure and carcinoma [6].Acute HCV infections are asymptomatic and about 15-20% of patients can clear acute infections, while the remaining majority develops into chronic infections [7]. Chronic HCV infection affects liver cells leading to cirrhosis, end-stage liver disease, and hepatic cancer. Chronic HCV infections caused about 350000 deaths per year [8].

HCV is a single-stranded RNA virus. A single HCV polyprotein is translated, and then cleaved by cellular and viral proteases into three structural proteins (core, E1, and E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B,

, NS5A and NS5B) [9]. There are at least 6 genotypes of HCV that have arisen from the fast viral replication enabling it to adapt to host immune response and antiviral drugs. Genotyping is used as treatment guide since some viral genotypes respond better than others to different therapies.

HCV transmission is mainly by blood; however rare cases are transmitted sexually especially when associated with human Immunodeficiency virus (HIV) or on having relationships with multiple partners. Blood transfusion and reuse of injection needles are the major transmission routes globally. Mother to child transmission during pregnancy occurs in 2-8% from infected mothers [10]. Sexual transmission of HCV to partner is very rare [11].

HCV diagnosis relies on HCV antibodies detection and tests that detect HCV RNA. Presence of HCV antibodies without detection of viral RNA resolved or treated infections, but detecting both simultaneously indicates current infection. HCV is a single stranded RNA virus. HCV single poly-protein of 3011 amino acids is translated then cleaved by viral and cellular proteases into three structural proteins and seven non-structural proteins that are (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) [9].

Seven genotypes of HCV were discovered. Genotype 1 is the most common worldwide genotype in about 46.2% of HCV infected population. Genotype 2 occurs to about 9.1% of HCV infections and was found to be the easiest for treatment. Genotype 3 occurs to about 30% of infected population globally and is the most difficult genotype in treatment. Genotype 4 is confined mostly to Africa. Egypt is considered the highest in prevalence of HCV in the world and 90% of infected Egyptians are infected with genotype 4. Genotypes 5, 6 and 7 occur in smaller prevalence [12].

HCV treatment

Early treatment of HCV was found to be much more effective than late stage treatments. Milestones in HCV treatment are summarized in (Figures 1 [13] and 2 [14]). In 1991, FDA approved the first interferon (Intron A) for treatment of HCV. Ribavirin was then approved in 1998 to be used in combination with interferon for treatment [15]. The leap forward in HCV treatment wasn't until the discovery of directly acting antiviral drugs (DAADs).

In 2011, FDA approved triple therapy using two DAADs, boceprevir and telaprevir, together with pegylated interferon and ribavirin. Unfortunately, the safety profile of this treatment was poor. In 2013, FDA approved three DAADs; Simeprevir (SMP) as new NS3 protease inhibitor, Sofosbuvir (SFS) as NS5B RNA polymerase inhibitor and daclatasvir (DAC) as NS5A inhibitor. These drugs were approved as combination therapy with peg-interferon and ribavirin for 12-24 weeks [10]. Ledipasvir (LDS) and velpatasvir (VLP) were approved as NS5A inhibitors in 2014 and 2015, respectively. It wasn't until late 2014 when interferon free regimen was approved.

The new regimens comprise a NS5B nucleoside inhibitor (eg. SFS) together with a protease inhibitor (eg.SMP) or NS5A inhibitor(eg.LDS,

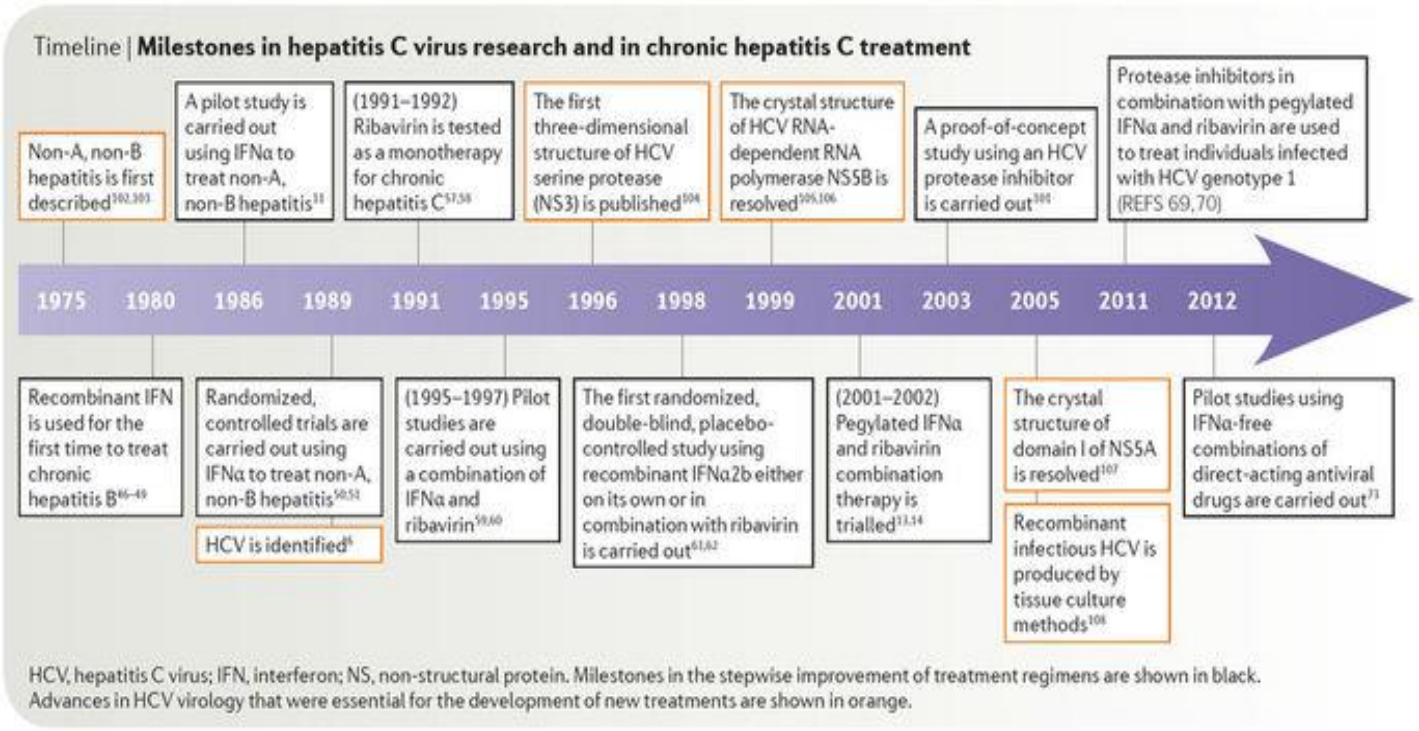

Figure 1: Milestones in HCV treatment.

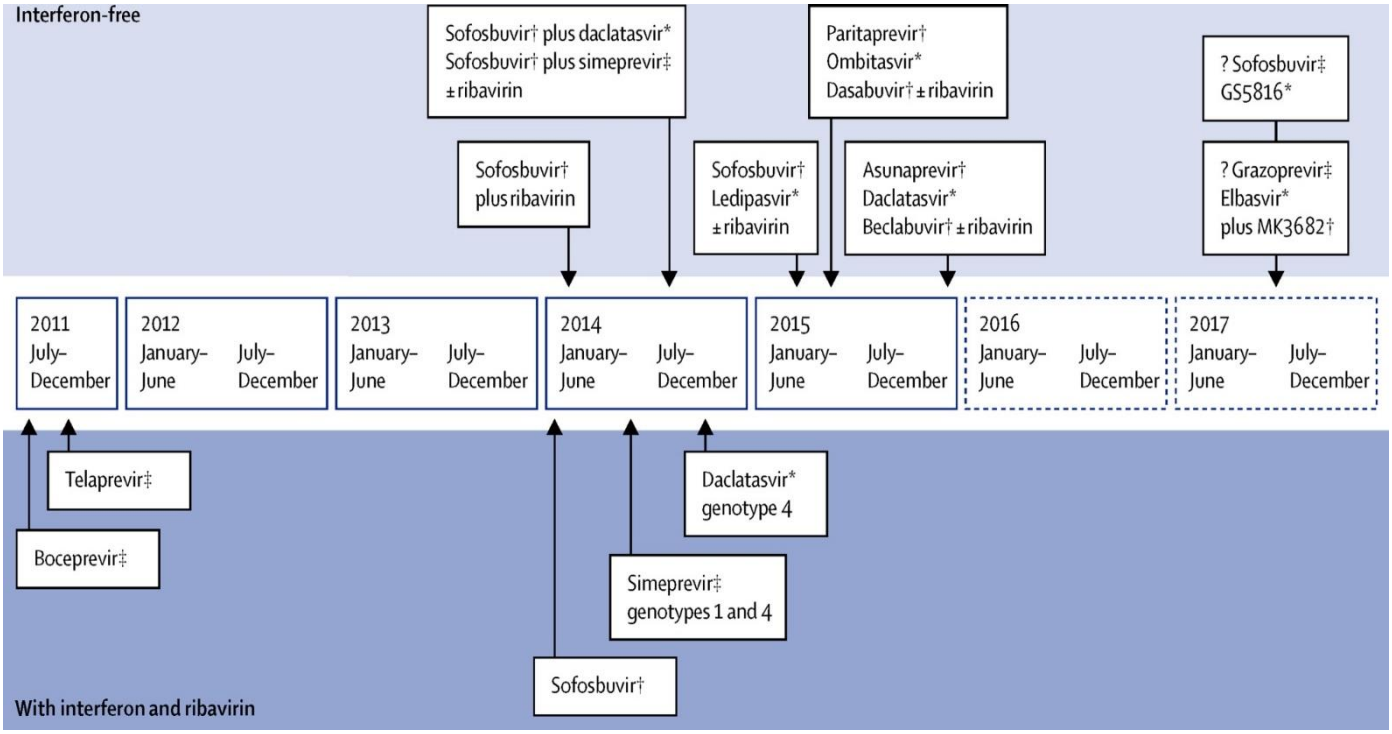

Figure 2: DAADs discovery milestone

Sofosbuvir (SFS) [16, 17]
* Molecular formula: $C_{22}H_{29}FN_3O_9P$.
* Molecular weight: 529.453 g/mol.
*IUPAC name: propan-2-yl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy-phenoxyphosphoryl]amino]propanoate (Figure 3).

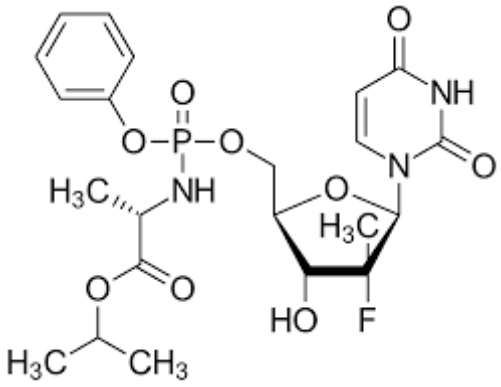

Figure 3: Sofosbuvir chemical structure

*Physical properties:
SFS is white to off-white crystalline powder which is slightly soluble in water and soluble in methanol. It has pKa values of 9.38 and 10.30.
*Mechanism of action:
SFS is a prodrug which is metabolized by body into its active form that act as defective nucleotide analog for HCV NS5B (non-structural protein 5B) RNA-dependent RNA polymerase. SFS prevents HCV viral replication by binding to HCV NS5B polymerase's active site and preventing further replication of HCV genetic material. After oral administration of the drug, SFS reaches its maximum plasma concentration in about 0.5 to 2 hours with a maximal concentration (C_{max}) of 567ng/mL.

*Literature review:
Reviewing literature revealed several methods for determination of SFS in pharmaceutical products and in plasma using liquid chromatography Table 1 summarizes LC methods for determination of SFS.
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Matrix	Column	Mobile phase	System	Ref. No
Plasma	C_{18}	0.1% formic acid: ACN (50:50)	UPLC-MS/MS	[18]
Plasma	C_{18}	0.1% formic acid: ACN (gradiant)	UPLC-MS/MS	[19]
Tablets	C_{18}	Water:MeOH (70:30)	HPLC-MS/MS	[20]
Plasma	C_{18}	0.5% formic acid: MeOH (30:70)	UPLC-MS/MS	[21]
Plasma	C_{18}	0.1% Phosphoric acid: ACN (68:32)	HPLC-UV 228nm	[22]
Tablets	C_{18}	Phosphate buffer pH 4.0: MeOH	HPLC-UV	[23]

		(50:50)	262nm	
Tablets	C_{18}	0.1% formic acid: ACN (60:40)	HPLC-UV 260nm	[24]
Tablets	C_{18}	MeOH: ACN (90:10)	HPLC-UV 260nm	[25]
Tablets	C_{18}	0.2% Phosphoric acid: ACN (gradiant)	UPLC-UV & MS/MS	[26]
Serum	C_{18}	0.05% Phosphoric acid: ACN (gradiant)	HPLC-UV	[27]

Table 1: Summary of reported LC methods for SFS determination

Other spectrophotometric methods were published [24, 25, 28]. The spectrophotometric methods were done at wavelength 260nm using methanol as organic solvent. The limits of quantitation for the reported spectrophotometric methods were higher than 2.5µg/mL and the determination ranges started from 5-100µg/mL .

Only one HPTLC and CE method for SFS determination was reported [24]. HPTLC method used range of 25-100µg/mL and had the lowest sensitivity that LOQ was 7µg/mL. The highest sensitivity was for CE method and LOQ was 0.3µg/mL

Simeprevir (SMP) [16, 17]
* Molecular formula: $C_{38}H_{47}N_5O_7S_2$.
* Molecular weight: 749.942 g/mol.
*IUPAC name: (1R,4R,6S,7Z,15R,17R)-N-(cyclopropanesulfonyl)-2-hydroxy-17-([7-methoxy-8-methyl-2-[4-(propan-2-yl)-1,3-thiazol-2-yl]quinolin-4-yl]oxy)-13-methyl-14-oxo-3,13-diazatricyclo[13.3.0.0] octadeca-2,7-diene-4-carboximidic acid (Figure 4).

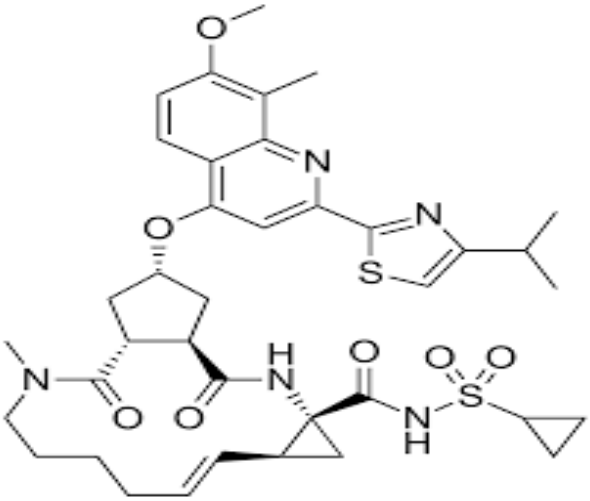

Figure 4: Simeprevir chemical structure

* Physical properties:
SMP is white to almost-white powder insoluble in water and slightly soluble in acetone and freely soluble in dichloromethane. It has pKa valueof 5.87. The drug is pharmaceutically available as water soluble sodium salt.
* Mechanism of action:
SMP inhibits HCV polyprotein cleavage via induced-fit binding to an NS3 catalytic site. After oral administration of the drug, SMP reaches its maximum plasma concentration in about 4 to 6 hours.
* Literature review:

Reviewing literature, A spectrophotometric method for determination of SMP in pharmaceutical dosage forms was published [29]. SMP was determined in presence of its oxidative degradation product at the range of 2.5-403µg/mL using different apectrophotometricmethods. Other LC determinations of SMP are summarized in Table 2.

Matrix	Column	Mobile phase	System	Ref. No.
Plasma	C_{18}	Phosphate buffer: ACN (30:70)	HPLC-UV	[30]
Tablets	C_{18}	ACN	HPLC-UV	[31]
Plasma	C_{18}	AmmoniumFormate: ACN: MeOH (gradiant)	HPLC-MS/MS	[32]

Table 2: Summary of reported LC methods for SMP determination

Ledipasvir (LDS) [16, 17]
* Molecular formula: $C_{49}H_{54}N_8O_6F_2$.
* Molecular weight: 889.0 g/mol.
*IUPAC name: methyl [(2S)-1-[(6S)-6-[4-(9,9-difluoro-7-[2-[(1R,3S,4S)-2-[(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl]-2-azabicyclo[2.2.1]hept-3-yl]-1H-benzimidazol-5-yl]-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-azaspiro[2.4]hept-5-yl]-3-methyl-1-oxobutan-2-yl]carbamate (Figure 5).

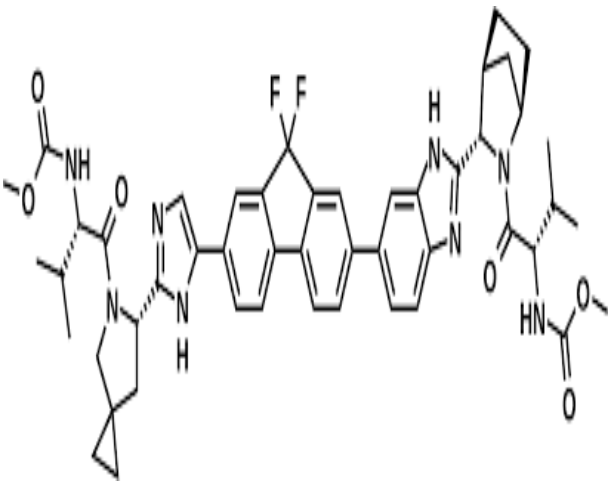

Figure 25: Ledipasvir chemical structure

* Physical properties:
LDS is white to beige powder. LDS is Insoluble in water, but soluble in acetonitrile and in methanol. It has pKa values of 4.0

and 5.0. The drug is pharmaceutically available as water soluble acetone solvate and Ledipasvircopovidone mixture.

*Mechanism of action:

LDS prevent hyperphosphorylation of NS5A polymerase which is required for viral protein production. It was found to be effective against HCV genotypes (1, 4, and 5) and with a lesser activity against genotypes (2 and 3). After oral administration of the drug, LDS reaches its maximum plasma concentration in about 4 to 4.5 hours with Cmaxof 323 ng/mL.

*Literature review:

Only two papers described the determination of LDS alone in tablet dosage forms [33, 34]. However; being only formulated in combination with sofosbuvir, several papers described TLC [35], spectrophotometric [36-40] and spectrofluorimetric [41, 42] besides chromatographic determinations of both drugs in several matrices. Table 3 summerizes some LC methods published for simultaneous determination of SFS and LDS.

Matrix	Column	Mobile phase	System	Ref.
Plasma	C_{18}	0.1% formic acid: ACN (gra	UPLC-M	[43]
Plasma	C_{18}	0.1% formic acid: ACN (50	UPLC-M	[44]
Plasma	C_8	Ammonium formatebuffer:ACN:MeOH (gradiant)	HPLC-M	[45]
Plasma	C_{18}	0.1% formic acid in MeOH:ACN: Acetic acid (63:25:12)	HPLC-M	[46]
Tablet	C_{18}	Ammonium acetate buffe (35:65)	HPLC-U 245nm	[47]
Tablets	C_{18}	Phosphate buffer : ACN (50	HPLC-U 254nm	[48]
Tablets	C_{18}	Trifluoroacetic in ACN:MeOH (30:50:20)	HPLC-U 267nm	[49]

Table 3: Summary of reported LC methods for LDS determination

Daclatasvir (DAC) [16, 17]

* Molecular formula: $C_{40}H_{50}N_8O_6$.

* Molecular weight: 738.89 g/mol.

* IUPAC name: Dimethyl N,N'-([1,1'-biphenyl]-4,4'-diylbis[1H-imidazole-5,2-diyl-[(2S)-pyrrolidine-2,1-diyl][(2S)-3-methyl-1-oxobutane-1,2-diyl]])dicarbamate (Figure 6).

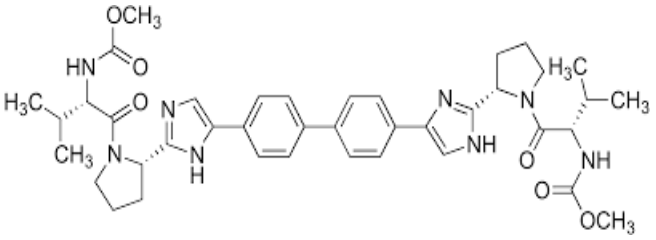

Figure 6: Daclatasvir chemical structure

*Physical properties:

DAC is white powder freely soluble in water. It has pKa values of 6.0 and 11.1. The drug is pharmaceutically available as dihydrochloride salt.

*Mechanism of action:

DAC inhibits HCV viral RNA replication and protein translation by directly inhibiting HCV protein NS5A polymerase. NS5A is critical for HCV viral transcription and translation, and as of 2014 it appeared that resistance can arise to daclatasvir fairly swiftly

After oral administration of the drug, DAC reaches its maximum plasma concentration in about 2 hours with bioavailability of 67%.

*Literature review:

A spectrophotometric and another spectrofluorimetric methods were published for determination of DAC [50, 51]. The spectrophotometric method used methanol as solvent for quantitation of DAC in range of 2-12µg/mL at wavelength 317nm. The spectrofluorimetric method was for simultaneous determination of DAC in presence of LDS.

Other LC methods for determination of DAC were reported either alone or in combination with sofosbuvir. Table 4 summarizes those reported chromatographic determinations.

Matrix	Column	Mobile phase	System	Ref. No.
Plasma	C_{18}	Ammonium formate bu (50:50)	UPLC-M	[52]
Tablets	C_8	Phosphate buffer: ACN (7	HPLC-U	[53]
powder	Amylose	ACN:MeOH diethylamine (gradiant)	HPLC-U	[54]
Tablets	C_{18}	Phosphate buffer: ACN (6	HPLC-U	[55]
Plasma	C_{18}	Ammonium acetate buf (56:44)	HPLC-U	[56]
Plasma	C_{18}	0.1% formic acid: ACN	UPLC-M	[57]
Plasma	C_{18}	Ammonium formate bu (50:50)	UPLC-M	[58]
Tablets	C_{18}	Phosphate buffer: ACN (	HPLC-U	[59]
Tablets	C_{18}	Phosphate buffer: ACN (	HPLC-U	[60]

Table 4: Summary of reported LC methods for DAC determination

Velpatasvir (VLP) [16, 17]

* Molecular formula: $C_{49}H_{54}N_8O_8$.

* Molecular weight: 883.019 g/mol.

*IUPAC name: Methyl [(2S)-1-[(2S,5S)-2-(9-[2-[(2S,4S)-1-[(2R)-2-[(methoxycarbonyl)amino]-2-phenylacetyl]-4-(methoxymethyl)-2-pyrrolidinyl]-1H-imidazol-4-yl]-1,11-

dihydroisochromeno[4',3':6,7] naphtho[1,2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl]carbamate (Figure 7).

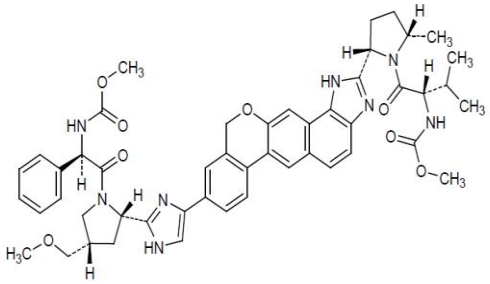

Figure 7: Velpatasvir chemical structure

*Physical properties:
VLP is off-white to yellow color powder insoluble in water and slightly soluble in methanol. It has pKa values of 3.8 and 6.0. The drug is pharmaceutically available as VLP copovidone mixture.
*Mechanism of action:
VLP is non-structural protein NS5A polymerase inhibitor. It inhibits HCV viral RNA replication and protein translation. After oral administration, VLP is 99.5% bound to plasma proteins with bioavailability of 25-30%.
*Literature review: Only two LC methods were reported for determination of VLP in tablet dosage forms as listed in Table 5.

Matrix	Column	Mobile phase	System	Ref. No.
Tablets	C_{18}	0.1% phosphoric acid in water:ACN (45:55)	HPLC-UV 240 nm	[61]
Tablets	C_{18}	0.1% phosphoric acid in water:ACN (45:55)	HPLC-UV 260 nm	[62]

Table 5: Summary of reported LC methods for VLP determination

Although some papers described validated methods for simultaneous determination of two DAADs (e.g. SFS/LDS, SFS/VLP, SFS/DAC), only two LC methods were developed for the quantification of some of the new DAADs using UHPLC-MS/MS [63, 64].

Conclusion:

This literature review is introducing brief summary about Hepatitis C disease, its treatment strategy, and instrumental analysis of recently used anti-HCV drugs specifically Sofosbuvir, Simeprevir, Ledipasvir, Daclatasvir and Velpatasvir in different matrices.

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