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A Review: Analytical method for determination of Elbasvir and Grazoprevir in bulk, pharmaceutical dosage form and biological fluid

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ABSTRACT:

Elbasvir/Grazoprevir (Zepatier) is a combination product with an FDA-approved indication for the treatment of chronic HCV genotypes (GTs)1and 4 in adults.Elbasvir is an NSSA inhibitor, preventing hepatitis C viral RNA replication and vision assembly.Median EC50 values range from 0.2 to 3600 pmol/L,based on genotype. Grazoprevir is a protease inhibitor of HCV NS3/4A that prevents cleavage of the polyprotein necnecessary for replication. Median EC50 values range from 0.16 to 0.8pmol/L.Analytical method play an important role in the physicochemical properties description. This review includes most recent analytical methods such as various spectroscopic methods (Simultaneous estimation, Mass Spectroscopy)and chromatographic methods (RP-HPLC, stability indicating HPLC)for determination of Elbasvir and Grazoprevir in various pharmaceutical dosage forms and biological fluid matrix were reported.

KEYWORDS: Elbasvir,Grazoprevir, RP-HPLC, UV-Visible spectroscopy, Synchronous Fluorescence spectroscopy

INTRODUCTION :

Hepatitis C is an infection caused by the hepatitis C virus (HCV) that attacks the liver and leads to inflammation. The World Health Organization estimates that about 3% of the world's population has been infected with HCV and that there are more than 170 million chronic carriers who are at risk of developing liver cirrhosis and/or liver cancer.[1] For almost 25 years, Pegylated interferon and ribavirin have been the cornerstone of treatment for this disease until the revolutionary development of protease inhibitors. This class of direct-acting antiviral agents has led to all oral HCV treatment regimens that have changed the strategies of hepatitis C treatment. [2]

Zepatieris a novel combination of two new Food and Drug Administration (FDA) approved drugs elbasvir (EBV) and grazoprevir (GRV).[3] It combines two direct-acting antiviral agents with distinct mechanisms of action that target HCV at multiple steps in the viral lifecycle. EBV (Figure 1) is an inhibitor of HCV NS5A, which is essential for viral RNA replication and virion assembly. On the other hand, GRV (Fig. 2) is an inhibitor of the HCV NS3/4 A protease which is necessary for the proteolytic cleavage of the HCV encoded poly protein and is essential for viral replication. [4]

Elbasvir is a highly potent and selective NS5A inhibitor of the hepatitis C virus NS5A replication complex3.The chemical name of elbasvir is methyl N-[(2S)-1-[(2S)-2-[5-[(6S)-3-[2-[(2S)-1-[(2S)-2-(methoxycarbonylamino)-3-methylbutanoyl]pyrrolidin-2-yl]-1H-imidazol-5-yl]-6-phenyl-6H-indolo[1,2-c]benzoxazin-10-yl]-1H-imidazol-2-yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate. [5]The molecular weight of elbasvir is 882.014 g/mol and molecular formula is C49H55N9O7.Chemical structure of Elbasvir is depicted in figure 1.

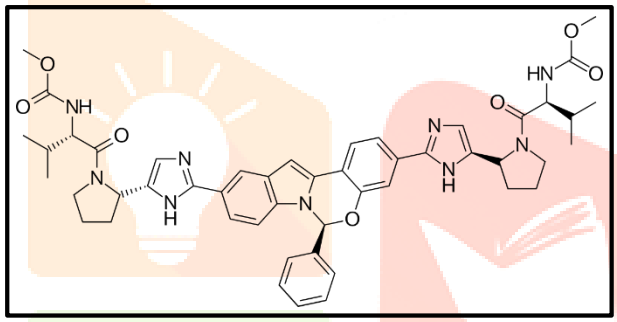

Figure No.1 : Structure of Elbasvir

Grazoprevir is a second generation hepatitis C virus protease inhibitor acting at the NS3/4a protease targets4. NS3/4a protease is an integral part of viral replication and mediates the cleavage of virally encoded polyprotein to mature proteins (NS3, NS4A, NS4B, NS5A and NS5B). [6] The chemical name of Grazoprevir is (1R,18R,20R,24S,27S)-N-{(1R,2S)-1-[(Cyclopropylsulfonyl)carbamoyl]-2-vinylcyclopropyl}-7-methoxy-24-(2-methyl-2-propanyl)-22,25-dioxo-2,21-dioxa-4,11,23,26-tetraazapentacyclo-nonacosa-3,5,7,9,11-pentaen-27-carboxamide. The molecular weight of Grazoprevir is 766.911 g/mol and molecular formula is C38H50N6O9S.Chemical structure of Grazoprevir is depicted in figure2.

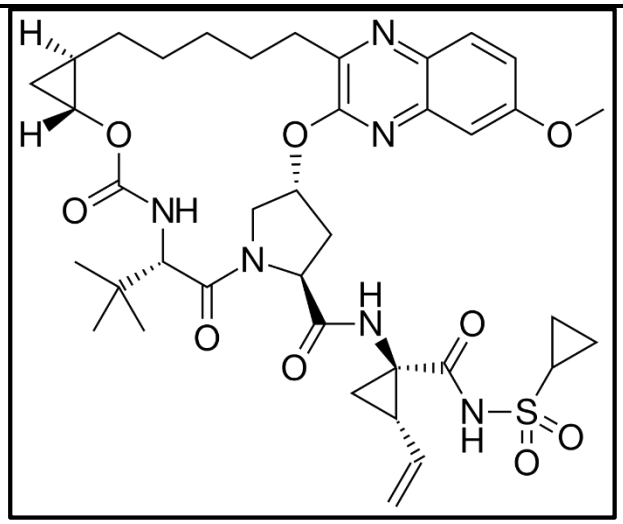

Figure No.2: structure of Grazoprevir

PHARMACOLOGY :

Elbasvir is an inhibitor of the HCV non-structural protein 5A. While the precise role of this protein is unknown, it is essential to viral replication and virion assembly.Synthesis Potential modes of action of NS5A inhibitors like elbasvir include blocking signaling interactions, redistribution of NS5A from the endoplasmic reticulum to the surface of lipid droplets, and modification of the HCV replication complex. [7]Computational target-based in silico research suggests that elbasvir may carry activity at several proteins required for replication of SARS-CoV-2 - namely RNA-dependent RNA polymerase, helicase, and papain-like proteinase. [8]

Elbasvir reaches peak plasma concentration 3-6 hours after administration and has an absolute bioavailability of 32%.Elbasvir has an estimated apparent volume of distribution of 680 liters.Elbasvir is more than 99.9% bound to plasma proteins.It binds both human serum albumin and α1-acid glycoprotein.The geometric mean apparent terminal half-life for elbasvir is 24 hours in HCV-infected subjects. [9]

Grazoprevir is a second generation NS3/4a protease inhibitor used to inhibit viral HCV replication. NS3/4a protease is an integral part of viral replication and mediates the cleavage the virally encoded polyprotein to mature proteins (NS3, NS4A, NS4B, NS5A and NS5B) Label. Grazoprevir inhibits the NS3/4protease enzymes of HCV genotype 1a, 1B, and 4 with IC50 values of 7pM, 4pM, and 62pM, respectively. [10]

Grazoprevir reaches peak plasma concentration 0.5-3 hours after administration. Grazoprevir has an absolute bioavailability of 27%.Grazoprevir has an estimated apparent volume of distribution of 1250 liters.The geometric mean apparent terminal half-life for Grazoprevir is 31 hours in HCV-infected subjects[11].

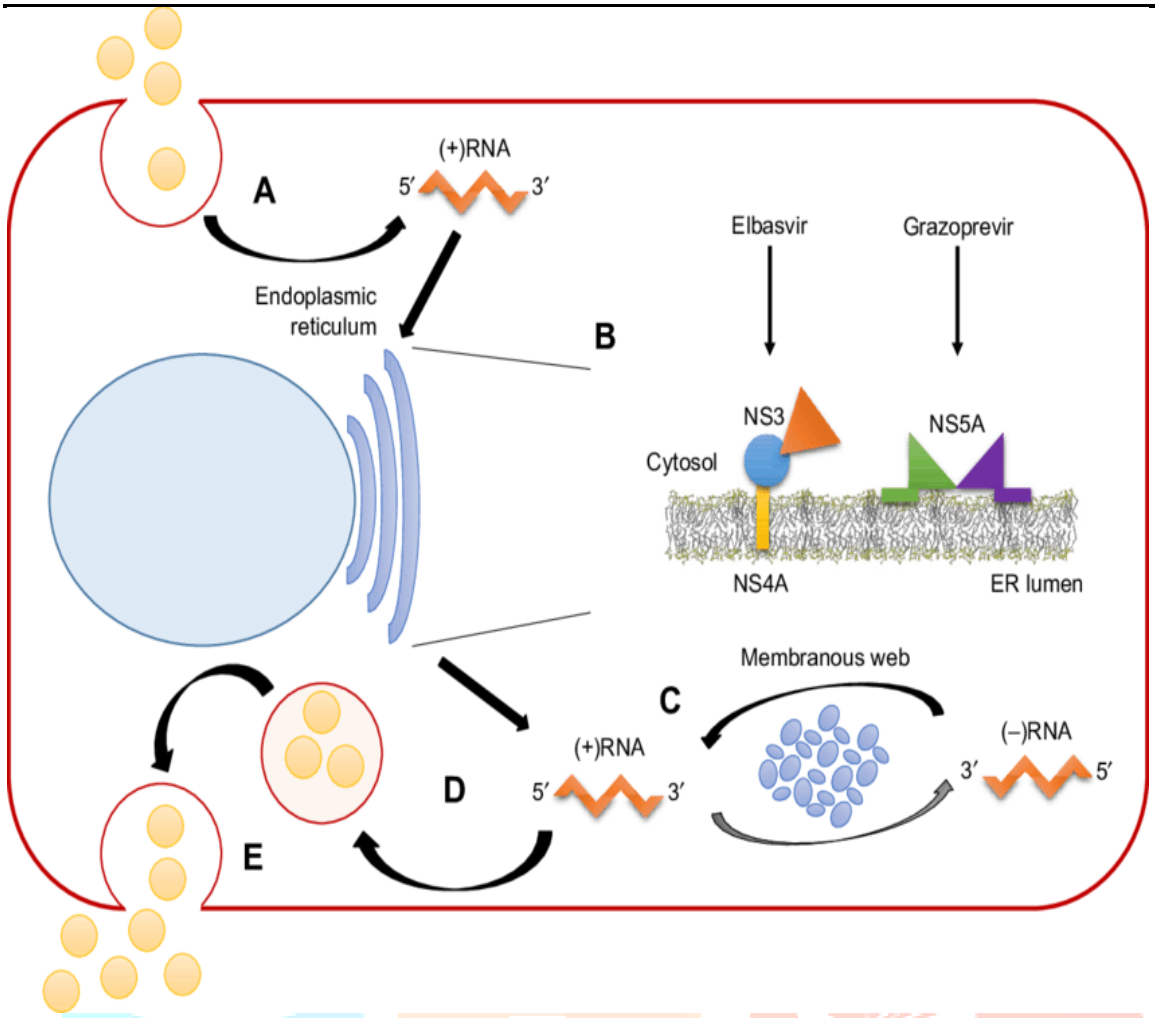

fig:no:1. pharmacology of elbasvir is an inhibitor of the hcv non-structural protein 5a

ANALYTICAL METHOD :

This all methods which are used for the determination of Elbasvir and Grazoprevir drug combination in Bulk, pharmaceutical dosage form and also in biological fluid like human plasma.[12] This all analytical method which are seen during the literature survey are reported. This article describes the review on the reported analytical method with specific conditions[14].

1. Chromatographic Method :

Various chromatographic methods are used for the determination and quantification of the Elbasvir and Grazoprevir drug combination in marketed formulation and in biological fluid. Chromatographic methods like High performance liquid chromatography (HPLC),Reverse phase High performance liquid chromatography (RP-HPLC),Liquid chromatography with tandem mass spectroscopy (LC-MS/MS) are used for determination of Elbasvir and Grazoprevir[15]. Below table describe the summary of the various chromatographic methods with the method description[16].

Table No.1: Summary of chromachromatographic method of Elbasvir and Grazoprevir

Title	Method	Mobile phase	Stationary phase	Wavelength	Detection (M/Z)	Ref.
Simultaneous determination of Elbasvir and Grazoprevir in their pharmaceutical preparation using HPLC method.	HPLC method	Acetonitrile :Methanol (50:50 v/v)	BDS Hypersil C18 column	253 nm	-	14
Rapid and precise RP-HPLC method has been developed for the validated of Elbasvir and Grazoprevir in it's pure form as well as in tablet dosage forms.	RP-HPLC method	Methanol: phosphate buffer PH 3.9 (55:45 v/v)	Zorbax C18 column	255 nm	-	15
The aim of study is to develop and validate a simple, accurate, precise RP-HPLC method for Simultaneous determination of Elbasvir and Grazoprevir in bulk and tablet formulation.	Isocratic RP-HPLC method	Methanol: water (80:20 v/v)	C18 column	260 nm	-	16
Picogram level quantification of Grazoprevir and Elbasvir with deuterated Internal standard in human	LC-MS/MS method	Ammonium acetate:acetonitrile (20:80v/v)	AgilentTC-C18,4.6×75mm,3.5 µm,80 A column	-	767.3/553.2 for Grazoprevir and 883.4/6	17

plasma samples by LC-ESI- MS/MS.					56.3 for Elbasvir	
RP-HPLC method development and validation for the Simultaneous estimation of Grazoprevir and Elbasvir in bulk and pharmaceutical dosage form .	RP-HPLC method	0.01n kh2po4 buffer and acetonitrile and Methanol (44:55v/v)	Kromosil (250 mm, 4.6mm,5µ)	260 nm	-	18

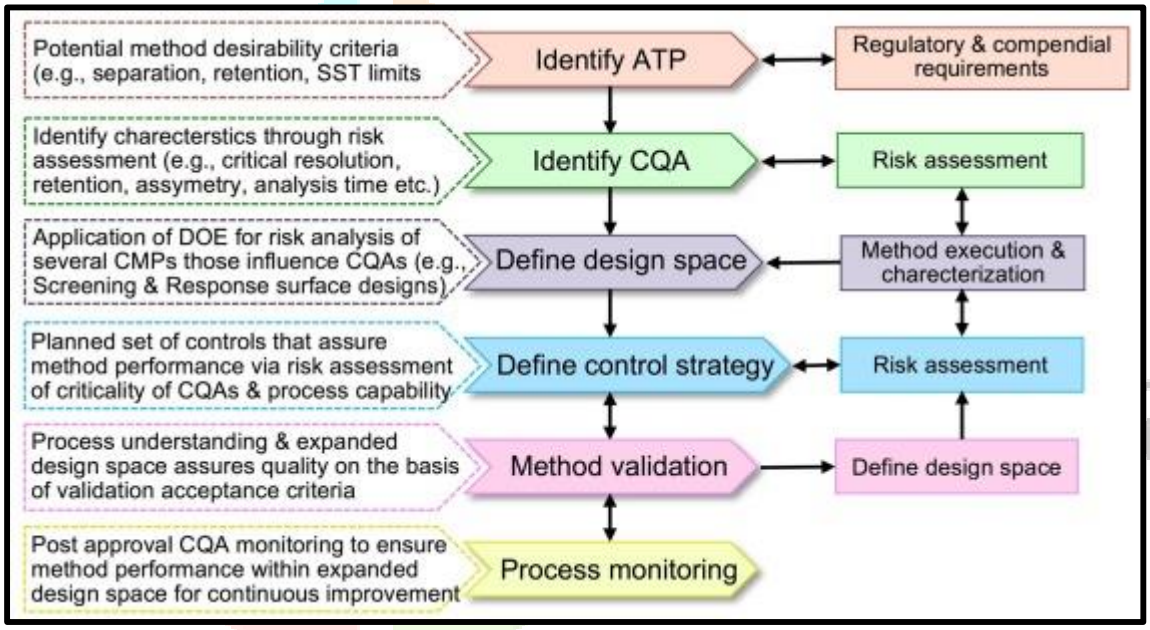

Fig:No:2. chromachromatographic method of Elbasvir and Grazoprevir

2. Spectroscopic Method :

Spectrophotometric method is economical and versatile particularly for developing countries. Spectrophotometric method has some advantages such as being easy, less time and less expensive consuming compared with most of the other methods[17]. A simple, precise and economical spectrophotometric method for the Simultaneous estimation of the Elbasvir and Grazoprevir in pharmaceutical bulk and tablet dosage form was developed and validated. Various method like Simultaneous estimation, dual wavelength, UV spectrophotometry,synchronous fluorescence spectroscopic method and derivative method are used for determination of Elbasvir and Grazoprevir drug combination in marketed formulation. Following table describe the different spectroscopic method with the method description and condition which are reported on review literature[18].

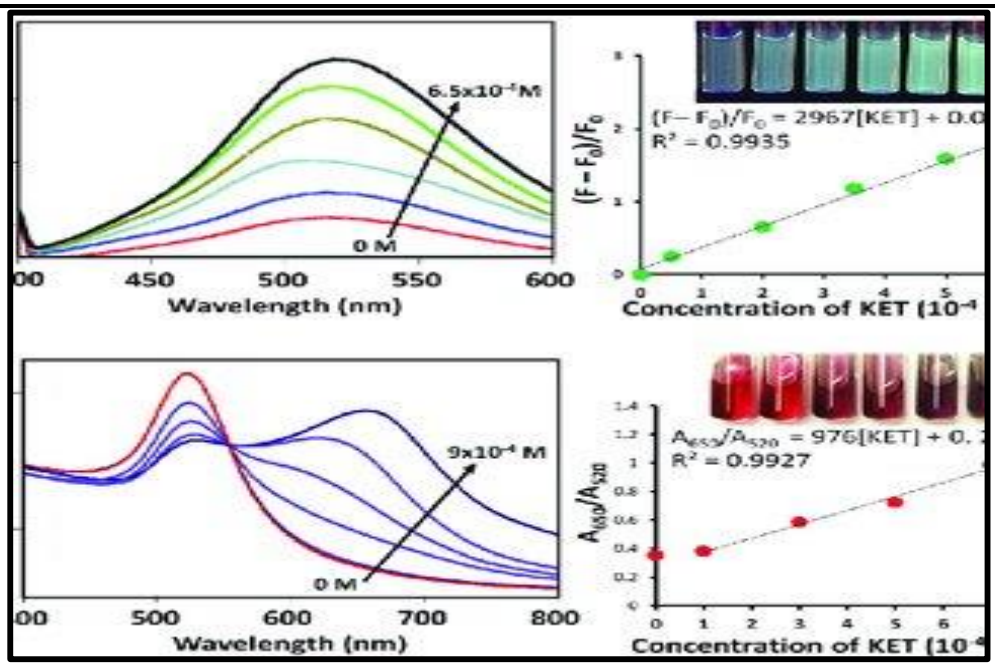

Fig:No:3. spectroscopic methods of Elbasvir and Grazoprevir

Table. No.2: Summary of spectroscopic methods of Elbasvir and Grazoprevir

Title	Method	Wavelength for Elbasvir	Wavelength for Grazoprevir	Ref.
Simultaneous spectrophotometric determination of Elbasvir and Grazoprevir in a pharmaceutical preparation	Ultraviolet spectroscopic method	351and 315 nm	375 and 334.5 nm	19
Simultaneous determination of Elbasvir and Grazoprevir in their pharmaceutical formulation by synchronous fluorescence spectroscopy coupled to dual wavelength method	Sensitive, selective and accurate synchronous fluorescence spectroscopic method	312 nm	390 and 372 nm	20
Application of different spectroscopic methods for Simultaneous determination of Elbasvir and Grazoprevir in pharmaceutical preparation	Simultaneous equation method	369 nm	253 nm	21
Development and validation of a highly sensitive second	Simultaneous determination	308 nm	389 nm	22

derivative synchronous fluorescence spectroscopic method for Simultaneous determination of Elbasvir and Grazoprevir in pharmaceutical preparation and human plasma				

3. Stability Indicating Method :

Stability indicating method is used to check out the stability of drug in different conditions like in acidic, basic, oxidative, photolytic and thermal degradation.Following table describes the various stability indicating method with the method description and condition which are reported on review literature[19,20,21].

Table No.3: Summary of stability indicating methods for Elbasvir and Grazoprevir[22,23,24,25]

Title	Method	Mobile phase	Stationary phase	Wavelength	Ref.
A new validated stability indicating RP-HPLC method for Simultaneous estimation of Grazoprevir and Elbasvir in tablet dosage forms	Stability indicating RP-HPLC method	0.1% orthophosphoric acid:acetonitrile (44:55 v/v)	BDS C18 column	260 nm	23
To develop accurate, precise stability indicating method for Simultaneous estimationof Elbasvir and Grazoprevir in bulkandpharmaceutical dosage form	Stability indicating method	OPA buffer(0.1%)and Acetonitrile (50:50 v/v)	Luna C18 column	258 nm	24
HPLC-MS/MS method development and validation for	HPLC-MS/MS method	0.1% formic acid:methanol	C18 column Ascentis Express	-	25

determining stability of Elbasvir in human plasma samples		(25:75 v/v)	(50mm×4.6 mm, 2.7µm)		

DISCUSSION :

The presented review highlights on various analytical methods reported for determination of Elbasvir and Grazoprevir in bulk, pharmaceutical dosage form and biological fluid like human plasma. UV, RP-HPLC and Stability indicating RP-HPLC method were found to be most commonly used methods.These methods are found to be rapid, accurate, sensitive, economical and reproducible for determination of Elbasvir and Grazoprevir.

CONCLUSION:

So,from all above information it should be concluded that various analytical methods such as chromatographic methods and spectroscopic methods were used for determination of Elbasvir and Grazoprevir ;which has been successfully used on a routine basis and allows the quantification of the drug in various bulk, pharmaceutical dosage form and in biological fluid .All these methods are simple, fast, accurate, sensitive, selective, reproducible and possess excellent linearity and precision characteristic.

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