IJBPAS, November, 2023, 12(11): 5220-5230

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND LEDIPASVIR: A COMPREHENSIVE REVIEW

BADELIYA SN[*1], CHAUHAN NF[2] AND DAVE SP[3]

[1,2]Department of Pharmaceutical Chemistry, [3]Department of Pharmacognosy,

[1,2,3]Saraswati Institute of Pharmaceutical Sciences, Dhanap, Di. Gandhinagar-382355, Gujarat, India

[*]**Corresponding Author: Dr. Sandip N Badeliya: E Mail: snb.success@gmail.com**

Received 19th Oct. 2022; Revised 16th Nov. 2022; Accepted 23rd March 2023; Available online 1st Nov. 2023

https://doi.org/10.31032/IJBPAS/2023/12.11.7585

ABSTRACT

Sofosbuvir is a newer antiviral drug candidate that well inhibits nucleotide polymerase virus. It can be given in the treatment of hepatitis C virus but it cannot prevent spread of the same to other people. Several ideal properties like limited daily dosing, less side effects, fewer drug-drug interactions, minimal drug resistance make this drug safer for the use. Ledipasvir is also a newer drug that was developed by Gilead Sciences and can be used in the treatment of Hepatitis C virus. This drug is an active inhibitor of hepatitis virus C protein NS5A. Common side effects of both these drugs include fatigue and headache. When combination of Sofosbuvir and Ledipasvir taken in a fixed dose, hepatitis C virus of genotypes 1, 4, 5, and 6 can be well prevented. Sofosbuvir-Ledipasvir therapy has been found to be safe and effective in the treatment of patient affected with hepatitis C virus. In this review, we have included several HPLC and RP-HPLC methods on Sofosbuvir and Ledipasvir either individually or in combination with another drug agent.

Key words: Hepatitis, Sofosbuvir, Ledipasvir, HPLC, RP-HPLC, UPLC

INTRODUCTION

Hepatitis, a disease of liver inflammation, can be well characterized by swelling upon tissue injury which occurs due to hepatitis virus. This infection can be short term (acute) or long term (chronic). Hepatitis virus can be classified in to five different classes. i) Hepatitis A virus (HAV), ii) Hepatitis B virus (HBV), iii) Hepatitis C virus (HCV), iv) Hepatitis D virus (HDV), and v) Hepatitis E virus (HEV).

Hepatitis A is the acute type of the liver inflammation which spread by the Hepatitis A virus. This is viral liver disease that can cause mild to severe illness. The Hepatitis A virus (HAV) can be transmitted either through ingestion of contaminated food and water or through direct contact of an infected person. Now-a-days, a safe and effective vaccine is available that can prevent Hepatitis A virus. Several effective ways like safe water supply, improved food safety, improved sanitation, hand washing and the vaccine of Hepatitis A virus (HAV) can well prevent this disease. **Hepatitis B** virus can be spread by Hepatitis B virus. It can cause acute and chronic both type of disease. The virus can be transmitted either through contact with the blood or some other body fluids of an infected person. Safer and effective vaccine is currently available that can prevent HBV.

Hepatitis C virus is major type of Hepatitis. It is a liver disease that occurs by the Hepatitis C virus (HCV). This virus can cause both type of Hepatitis infection, acute as well as chronic. In this infection, the patient may be having mild illness that lasts a few weeks or a serious lifelong illness. This virus is a blood borne virus that occurs through use of unsafe injection, lacking of medical equipment sterilization, and the unscreened blood and blood products transfusion. Liver cirrhosis or liver cancer can also be developed in chronically infected patients. Usually, acute HCV infection is asymptomatic and associated with life-threatening disease in a very rare case. Among the Hepatitis C virus infected patients, about 15-45% persons can clear the virus within 5 to 6 months without any kind of treatment. The remaining 55-85% of persons will develop chronic HCV infection. The patients with chronic HCV infection, the risk of cirrhosis of the liver is in between 15-30% within 20 years. It is a blood borne virus and most common reasons for transmission of this disease are injecting drug through the sharing of unsafe injection equipment, inadequate sterilization of

medical equipment especially syringes and needles in healthcare settings, transfusion of unscreened blood and blood products. It can also be transmitted sexually and can be passed from an infected mother to her baby; however these modes of transmission are less common.

Hepatitis D virus (HDV) is a ribonucleic acid (RNA) virus and for replication, it requires Hepatitis B virus (HBV). This infection occurs either simultaneously or as a super-infection with HBV. This virus can be transmitted either by contact with the blood or other body fluids of an HDV infected person. Currently there is no any safe and effective antiviral drug medication available for Hepatitis D but it can be prevented by Hepatitis B immunization. **Hepatitis E** is type of Hepatitis virus which is caused by Hepatitis E virus. Hepatitis E is usually self-limiting but sometime it may results in liver failure. It occurs by use of unsafe water and its transmission occurs via fecal-oral route, principally via contaminated water. Hepatitis E can be found worldwide, but its spreading is comparatively more in East and South Asia. A vaccine to prevent Hepatitis E virus infection has been developed and is licensed in China, but is not yet available elsewhere **[1-3]**.

Symptoms

Generally, incubation period for Hepatitis C virus is 2 weeks to 6 months. After initial infection, about 80% of the persons do not show any kind of symptoms. Fever, fatigue, decreased appetite, nausea, vomiting, abdominal pain, dark urine, grey-coloured faeces, joint pain and jaundice can be seen in the acutely infected persons. Severe symptoms include liver failure **[4, 5]**.

Causes

Causes for hepatitis are either may be infectious, metabolic, ischemic, genetic, autoimmune, or other. Several infectious agents like bacteria, viruses, and parasites are responsible for hepatitis. Prescription medications, toxins, and non-alcoholic fatty liver disease are metabolic causes. Autoimmune and genetic causes include genetic predispositions.

Treatment

Hepatitis C can be well treated by direct acting antiviral medicines (DAA) which are very safe and most effective medications. DAA medicines should be taken for 8 to 12 weeks. NHS has approved sofosbuvir, or combination of sofosbuvir and ledipasvir, or combination of ombitasvir, paritaprevir and ritonavir with or without dasabuvir, or combination of elbasvir and grazoprevir, or combination of sofosbuvir and velpatasvir, or combination of sofosbuvir, velpatasvir

and voxilaprevir, or combination of glecaprevir and pibrentasvir, or ribavirin for the treatment of hepatitis C. These DAA medications have fewer side effects like little sickness and trouble in sleeping. In very rare case, side effects like skin irritation, insomnia, anorexia, tiredness, depression, anxiety, hair loss, and aggressive behaviour can be seen in the patients.

The purpose of the study

In the present review, we have enumerated some reported analytical methods for the estimation of Sofosbuvir and Ledipasvir, as well as simultaneous estimation of Sofosbuvir and Ledipasvir in combined dosage form. Physicochemical characteristics and reported analytical techniques of newly invented drugs for the treatment of hepatitis virus infection will be surely beneficial for the further research. Researcher can take benefits and advantages from this review for the further development of analytical methods for simultaneous estimation of this drug combination as well as combination with some other drug candidates.

Physicochemical properties of Sofosbuvir and Ledipasvir [6]

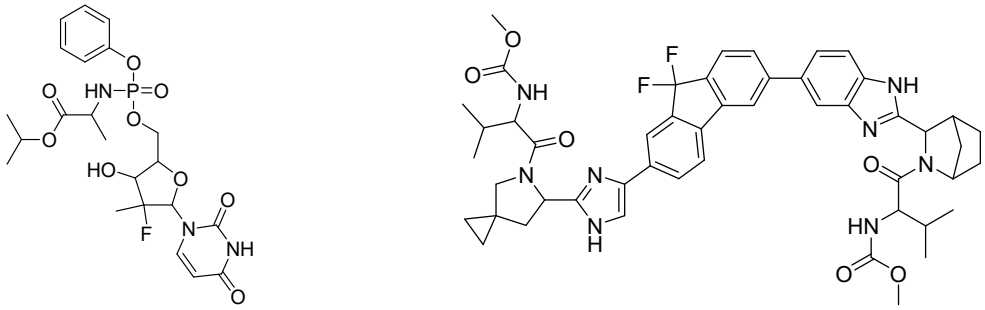
1. Sofosbuvir

Sofosbuvir is white to off-white crystalline solid powder. Chemically, it is Propan-2-yl-(2S)-2-{[(S)-{[(3R,4R,5R)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy(phenoxy)phosphoryl]amino}propanoate. This drug has molecular formula $C_{22}H_{29}FN_3O_9P$ and molecular weight 529.45 gm/mol. It is slightly soluble in water and freely soluble in acetone and ethanol. Melting point, log P, and pKa values of this drug are 93.9-104.7 °C, 1.62, and 9.3 respectively. It is basic compound and should be stored at the temperature below 30 °C. Biological half life of this drug is 0.4 hours. Approximately 61-65% portion of this drug can be bound to plasma proteins.

2. Ledipasvir

Ledipasvir is solid white to tinted powder. Chemically, it is (2S)-1-[(6S)-6-[5-(9,9-Difluoro-7-{2-[(1R,3S,4S)-2-[(2S)-2-{[hydroxy(methoxy)methylidene]amino}-3-methylbutanoyl]-2-azabi cyclo[2.2.1]heptan-3-yl]-1H-1,3-benzodiazol-6-yl}-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-aza spiro[2.4]heptan-5-yl]-2-{[hydroxy(methoxy)methylidene]amino}-3-methylbutan-1-one. This drug has molecular formula $C_{49}H_{54}F_2N_8O_6$ and molecular weight 888.99 gm/mol. It is practically insoluble in water and freely soluble in ethanol. Melting point, log P, pKa_1, and pKa_2 values of this drug are 204-220 °C, 3.8, 4.0, and 5.0 respectively. It should be stored at 25 °C with

60 %RH and protected from light. Biological half life of this drug is 47 hours. More than 99.8% portion of this drug can be bound to plasma proteins.



(A) **(B)**

Figure 1: (A) Structure of Sofosbuvir (B) Structure of Ledipasvir

Official and reported methods for the estimation of Sofosbuvir and Ledipasvir

Official method for the estimation of Sofosbuvir

No any official analytical method available for the estimation of Sofosbuvir in Pharmacopoeia.

Reported methods for the estimation of Sofosbuvir

Table 1: Reported methods for estimation of Sofosbuvir

Sr. No.	Method	Description	Ref. No.
1	Guguloth RA, *et al*. have reported analytical method development and validation of Sofosbuvir tablets by RP-HPLC	Column: Agilent C18 (4.5×100 mm, 3.0 µm) Mobile phases: 60:40 of Methanol: Water Flow rate: 1.0 mL/min Wavelength: 235 nm Retention times : 2.351 min	7
2	Abdel-Gawad SAN have reported simple chromatographic and spectrophotometric determination of Sofosbuvir in pure and tablet forms	Column: Hypersil TM ODS C18 column (150×4.6 mm, 5 µm) Mobile phase: Methanol: Acetonitrile (90:10, %v/v) Flow rate :1 mL/min Wavelength: 260 nm Retention time: 1.99 min	8
3	Vikas PM, *et al*. have reported development and validation of new RP-HPLC method for the determination of Sofosbuvir in pure form	Column: Hisil C18 (4.6 x 250mm, 5 µm) Mobile phase: Phosphate buffer (4.0 pH): Methanol (50:50 %v/v) Flow rate : 0.8 mL/min Wavelength: 262 nm Retention time : 1.01 min	9
4	Nebsen M, *et al*. have reported stability-indicating method and LC–MS-MS characterization of forced degradation products of Sofosbuvir	Column: Inertsil ODS-3 C18 (250 mm × 4.6 mm i.d., 5 µm) Mobile phase: Methanol: Water (70:30 %v/v) Flow rate: 1.3 ml/min Wavelength: 254 nm Retention time: 4.5 min	10
5	Vejendla1 R, *et al*. have reported estimation and validation of Sofosbuvir in bulk and tablet dosage form by RP-HPLC	Column: Phenomenex prodigy ODS-3V (150 mm x 4.6 mm, 5 µm) Mobile phase: Methanol and 0.1% Tri-fluro acetic acid as buffer having pH of 3.2 in the ratio of (30:70 %v/v) Flow rate: 1.0 mL/min Wavelength: 260 nm Retention time: 2.990 min	11
6	Rezk MR, *et al*. have reported development of a sensitive UPLC-ESI-MS/MS method for quantification of Sofosbuvir and its metabolite, GS-331007, in human plasma: application to a bioequivalence study	Column: UPLC HSS C18 (50 mm×2.1 mm, 1.8 µm) Mobile phase: 0.1% Formic acid: Acetonitrile (50:50 %v/v) Flow rate: 0.3 mL/min Retention time: 0.65 min	12

Official method for estimation of Ledipasvir

No any official analytical method available for the estimation of Ledipasvir in Pharmacopoeia.

Reported methods for the estimation of Ledipasvir

Table 2: Reported methods for estimation of Ledipasvir

Sr. No.	Method	Description	Ref. No.
1	Devilal J, *et al*. have reported new method development and validation for the determination of Ledipasvir in bulk drug form by using RP-HPLC technique	Column: BDS column (250 mm, 4.6 mm, 5 µm) Mobile phase: 0.05% Triflouroacetic acid in methanol + 0.05% Triflouroacetic acid in acetonitrile (55:45 %v/v) pH(3.0). Flow Rate: 1 mL/min Wavelength: 270 nm Retention time: 2.75 min	13
2	Zhang K, *et al*. have reported UPLC-MS/MS method for the quantitation of Ledipasvir in rat plasma: application to a pharmacokinetic study	Column: UPLC BEH C18 Column (2.1 mm × 50 mm, 1.7 µm) Mobile phase: Acetonitrile and 0.1% Formic acid in water Flow rate: 0.40 mL/min Retention time: 3 min	14
3	Suryawanshi R, *et al*. have reported development and validation of simple UV spectrophotometric method for the determination of Ledipasvir in bulk form and stress degradation studies	Solvent: Methanol λ_{max}: 333 nm	15

Reported analytical methods for the simultaneous estimation of Sofosbuvir and Ledipasvir in combined dosage forms

Table 3: Reported methods for estimation of Sofosbuvir and Ledipasvir

Sr. No.	Method	Description	Ref. No.
1	Rezka MR, *et al*. have reported quantification of Sofosbuvir and Ledipasvir in human plasma by UPLC–MS/MS method: application to fasting and fed bioequivalence studies	Column: BEH C18 column Mobile phase: 0.1% Formic acid and Acetonitrile (50:50 %v/v) Flow rate: 0.4 mL/min Retention time: 0.50 min	16
2	Chenwei P, *et al*. have reported simultaneous determination of Ledipasvir, Sofosbuvir and its metabolite in rat plasma by UPLC–MS/MS and its application to a pharmacokinetic study	Column: BEH C18 chromatography column (2.1 mm × 50 mm, 1.7 µm) Mobile phase: Acetonitrile and 0.1% Formic acid in water Flow rate: 0.4 mL/min	17
3	Elkady EF, *et al*. have reported a rapid and optimized LC-MS/MS method for the simultaneous extraction and determination of Sofosbuvir and Ledipasvir in human plasma	Column: C18 Zorbax eclipse plus column (5 µm, 100 × 4.6 mm; Agilent) Mobile phase: 10 mM Ammonium acetate (pH 4.0 by acetic acid): Acetonitrile: 0.1% Methanolic formic acid (12:25:63 %v/v/v) Flow rate: 0.6 mL/min Retention time: 2.8 min	18
4	Ariaudoa A, *et al*. have reported a UHPLC–MS/MS method for the quantification of direct antiviral agents Simeprevir, Daclatasvir, Ledipasvir, Sofosbuvir/GS-331007, Dasabuvir, Ombitasvir and Paritaprevir together with Ritonavir in human plasma	Column: BEH C18 1.7 µm, 2.1 mm × 50 mm column Mobile phase: ammonium acetate 5 mM (pH 9.5) and acetonitrile Flow rate: 0.4 mL/min	19
5	Zaman B, *et al*. have reported RP-HPLC method for simultaneous determination of Sofosbuvir and Ledipasvir in tablet dosage form and its application to in vitro dissolution studies	Column: Luna analytical column C_8 (250×4.6 mm), 5 µm, octyl silica packing (Si-$[CH_2]_7$-CH_3) Mobile phase: Ammonium acetate buffer solution pH 7.0: Acetonitrile (35:65 %v/v) Flow rate: 0.7 mL/min Wavelength: 240 nm Retention time: 4.468±0.013 min (Sofosbuvir), 8.242±0.012 min	20

6	**Pavankumar V, *et al*. have reported RP-HPLC method development and validation for the simultaneous estimation of Ledipasvir and Sofosbuvir in fixed dosage form**	**Column: Kromasil C18 (250×4.6 mm, 5 µm) Mobile phase: Phosphate buffer pH 3.5: Methanol (45:55 %v/v) Flow rate: 1 mL/min Wavelength: 259 nm Retention time: 3.294 min (Sofosbuvir), 4.630 min (Ledipasvir)**	**21**
7	**Bandla J, *et al*. have reported development and validation of a stability-indicating method for the simultaneous estimation of Sofosbuvir and Ledipasvir by RP-HPLC**	**Column: Discovery C18 (250×4.6 mm, 5 µm) Mobile phase: 0.1 % ortho phosphoric acid and acetonitrile (45:55 % v/v) Flow rate: 1.0 mL/min Wavelength: 270 nm Retention time: 2.08 min (Sofosbuvir), 3.06 min (Ledipasvir)**	**22**
8	**Mastanamma SK, *et al*. have reported development and validation of stability indicating RP-HPLC method for the simultaneous estimation of Sofosbuvir and Ledipasvir in bulk and their combined dosage form**	**Column: Luna C18 (250 mm × 4.6 mm, 5 µm) Mobile phase: Acetonitrile: Triethylamine Buffer (pH-2.5) (50:50 %v/v) Flow rate: 1.0 mL/min Wavelength: 227 nm Retention time: 4.905 min (Sofosbuvir), 2.751 min (Ledipasvir)**	**23**
9	**Rote AP, *et al*. have reported development and validation of RP-HPLC method for the simultaneous estimation of Ledipasvir and Sofosbuvir in bulk and pharmaceutical dosage form**	**Column: C18 column (250 mm × 4.6 mm, 5 µm) Mobile phase: Methanol: Water 83:17 %v/v pH 3.0 with 0.05% acetic acid Flow rate: 1.0 mL/min Wavelength: 245 nm Retention time: 7.45 min (Sofosbuvir), 3.50 min (Ledipasvir)**	**24**
10	**Farid NF, *et al*. have reported chromatographic analysis of ledipasvir and sofosbuvir: New treatment for chronic hepatitis C infection with application to human plasma**	**Column: Zorbax® Eclipse C18 Mobile phase: Acetonitrile: Water Flow rate: 1.0 mL/min Wavelength: 260 nm (Sofosbuvir), 330 nm (Ledipasvir)**	**25**
11	**Hemdan A, *et al*. have reported simultaneous chromatographic analysis of Sofosbuvir/Ledipasvir in their combined dosage form: an application to green analytical chemistry**	**Column: Inertsil C18 column (150 × 4.6 mm, 5 µm) Mobile phase: 20 mM Potassium dihydrogen orthophosphate (adjusted to pH = 3 using acetic acid): Ethanol (60:40 %v/v) Flow rate: 1 mL/min Wavelength: 265 nm**	**26**
12	**Prasad R, *et al*. have reported HPLC method for simultaneous estimation of drug release of Ledipasvir and Sofosbuvir in Ledipasvir and Sofosbuvir tablets**	**Column: Zorbax Eclipse Plus C18 (100 × 4.6 mm, 3.5 µm) Mobile phase: Buffer pH 3.0 and Acetonitrile Flow rate: 1 mL/min Wavelength: 260 nm**	**27**

CONCLUSIONS AND FUTURE PROSPECTIVES

Ledipasvir and Sofosbuvir are newely invented safer and effective drug candidates for the treatment of Hepatitis virus infection. Till date, official analytical method for the estimation of Sofosbuvir and Ledipasvir is not available in the Pharmacopoeia. In this present review, we have enumerated various analytical methods reported for the estimation of Sofosbuvir and Ledipasvir as well as simultaneous estimation of Sofosbuvir and Ledipasvir drug combination in various dosage forms. This review will help the researchers for further analytical studies in method development and validation purpose.

Abbreviations

HAV= Hepatitis A Virus

HBV= Hepatitis B Virus

HCV= Hepatitis C Virus

HDV= Hepatitis D Virus

HEV= Hepatitis E Virus

RH= Relative Humidity

Ref= Reference

DAA= Direct Acting Antiviral drugs

NHS= National Health Service

Human and animal rights

No animals/humans were employed in the studies interpreted in this review.

Conflict of Interest

The authors asserted no conflict of interest, financial or otherwise.

REFERENCES

[1] Theo V, Christine A, Arora M, Barber RM, Bhutta ZA, Brown A, *et al*. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet 2015;388(10053):1545–1602.

[2] Basra S. Definition, epidemiology and magnitude of alcoholic hepatitis. World J Hepatol 2011;3(5):108–13.

[3] Haidong W, Mohsen N, Christine A, Barber RM, Bhutta ZA, Carter A, *et al*. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet 2016;388(10053):1459–1544.

[4] Dienstag, JL. Acute Viral Hepatitis. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J, editors. Harrison's Principles of Internal Medicine. 19th ed. New York: NY: McGraw-Hill; 2015. ISBN 978-0-07-180215-4.

[5] Afdhal N, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, *et al*. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med 2014;370(20):1889–98.

[6] Sathiya CH, Badeliya SN. Analytical method development and validation for simultaneous estimation of Sofosbuvir and Ledipasvir in tablet dosage form. World J Pharm Pharm Sci 2018;7(7):897-914.

[7] Guguloth RA, Madhukar AB, Kannappan NC, Ravinder AD, Sravanthi KB. Analytical method development and validation of Sofosbuvir tablets by RP-HPLC. J Pharm Res 2016;5(7):161-3.

[8] Abdel-Gawad SAN. Simple chromatographic and spectrophotometric determination of

Sofosbuvir in pure and tablet forms. Eur J Chem 2016;7(3):375-9.

[9] Vikas PM, Satyanarayana T, Vinod Kumar D, Mounika E, Sri Latha M, Anusha R, *et al*. Development and Validation of new RP-HPLC method for the determination of Sofosbuvir in pure form. World J Pharm Pharm Sci 2016;5(5):775-81.

[10] Nebsen M, Elzanfaly ES. Stability indicating method and LC–MS-MS characterization of forced degradation products of Sofosbuvir. J Chromatogr Sci 2016;54(9):1631-40.

[11] Vejendla1 R, Subramanyam CVS, Veerabhadram G. Estimation and validation of Sofosbuvir in bulk and tablet dosage form by RP-HPLC. Int J Pharm 2016;6(2):121-7.

[12] Rezk MR, Basalious EB, Karim IA. Development of a sensitive UPLC-ESI-MS/MS method for quantification of Sofosbuvir and its metabolite, GS-331007, in Human Plasma: Application to a bioequivalence study. National center for biotechnology information (NCBI) 2015;114:97-104.

[13] Devilal J, Durgaprasad B, Pal N, Srinivasa Rao A. New method development and validation for the determination of Ledipasvir in bulk drug form by using RP-HPLC technique. World J Pharm Pharm Sci 2016;5(8):1312-21.

[14] Zhang K, Ma XQ, LI ZH, Zhang YL, Song JJ. An UPLC-MS/MS method for the quantitation of Ledipasvir in rat plasma: Application to a pharmacokinetic study. Lat Am J Pharm 2016;35(1):1116-21.

[15] Suryawanshi R, Shinde N, Todkar G, Gholve SB. Development and validation of simple UV spectrophotometric method for the determination of Ledipasvir in bulk form and stress degradation studies, Inventi Rapid: Pharm Analysis & Quality Assurance 2016;2016(3):1-5.

[16] Rezka MR, Bendasb ER, Basaliousc EB, Karim IA. Quantification of Sofosbuvir and Ledipasvir in human plasma by UPLC–MS/MS method: Application to fasting and fed Bioequivalencestudies. J Chromatogr B 2016;1028:63-70.

[17] Chenwei P, Yongping C, Weilai C, Guangyao Z, Lingxiang J,Yi Z, *et al*. Simultaneous determination of Ledipasvir, Sofosbuvir and its metabolite in rat plasma by UPLC–MS/MS and its application to a pharmacokinetic study. J Chromatogr B 2016;1008:255-9.

[18] Elkady EF, Aboelwafa AA. A rapid and optimized LC-MS/MS method for the simultaneous extraction and determination of Sofosbuvir and Ledipasvir in human plasma. J AOAC Int 2016;99(5):1552-9.

[19] Ariaudoa A, Favataa F, De Nicoloa A, Marco SB, Luca PA, Lucio B, *et al*. A UHPLC–MS/MS method for the quantification of direct antiviral agents Simeprevir, Daclatasvir, Ledipasvir, Sofosbuvir/GS-331007, Dasabuvir, Ombitasvir and Paritaprevir, together with Ritonavir, in Human Plasma. J Pharm Biomed Anal 2016;125:369-75.

[20] Zaman B, Siddique F, Hassan W. RP-HPLC Method for simultaneous determination of Sofosbuvir and Ledipasvir in tablet dosage form and its application to in vitro dissolution studies. Chromatographia 2016;79(23-24):1605-13.

[21] Pavankumar V, Harikrishnan N, Subramanian G. RP-HPLC method development and validation for the simultaneous estimation of Ledipasvir and Sofosbuvir in fixed dosage form. Int J Pharm Sci Res 2021;12(7):3852-7.

[22] Bandla J, Ganapaty S. Development and Validation of a Stability-indicating Method for the Simultaneous Estimation of Sofosbuvir and Ledipasvir by RP-HPLC. Indian J Pharm Sci 2018;80(6):1170-6.

[23] Mastanamma SK, Chandini SK, Reehana SK, Saidulu P. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of Sofosbuvir and Ledipasvir in bulk and their combined dosage form. Future J Pharm Sci 2018;4(2):116-23.

[24] Rote AP, Alhat J, Kulkarni AA. Development and validation of RP-HPLC method for the simultaneous estimation of Ledipasvir and Sofosbuvir in bulk and pharmaceutical dosage form. Int J

Pharm Sci Drug Res 2017;9(6):291-8.

[25] Farid NF, Abdelwahab NS. Chromatographic analysis of Ledipasvir and Sofosbuvir: New treatment for chronic hepatitis C infection with application to human plasma. J Liq Chromatogr Relat Technol 2017;40(7):327-32.

[26] Hemdan A, Eissa MS. Simultaneous chromatographic analysis of Sofosbuvir/Ledipasvir in their combined dosage form: an application to green analytical chemistry. J Anal Sci Technol 2019;10(39).

[27] Prasad R, Bhatt S, Singh K. HPLC method for simultaneous estimation of drug release of Ledipasvir and Sofosbuvir in Ledipasvir and Sofosbuvir tablets. Int J Pharm Sci Res 2019;10(2):634-41.