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REVIEW ARTICLE

Analytical Review: Methods of Determination for Ledipasvir and Velpatasvir in Pharmaceutical and Biological samples

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ABSTRACT

Infection due to hepatitis C virus (HCV) is a leading cause for severe chronic liver disease, which can result in progressive liver damage such as cirrhosis and hepatocellular carcinoma. Thus, it is considered to be a great worldwide health problem specifically in Egypt, which has the greatest prevalence of the epidemic problem of HCV in the world in accordance with the reported Egyptian Demographic Health Survey that had reached 14.7%. Hence, prevention of HCV becomes a national priority. Various techniques were used for the analysis of LDS in pure forms, in their pharmaceutical formulations and in biological fluids. This literature review represents an up-to-date survey about all reported methods that have been developed for the determination of LDS and VLP in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as liquid chromatography, spectrophotometry, spectrofluorimetry, and electrochemistry.

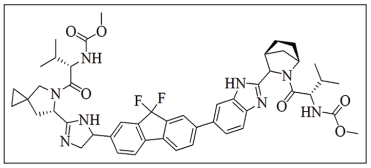
Keywords: Biological samples, Ledipasvir, Velpatasvir

INTRODUCTION

Infection due to hepatitis C virus (HCV) is a leading cause for severe chronic liver disease, which can result in progressive liver damage such as cirrhosis and hepatocellular carcinoma. Thus, it is considered to be a great worldwide health problem specifically in Egypt, which has the greatest prevalence of the epidemic problem of HCV in the world in accordance with the reported Egyptian Demographic Health Survey that had reached 14.7%. Hence, prevention of HCV becomes a national priority. The available treatment options for HCV infection until 2011 were restricted to ribavirin with pegylated interferon combination. This drug regimen has limited efficacy, especially in genotype 1 infected patients, and was also accompanied with dangerous side effects. In 2014, the directly acting antivirals (DAAs) were introduced in the market as a new anti-HCV generation. The main goal of these new drug therapies is decreasing the incidence of possible side effects for HCV patients. These powerful drugs encompass non-nucleoside inhibitors (NIs) and NIs of HCV RNA polymerase (non-structural protein 5A [NS5A]/5B) and protease inhibitors.

As such, in this literature review, we will introduce most of up-to-date reported methods that have been developed for the determination of two important anti-HCV drugs which are ledipasvir (LDS) and velpatasvir (VLP) in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples.

LDS



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Pharmacological action

LDS is DAAs drugs (DAAD) used as part of combination therapy to treat chronic hepatitis C, an infectious liver disease caused by infection with HCV. HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1 being the most common in the United States, and affecting 72% of all chronic HCV patients. Treatment options for chronic hepatitis C have advanced significantly since 2011, with the development of DAAs such as LDS. More specifically, LDS is an inhibitor of the HCV NS5A, which is required for viral RNA replication and assembly of HCV virions. Although its exact mechanism of action is unknown, it is postulated to prevent hyper-phosphorylation of NS5A which is required for viral protein production. It is effective against genotypes 1a, 1b, 4a, and 5a and with a lesser activity against genotypes 2a and 3a of HCV. LDS and other DAAs are very potent options for the treatment of hepatitis C, as they exhibit a high barrier to the development of resistance. This is an important advantage relative to HCV drugs that target other viral enzymes such as the protease, for which rapid development of resistance has proven to be an important cause of therapeutic failure.[1]

In a joint recommendation published in 2016, the American Association for the Study of Liver Diseases and the Infectious Diseases Society of America recommend LDS as a first-line therapy option in combination with spasm frequency scale (SFS) for the treatment of HCV genotypes 1a, 1b, 4, 5, and 6.[2] Treatment with LDS is used with the intent to cure, or achieve a sustained virologic response, after 12 weeks of daily therapy. A sustained virologic response and eradication of HCV infection is associated with significant long-term health benefits including reduced liver-related damage, improved quality of life, reduced incidence of hepatocellular carcinoma, and reduced all-cause mortality.[3] Treatment with DAAD such as LDS is associated with very minimal side effects, with the most common being headache and fatigue. Lack of significant side effects and short duration of therapy is a considerable advantage over older interferon- and ribavirin-based regimens, which were limited by infusion site reactions, reduced blood count, and neuropsychiatric effects.[4]

Since 2014, LDS has been available as a fixed dose combination product with SFS (trade name Harvoni) used for the treatment of chronic hepatitis C. Approved in October 2014 by the FDA, Harvoni is indicated for the treatment of HCV genotypes 1, 4, 5, and 6 with or without ribavirin depending on the level of liver damage or cirrhosis. When combined together, LDS and SFS as the combination product Harvoni have been shown to achieve a sustained virology response between 93 and 99% after 12 weeks of treatment. Its use has also proven successful in the treatment of HCV in patients coinfected with HIV.[5]

Review of analytical methods

Various techniques were used for the analysis of LDS in pure forms, in their pharmaceutical formulations and in biological fluids. The available reported methods in the literature can be summarized as follows:

Spectroscopic methods

Spectrophotometric methods

Drugs	Method or reagent	λ_{max}	Ref.
SFS and LDS	First derivative spectrophotometry	276 nm (SFS) and 324, 338 nm (LDS)	[6]
SFS and LDS	Zero-order spectrophotometric method	261 nm and 333.0 nm	[7]
SFS and LDS	First derivative spectrophotometric method	285 nm and 333.0 nm	[8]
SFS and LDS	Third derivative ratio	265 nm and 333.0 nm	[9]
SFS and LDS	First derivative spectrophotometric method	261 nm and 314.0 nm	[10]
SFS and LDS	Measurement of UV absorbance ratio spectra	325 nm (LDS) 262nm (SFS), 325 (LDS)	[11]

SFS: Spasm frequency scale, LDS: Ledipasvir, UV: Ultraviolet

Spectrofluorometric methods

Drugs	Fluorogenic reagent (method)	λ_{ex} (nm)	λ_{em} (nm)	Ref.
DAC and LDS	Native fluorescence	315 (DAC) 332 (LDS)	381 (DAC) 387 (LDS)	[12]
DAC and LDS	Native fluorescence in methanol	318 (DAC) 340 (LDS)	384 (DAC) 402 (LDS)	[13]
LDS	Native fluorescence in ethanol	340 nm	405 nm	[14]
LDS	Polyoxyethylene 50 stearate	340 nm	430 nm	[15]

LDS: Ledipasvir, DAC: Daclatasvir

Chromatographic methods

High-performance liquid chromatography (HPLC)

Matrix	Column	Mobile phase	Detector	Ref.
Tablets	Luna C_{18}	Ammonium acetate buffer solution (pH 7.0) and acetonitrile 35:65% v/v	HPLC–UV 245 nm	[16]
Plasma	Zorbax eclipse plus C_{18}	10 mM ammonium acetate pH 4.0–acetonitrile–0.1% methanolic formic acid (12/25/63, v/v/v)	HPLC–MS/MS	[17]
Tablet	Eclipse XDB C_{18}	Buffer solution of pH 3.0 containing 0.02 M potassium dihydrogen phosphate and 5.7 mM hexane sulfonate: acetonitrile (50:50 v/v)	HPLC-UV 254 nm	[18]
Plasma	UPLC BEH C_{18}	Acetonitrile and 0.1% formic acid in water (gradient elution)	UPLC–MS/MS	[19]
Plasma	UPLC HSS C_{18}	0.1% formic acid and acetonitrile (50:50, v/v)	UPLC–MS/MS	[20]
Plasma	UPLC BEH C_{18}	0.1% formic acid and acetonitrile (50:50, v/v)	UPLC–MS/MS	[21]
Plasma	UPLC BEH C_{18}	Ammonium acetate 5 mM (pH 9.5) and acetonitrile (gradient elution)	UHPLC–MS/MS	[22]
Tablet	C_{18}	0.05% trifluoroacetic acid in methanol and 0.05% trifluoroacetic acid in acetonitrile (55:45)	HPLC-UV 270nm	[23]
Tablet	Hypersil C_{18}	Acetonitrile: 0.1% tetrafluoroacetic acid (32:68 v/v)	HPLC-UV 260nm	[24]
Tablet	Intersil C_{18}	Tetrafluoroacetic acid buffer (pH 2.0), acetonitrile, and methanol (30:50:20% v/v/v)	HPLC-UV 267nm	[25]
Plasma	Xterra MS C_8 column	10 mM ammonium formate buffer (pH 3.5) acetonitrile and methanol (gradient elution)	LC–MS/MS	[26]
Plasma	Zorbax C_{18}	Acetonitrile: water (80:20 v/v)	HPLC-UV 260nm, 330nm	[27]
Tablet	Thermohypersil C_8	0.01 M sodium dihydrogen phosphate (pH 2.5):methanol (20:80 v/v)	HPLC-UV 262nm, 332nm	[6]
Plasma	UPLC BEH C_{18}	Acetonitrile and 0.1% formic acid in water (gradient elution)	UPLC–MS/MS	[28]
Tablet	Zodiac C_{18}	Acetonitrile: methanol: water (60: 20: 20)	HPLC-UV 270 nm	[29]
Tablet	Luna C_{18}	Acetonitrile: triethylamine buffer (pH-2.5) (50:50)	HPLC-UV 227 nm	[30]
Tablet	YMC C_{18} analytical column	Disodium hydrogen phosphate solution (0.1 M) pH 6.5 and acetonitrile (60:40 v/v)	HPLC diode array detector 282 nm	[31]
Tablet	C_{18} column	Phosphate buffer (pH-4):methanol: triethylamine (pH adjust to 4 by adding 1% o-phosphoric acid) in a volume (40:60:0.1% v/v/v)	HPLC-UV 240 nm	[32]
Tablet	C_{18} column	A gradient elution with 50 mM ammonium formate pH 3.5: CAN	HPLC-UV 325 nm	[33]
Tablet	C_{18} column	CAN: 0.1% orthophosphoric acid (35:65 v/v)	HPLC-UV 272 nm	[34]
Tablet	C_{18} column	CAN: methanol: 0.01% TEA (pH 3) (35: 35:30) (v/v/v).	HPLC-UV 261nm, 333 nm fluorescence detector 404 nm	[35]
Tablet	Discovery C_{18}	0.1% orthophosphoric acid and acetonitrile 45:55 (% v/v)	HPLC-UV 270 nm	[36]
Tablet	Intersil C_{18} column	Methanol and 0.01 M ammonium acetate buffer (70:30 v/v)	HPLC-DAD 254 nm	[37]
Tablet	Zorbax Eclipse C_{18} Column	Gradient elution with buffer solution (0.1% triethylamine solution with pH 3.0):CAN.	HPLC-UV 260 nm	[38]
Tablet	C_{18} column	0.1% orthophosphoric acid buffer: CAN (60:40 v/v)	UPLC-UV 220 nm	[39]
Plasma	UPLC C_{18} column	A gradient elution with 10 mM AF with 0.005% formic acid in water at pH 4.5:10 mM AF with 0.005% FA in methanol: 10 mM AF with 0.005% FA in a mixture of 10 mM AF with 0.005% FA in a mixture of methanol and CAN (10:90)	HPLC-MS/MS	[40]

HPLC: High-performance liquid chromatography, UV: Ultraviolet, UPLC: Ultra-performance liquid chromatography

High-performance thin-layer chromatography (HPTLC)

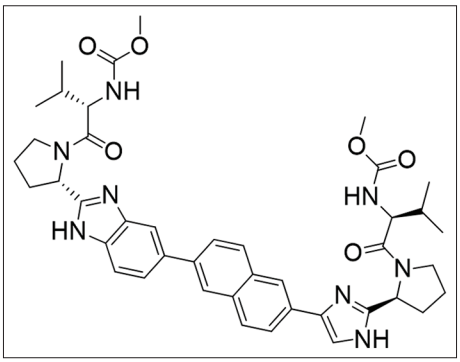
Matrix	Stationary phase	Mobile phase	Detector	Ref
Tablet	Silica gel 60 F_{254} plate	Methylene chloride/methanol/ethyl acetate/ammonia (25%) (6:1:4:1, v/v/v/v)	UV – 275 nm	[41]
Plasma	Silica gel 60 F_{254} plate	Ethyl acetate-glacial acetic acid (100:5 v/v)	UV – 265 nm, 327 nm	[42]

HPLC: High-performance liquid chromatography, UV: Ultraviolet, UPLC: Ultra-performance liquid chromatography

Other methods

Chemometry,[43-45] electrochemical,[46] and capillary electrophoresis.[47]

VLP



Pharmacological action

VLP is DAAD used as a part of combination therapy to treat chronic HCV, an infectious liver disease caused by infection with HCV. VLP acts as a defective substrate for NS5A, a non-enzymatic viral protein that plays a key role in HCV replication, assembly, and modulation of host immune responses.[48] Treatment options for chronic HCV have advanced significantly since 2011, with the development of DAAD such as VLP. Notably, VLP has a significantly higher barrier to resistance than the first-generation NS5A inhibitors, such as LDS and Daclatasvir, making it a highly potent and reliable alternative for the treatment of chronic HCV.[49]

In a joint recommendation published in 2016, the American Association for the Study of Liver Diseases and the Infectious Diseases Society of America recommend VLP as first-line therapy in combination with SFS for all six genotypes of hepatitis C.[50] VLP is currently only available within a fixed-dose combination product as Epclusa with SFS, another DAAD. Goals of therapy for Epclusa include the intent to cure or achieve a sustained virologic response, after 12 weeks of daily therapy. Sustained virologic response and eradication of HCV infection is associated with significant long-term health benefits including reduced liver-related damage, improved quality of life, reduced incidence of hepatocellular carcinoma, and reduced all-cause mortality and risk of requiring a liver transplant.[3]

Review of analytical methods

Various techniques were used for the analysis of VLP in pure forms, in their pharmaceutical formulations, and in biological fluids. The available reported methods in the literature can be summarized as follows:

Spectroscopic methods

Spectrophotometric methods

Drugs	Method or reagent	λ_{max}	Ref.
SFS and VLP	Zero-order spectrophotometry	251 nm, 339 nm	[51]
SFS and VLP	Zero-order spectrophotometry dual wavelength, ratio subtraction, ratio difference, and first derivative of ratio spectra method	302.5 and 337.0 nm	[52]

VLP: Velpatasvir, SFS: Spasm frequency scale

Spectrofluorometric methods

Drug	Fluorogenic reagent (method)	λ_{ex} (nm)	λ_{em} (nm)	Ref.
VLP	Native fluorescence of in methanol	385 nm	400 nm	[53]
VLP	Native fluorescence of in methanol	339 nm	383 nm	[54]

VLP: Velpatasvir

Chromatographic methods

HPLC

Matrix	Column	Mobile phase	Detector	Ref.
Tablet	Discovery C_{18}	Orthophosphoric acid in water and CAN (60:40 v/v)	HPLC-UV 240 nm	[55]
Tablet	Hypersil C_{18}	CAN: water (50:50% v/v)	HPLC-UV 235 nm	[56]
Tablet	Primesil C_{18}	Acetonitrile: 0.1%perchloricacid (50:50 v/v)	HPLC-UV 262 nm	[57]
Tablet	Inertsil ODS C_{18}	0.05M potassium dihydrogen phosphate buffer (pH – 3.5): CAN (60:40 v/v)	HPLC DAD 270 nm	[58]
Tablet	Inertsil C_{18}	Methanol: buffer (60: 40 V/V)	HPLC-UV 254 nm	[59]
Tablet	Water's C_{18}	Sodium dihydrogen orthophosphate (pH 4.2): CAN (85:15 V/V)	HPLC DAD 292 nm	[60]

Tablet	Purospher C_{18} column	CAN: phosphate buffer (pH 3.0):methanol (60:30:10 v/v/v)	HPLC DAD 262 nm	[61]
Tablet	Acquity UPLC C_{18}	Orthophosphoric acid buffer: CAN (45:55 v/v)	UPLC-UV 250 nm	[62]
Plasma	C_{18} Zorbax Eclipse Plus	0.1% formic acid in water: acetonitrile: methanol (30:60:10, v/v/v)	LC–MS/MS	[63]
Plasma	UPLC C_{18} column	A gradient elution with 10 mM AF with 0.005% formic acid in water at pH 4.5:10 mM AF with 0.005% FA in methanol: 10 mM AF with 0.005% FA in a mixture of 10 mM AF with 0.005% FA in a mixture of methanol and CAN (10:90)	HPLC-MS/MS	[40]
Plasma	Acquity UPLC C_{18}	CAN: 0.1% FA (50: 50, v/v)	LC MS/MS	[64]
Tablet	a Symmetry C_{18} column	Methanol: TEA pH 4.2 (40:60 v/v)	HPLC-UV 272 nm	[65]
Tablet	Kromasil C_{18} column	Buffer: CAN (45:55 v/v)	HPLC-UV 260 nm	[66]
Tablet	Discovery C_{18}	0.1% orthophosphoric acid: CAN (60:40%v/v)	HPLC-UV 220 nm	[67]
Tablet	Waters C_{18}	Buffer: CAN (50:50 v/v)	HPLC-UV 220 nm	[68]

HPLC: High-performance liquid chromatography, UV: Ultraviolet

HPTLC

Matrix	Stationary phase	Mobile phase	Detector	Ref
Tablet	Silica gel 60 F_{254} plate	Ethyl acetate-isopropanol (90:10, v/v).	UV – 260 and 302 nm	[69]
Tablet	Silica gel 60 F_{254} plate	Methylene chloride–methanol–ethyl acetate–ammonia (25%) at a ratio of 5:1:3:1 (v/v).	UV – 275 nm	[70]

UV: Ultraviolet

Other methods

Electrophoresis.[51]

CONCLUSION

This literature review represents an up-to-date survey about all reported methods that have been developed for the determination of LDS and VLP in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as liquid chromatography, spectrophotometry, spectrofluorimetry, and electrochemistry.

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