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A Review: Hepatitis C and its overview of analytical methods

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ABSTRACT:
Chronic hepatitis C infection affects millions of people worldwide and confers significant morbidity and mortality. Effective treatment is needed to prevent disease progression and associated complications. The era of direct acting antiviral (DAA) therapies began with the development of first-generation NS3/4A protease inhibitor in 2011. They vastly improved outcomes for patients. Daclatasvir is an inhibitor of hepatitis C virus NSSA protein that is used for the therapy of chronic hepatitis. Daclatasvir used with combination of other drug
like sofosbuvir, ledipasvir for the treatment of chronic hepatitis C infection. There is need to developed and validate a simple, but still reliable and sensitive different analytical methods for the quantification of Daclatasvir and its combination. This review presents the effect off Daclatasvir, sofosbuvir and ledipasvir on the treatment of HCV with the help of spectrophotometric method, analytical techniques and separation method.

KEYWORDS: Hepatitis c, Daclatasvir, Analytical methods, HCV

I. INTRODUCTION

Hepatitis C is an infection to the liver which is caused by the hepatitis c virus (HCV). Now a days; many people's get infected by this hepatitis c virus by sharing the needles or other equipment's to inject the drugs. To some of the peoples, hepatitis c is a short-term illness but for 80-85% of peoples who get infected with this virus hepatitis c it becomes long term chronic infection WHO's updated around 2018 guidelines which recommend the therapy with the panic-genotypic direct acting antiviral (DAAs)[1]

Chronic hepatitis c is a danger disease which can produce long term problems related to health, even death too. There is unavailability's of the vaccines for hepatitis c. Hepatitis c is an infection to the liver which can ultimately lead to damage to the liver. Around 4 millions peoples in the US have this disease. This virus is spread through the infected persons blood or fluids of body. Hepatitis c virus is the blood borne virus. In the world, 71 million peoples have this chronic hepatitis c virus infection. From these peoples significantly number of these has developed cirrhosis or liver disease.[2]

DAAs can cure the many people with the infection of HIV and duration of treatment is mostly short that is (usually 12 to 24 months), which depends upon absence or presence of the cirrhosis. Interferon, ribavarin and peg interferon is the main which is mainly used in the treatment for hepatitis c. This can also have some side effects such as fatigue, anemia, skin rash, depression and diarrhea. But in recent years the treatment for the hepatitis c is changed rapidly. [3]

Now you are most likely seen availabilities of this kind of medication: DACLASTASVIR (Daklinza) this pills once in a day with the sofosbuvir for the 12 weeks. SOFOSBUVIR-VELPATASVIR this pills which have to take for the 12 weeks. [3] LEDIPASVIR- SOFOSBUVIR (Harvoni) this pills cures the disease in many peoples in 8-12 weeks in genotype 1,4,5,6. DAAs which mainly target HCV proteins in such a way as the nonstructural (NS) 3 protease, NS5B polymerase and NS5A proteins and those other factors which are requires for replication of the HIV. DAAs have the characteristics which are increased the potency, the higher barriers to the resistance, more favorable profile.

II. ANALYTICAL METHOD

The worldwide use of these HCV antiviral agents in single or in the combined dosage form and there clinical or pharmacological studies which make enhance for the need of development of the fast and sensitive analytical techniques or method to assay this kind of the drugs. There are several

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chromatographic or spectroscopic method has been used to resolve the mixture of these compounds with the most of overlapping spectra without the separation of such spectrophotometry derivatives.[4] In the current situation, the development of effortless and the rapid spectrophotometric method to estimate the single or combined pharmaceuticals formulation or achieving the high accuracy and that too without the prior separation.Analytical techniques which are defined as the set of techniques or set of methods which allow us to know qualitatively or/and quantitatively the composition of any of the material and the chemical state in which it is located. Now a days, the method of assay in the monographs which includes spectrometry, chromatography, titrimetric and capillary electrophoresis and one of the electro analytical method also can been seen. From the starting of the drug development stage to the marketing and the post marketing, analytical techniques play a key role, it mainly use for understanding the chemical stability and physical stability of the drug, for the stability of any drug molecules identifying the impurities.

Various analytical methods are available that are as follows-

A. Spectroscopic method–
1. UV
2. IR
3. NMR
4. Massspectrometry

B. Separation methods
1. TLC
2. GC
3. HPLC

C. Hyphenated methods-
1. GC–MS
2. LC–MS
3. LC–DAD–MS
4. LC–NMR
5. LC–MS–MS
6. HPLC–DAD–MS
7. HPLC–DAD–NMR–MS

Analytical method for hepatitisc drugs

A. Daclatasvir(single drug):

Daclatasvir is sold under the trade name of Daklinza, a medication used single or in combination with other medication to treat hepatitis c. [7] The other medication used with the daclatasvir are sofosbuvir, ribavirin and interferon, they are vary depending on the virus type. It is taken orally once a day. Molar mass of daclatasvir is 738.89 g/mol. Bioavailability of daclatasvir is 67%. Protein binding is 99%. Elimination half-life is 12-15 hours.

Mechanism of action:

Daclatasvir is used to stop HCV viral RNA replication and protein translation by directly inhibiting HCV protein NS5A. [18]

B. Sofosbuvir and Ledipasvir (combination drug)

The fixed dose of combination of ledipasvir-sofosbuvir which is provide an effective and well tolerance one pill in a day for the treating of genotypes 1,4,5,6, chronic hepatitis (HCV) infection. The combination of sofosbuvir and ledipasvir are used in alone or in combination with ribavirin (copegus, Ribasphere, Rebetol) to treat the certain types of combination of the chronic hepatitis C in the adults and in the children of 3 years of age and the older.[6]

HARVONI is a fixed dose combination of tablets containing sofosbuvir and ledipasvir for oral administration. Ledipasvir is an HCV NS5A inhibitor and that of sofosbuvir is the nucleotide analog inhibitor of HCV NS5B polymerase.[7]

Mechanism of action:

Ledipasvir inhibit a important viral phosphoprotein NS5A, which is an involved in the viral replication, and the secretion

Sofosbuvir on the other hand it is metabolized to an uridine triphosphate mimic, which ultimately act as an a RNA chain terminator when it is incorporated with RNA by NS5B polymerase.

C. Sofosbuvir-

Sofosbuvir, It is mainly sold under the brand name of ‘Sovaldi’ like others it is a medication used for the treating Hepatitis C . It can recommend with another combination with ribavarin, ledipasvir, daclatasvir, etc. The cure rate is 30 to 97% which is depend on the type of hepatitis c virus involved. The bioavailability of the drug is 92%. The metabolism of it mainly activate to triphosphate (cat A) CES1 which is mainly help your liver recovery. It is mainly discovered in 2007, administeredorally. The peak concentration after the administered orally of sofosbuvir is 0.5-2hrs. post dose.[9]

Plasma protein binding of sofosbuvir is 61-67%.\

It is discovered by Michael Sofia

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2) Daclatasvirand Sofosbuvir(combination) –

Daclatasvir and Sofosbuvir combine therapy (SOF/DCV) is an effective and safe for the real world treatment of liver patients associated with liver cirrhosis with hepatitis c virus (HCV) genotype 4. A study took place in 2016 at 4 different clinical setting in Egypt. A total of 551 patients with liver cirrhosis having hepatitis c genotype 4. [15] They get treated by giving sofosbuvir 400mg and daclatasvir 60mg once daily for the 12 week treatment. [19] Sofosbuvir and daclatasvirrepresent the antiviral pan genotypic regimen with pharmacokinetics in hemodialyzed patients. In the treatment of genotype 3a chronic Hcv in that the safety and the efficacy of combination of sof and dcv in 6 male patients.[20]These patients were treated with a reduced dose of SOF 400mg and 60mg of DCV once daily for 12 weeks Sofosbuvir 400mg + daclatasvir 60mg Both are water soluble and they can be absorbed into the bloodstream through stomach. Sofosbuvir and daclatasvir in range of 93% for genotype 3. For genotype 2, 5 and genotype 6 they cure rates are 100% and hepatitis c genotype of cure rate is 97% came from 12-week treatment.

Table1: Different types of method used by different authors in their study of Daclatasvir.

SR.NO.	NAMEOFAUTHOR(FIRSTAUTHOR)	TYPEOFSTUDY	MATERIALUSEDINTHEIRSTUDY.	RESULT.	YEAR(REFERENCE)
1.	M.M.BAKER, D.S.ELKAFRAWY	StabilityindicatingHPLC-DAD method.	Waters C8 column (4.6x250mm,5µmparticlesize)DAD Detector MILLEN-NIUM32login version 4.00 software.Injector model SM7 withloop capacity 100µL.Flow rate: 1.2 mL/minMobile phase: Phosphatebuffer(PH2.5) andacetonitrileratioof75:25.	Retentiontimeis5.4min.Linearity range was 0.6-60µg/mL. Correlation coefficient>0.999. Daclatasvir exhibitsconsiderable absorptionallover the range 210-350nmwithmaximumat306nm. Retention factor is 4.42Tailingfactoris1.15andN is6957.	2017(21)
2.	ASIA NAZ, NAWABSHER.	Threeorganicimpuritiesandstabilitystudies usingHPLC method	HPLC columnHypersilC18(4.6×100mm×5µm). Flow rate: 1 ml/minInjection samplevolumnwas10.0 µL Mobilephase(A):acetonitrile and buffer (25:75) Mobilephase(B):100% acetonirile. PH5	Wavelength318nmRuntime:23 Mean%recovery:99.35SD:1.6 RSD%:1.3 Tailingfactor:1.002-1.03 Theoreticalplates24000-60000 Correlation:0.9996	2019(22)

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SR.NO.	NAMEOFAUTHOR(FIRSTAUTHOR)	TYPEOFSTUDY	MATERIALUSEDINTHEIRSTUDY.	RESULT.	YEAR(REFERENCE)
3.	YANDEHUANG	Forcedoxidativedegradationpathwayofimidazolemoiety usingHPLC	A sunfire C18Columnwaters Flowrate:1ml/min Mobile phase: water/ACN/TFA(1900:100:1V/V/V) Wavelenth306	Threesample carried outandthereretentiontimearecarried out 1st sample : RT -7.22min2nd sample: RT -9.62 min3rdsample:RT-11.63min	2019(23)

Table2: Different types of method used by different authors in their study of Sofosbuvir and Ledipasvir

SR NO.	NAME OFAUTHOR (FIRST)	TYPEOFSTUDY	MATERIALUSEDINTHERESTUDY	RESULT	YEAR(REFERENCE)
1.	S.K.MASTANAMMA	Stability indicatingRP-HPLCmethodfor the simultaneousestimation indosageform	Luna C18 columnWaters2996 Photodiodearraydetector EMPOWERPDA2 software Flow rate 1ml/minMobilephase:ACN:TEA(50:50) Wavelength:227nm	Runtime:7minRT: 2.75(LED)4.905(SOF) Sofosbuvirmeanamountestimated5.53 Ledipasvirmeanamountestimated1.26 %assay:100.8 %RSD:0.52	2017(24)
2.	SAMIA M.EL-GIZAWY	HPTLC method for simultaneous determination of anti hepatitis in rabbit plasma.	CAMAG HPTLC WinCATSversion 1.4.10.software silica gel 60 F254 aluminium sheet 100µL syringe Scanningspeed20 mm/s Mobilephase: Ethylacetate:GAA (100:5)	Recovery of sofosbuvir- 100.91 SD: 1.64 Recovery of LDS-100.63 SD: 0.52 RSD%: 2.51 &3.18 Cmax : 1662.30& 355.84	2018(25)

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SR NO.	NAME OFAUTHOR (FIRST)	TYPEOFSTUDY	MATERIALUSEDINTHERESTUDY	RESULT	YEAR(REFERENCE)
3.	OLA M.ABDALLAH	LC-MS/MS method and its major metabolite GS-331007 in human plasma	Agilent 1260 HPLC with API4000 mass spectroscopyAnalyst 1.62 software Hotfixes.Waters Xterra MS C8 columnInjection volume- 5 µL Mobile phase-Amm.formate buffer (PH 3.5)Oracetonitrile: methanol(40:60)	Flow rate- 0.7 Ml/min Intraday-Ledipasvir %recovery- 107.6 RSD%- 7.01 Sofosbuvir Recovery%-101.33 RSD%- 9.70 Inter day- Ledipasvir Recovery%-104.06 RSD%- 6.61 Sofosbuvir Recovery%- 93.65 RSD% 8.8-	2017(26)
4.	M.M.BAKER	Validatedspectrophotometricandchromatographic method for analysis of hepatitis c antiviral.	Shimadzu1601PCUV-VIS spectrophotometryWithUV1601PC software HPTLC includeCAMAGmicrolitersyringe(100µL) TLCsilicagel aluminiumplates60F254 HPLC system withDAD consistofwaters 2695 allianceThermohypersil C8 column M0BILEPHASE-Chloroform and methanol(94:6)forHPTLC MOBILEPHASE-0.01M sodiumdihydrogen phosphate(PH2.5)-methanl(20:80) Flow rate 1.2 MlWavelength-332and262	HPTLC-Rf value-sofosbuvir-0.19±0.03 and ledipasvir-0.44±0.03 HPLC-DAD: Sofosbuvir-RT-2.78±0.02 Tailing factor-1.16Theoretical plates-4812 Ledipasvir-RT-3.87±0.003 Tailing factor-1.09Theoretical plates-4860	2017(27)

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Table3: Different types of method used by different authors in their study of Sofosbuvir.

SR. NO.	NAMEOFTHE AUTHOR	TYPEOFSTUDY	MATERIALUSEDINTHEIRSTUDY	RESULT	YEAR(REFERENCE)
1	SHAHRAMMIRAGHAEI	HPLC-DAD methodinhuman serum & itscomparison withLC-MS/MS technique	-Agilent1200series LCsystem -Manualinjectorvalve20µl -261nmwavelength -Merck Microspore RP 18Column(250×4mmID,5 µm) -c18guardcolumn -Mobile phase-water:Acetonitrile(57:43 V/V) -flowrate-0.8ml/min	-R.T.-5.3&6.4 -UV-LOD &LOQ-10 &25ng/mlmass detection -Linear regressionColumnY=0.224x+1.874 -Recovery%-80.5-85.2±3 -m/z-287	2017(28)
2	B.M.GHANDHI	UPLC-MS/MS Method fordetermination ofhumanplasma	-UPLCH-CLASS-XevoTQD system -Electronsprayionization -GeminiC-18column - Mobilephase0.5%Formic acid:methanol(30:70) - FlowRate-0.5ml/min3.0kv capillaryvoltageDesolvationTemp.-450°c	Runtimeofmethod-1.60min -R.T.of SOFIS0.79 min Mass spectra SOF is(M+H)+ Ion is m/z -425.85tom/z–431.38 -morethan0.99 %recovery-76.26±5.70	2017(29)
3	MARIADELMAZ.CONTRERAS	RP-UHPLC-DAD - MSforqualitativeand quantitativeanalysis ofsofosbuvir	Agilent1200series -6540AgilentUHD -QToF -Mobilephase-0.2%formicacid:Acetonitrile(Gradientsystem) -ZORBAXECLIPSE -XDB-C18column -Columntemp -24℃ -Injectionvolume-2μl - Absorbanceat190to600nm Wavelength-260±4nm	Tailingfactorislowerthan2 R^2-0.998 CV-1.8% LOD-0.07μg/mlLOQ-0.36 μg/mlRSD-1.5% m/zatblankwas 205.068 m/zat(m+Na)+ was181.0727 Recovery-101±8Accuracy -Intraday-99.4Interday-101	2017(30)

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Table 4: Different types of method used by different authors in their study of Sofosbuvir and Daclatasvi

SR. NO.	NAMEOFAUTHOR(FIRSTAUTHOR)	TYPE OFSTUDY	MATERIALUSEDINTHEIRSTUDY	RESULT	YEAR(REFERENCE)
1.	Mohamed AAbdel-lateef	Fouriertransforminfraredspectroscopyfor Determinationanti-HCV	FTIR measurementNicolet-6700 FTIRspectrometerPotassium bromidedisc of sof and DACwereprepared. vm-300supermixedvortex electronicsinglepan balance -falcon-50ml conicaltubes -midIR-region(4000-400cm)	-correlationcoeff.0.9995,0.9993for both -LOD-0.049&0.057resp. -LOQ=0.149&1.72 FORDAC&SOF. -standard deviationforDAC-2.20&SOF-1.71	2019(8)
2.	Stefanianotari	UPLC-MS/MS methodfor simultaneousquantification in plasmaofHIC/HCV Co-infectedpatients	-UPLCfromwaters\ -c18Luneomegacolumn(50mm*2.1mmI.D.) -particlesizeof1.6um50ccolumn oven -Mobile phase-A.0.1%formicacidandphaseBacetonitrile -flowrate0.4ml/min -Runtime0.4ml/min -Runtime3.5min -UPLCcoupledwith*EVD TQD	Retentiontime -Sof-1.27minDIC-1.42min M/Z—530.098forSOF & 313.03DAC -LOQ—11.71ng/ml19.53mg/ml -Recovery-SOF-95%DAC-98% Carryover-(n=6)Capillaryvoltage3.5kvTemp-60^{0}C Desoluation gasflow—800L/H	2018(10)
3.	Mohammadnabil abo-zeid	HPTLC-Dualwavelengthspectrodensito-metricmethod	CAMAG- HPTLC systemconsistoflinomat-5automaticsampleinjector. HPTLCsilicagel60F254aluminiumsheets. -particlesize-150-200um -bandsize-6mm -100ulsamplesyringe -slitdimension5*0.45mmUltrasonic cleaner centrifugemegafuge‖	-absorption spectraof sof and DCSinrange200to400cm -maxforSOF&DAC-265&DAC-265&311nm -Rf value-Sof->0.80 DAC- 0.41 LOD-11.3&6.5ng -LOQ-34.2&19.7	2018(12)

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				ng -Recovery– 96.6–100.2% RSD– 2.39 &1.29	
4.	NOHA N Atia	HPLC-VV methodforpharmacokineticstudyinrabbits	Youngline HPLCsystem with uv-detectorandrheodyneinjectorYounglinAutochro3000software. C18columnMobilephasacetonitrileand10mm sodium acetatebuffer PHS-(50:50v/v)Flow rate 1.0ml/minWavelength 260nm &313nm Ultrasonic cleanerCentrifugemegafuellPH meter model HI-4222	>(nm)-sof-260 -DAC-313 RT(4.25&5.07) Retention factor- 1.13 &1.54 -No.- 4447&3010 oftheoreticalplate -Resolution– 2.40 -LoQ– 0.62&0.57 -LOD- 0.20 &0.19 -r –0.9995 -Cmax–1.18+- 0.26 1.40+-0.19	2018(13)
5.	Dalia W.zidan	Humanplasma &human wineusingmicellarmonolithicHPLC-UV	HPLCsystemconsistof a PerkinElmerseries 200 uv/visdetector C8monolithiccolumn0.45um membranefiller Degasser DGU –20A5 Flowrate–0.5ml/minat25c Mobilephase0.1msodium dodecylsulfate and 0.3%triethylamine pH– 6.5 > =226nm Injectionvolume–20ul	Linearity– sofandDAC Plasma–(60-300) (50-300) Urine– (50-400) (40-400) Retentionfactor- 0.655&1.241 Resolution – 1.895 No. of theoreticalplate –1474,2704rezp Accuracy– 0.222, 0.343 pHofmobilephase 6.4-recovery 99.56 6.6 – 100.05	2018(14)

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				recovery	

III. CONCLUSION:

The method was applied to therapeutic monitoring of patients for hepatitis C. Thus, this method provides a simple,sensitive, precise and reproductive assay for dosing of their drug that can be readily adoptable to routine use.

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