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Analytical Method Development and Validation of Elbasvir and Grazoprevir in Bulk and Tablet Formulations by Rp- HPLC

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Abstract: *The aim of the study is to develop and validate a simple, accurate, precise and rapid isocratic reverse-phase high-performance liquid chromatographic method for simultaneous determination of Elbasvir and Grazoprevir in bulk and tablet formulations. Chromatographic separation was supported on Inertsil ODS, 5µm C18(150x4.6 ID) column with a blend of methanol: water (80:20) as mobile phase at a flow rate of 1 ml/min. UV detection was performed at 260 nm using HPLC Shimadzu (LC 20 AT VP) with LC solutions software. The retention times were found to be 2.420 minutes ad 4.270 minutes for Elbasvir and Grazoprevir respectively. Calibration plots were linear for both the drugs (r^2=0.999) over the concentration range 36-84 µg/ml for Elbasvir 6-14 µg/ml for Grazoprevir. The LOD and LOQ values for Elbasvir and Grazoprevir were found to be 0.81 µg/ml, 2.46µg/ml and 0.53µg/ml, 1.633µg/ml respectively. The optimized method was validated in accordance with ICH guidelines for accuracy, precision, specificity, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of Zepatier tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. As the method shows high recovery and low relative standard deviation which confirms the suitability of the method for routine determination of Elbasvir and Grazoprevir in bulk drug and tablet dosage forms.*

Key words: *Elbasvir, Grazoprevir, simultaneous, RP-HPLC method and validation, ICH.*

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I. Introduction

Elbasvir is chemically 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][1,3]benzoxazin-10-yl]-1H-imidazol-2-yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate. Elbasvir is an inhibitor of the Hepatitis C Virus (HCV) Non-Structural protein 5A (NS5A), which is essential for viral RNA replication and virion assembly. Elbasvir is a compound with molecular formula $C_{49}H_{55}N_9O_7$ and molecular weight of 882.035 gm/mol1. It is sparingly soluble in methanol and insoluble in water. Grazoprevir is chemically(1aR,5S,8S,10R,22aR)-5-tert-butyl-N-{(1R,2S)-1-[(cyclopropanesulfonyl)carbamoyl]-2-ethenylcyclopropyl}-14-methoxy-3,6-dioxo1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8H-7,10methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-b] quinoxaline-8-carboxamide.Grazoprevir is a direct acting second generation antiviral medication used as part of combination therapy to treat chronic Hepatitis C, which is a liver disease caused by infection with Hepatitis C Virus (HCV). Grazoprevir is a compound with molecular formula $C_{38}H_{50}N_6O_9S$ molecular weight of 766.903 gm/mol[2]. Grazoprevir is very slightly soluble in water, soluble in methanol and acetonitrile. Elbasvir, when used in combination with grazoprevir as the combination product Zapatier[3,4], is indicated for use with or without ribavirin for the treatment of chronic hepatitis C virus (HCV) genotypes 1 or 4 infections in adults.

The literature survey indicates that Elbasvir and Grazoprevir estimation were carried by various UV, HPLC and HPTLC analytical techniques[5-9]. As Zapatier is a new combination product recently entered into the market, there are no suitable simple RP- HPLC methods for simultaneous estimation of Elbasvir and Grazoprevir were reported. Hence, this study was performed to develop a specific method for estimation of Elbasvir and Grazoprevir simultaneously using RP-HPLC.

II. Materials And Methods

All the chemicals and reagents procured and used were of AR/HPLC grade. Pure standards of Elbasvir, Grazoprevir gift samples obtained from Merck India. HPLC grade methanol, acetonitrile as well as AR grade Potassium dihydrogen phosphate were obtained from Merck India (Mumbai, India). Chromatographic analysis was done using Shimadzu HPLC (LC 20 AT VP) with LC solutions software.

Preparation of mobile phase:

Isocratic mixture of 80 volumes of methanol (80%) and 20 volumes of water (20%) were taken and degassed for 5 minutes in ultra sonicator and vaccum filtered through 0.45 µ filter.

Preparation of standard stock solution of Elbasvir

Accurately 10 mg of Elbasvir was weighed and transferred in to a clean dry 100ml volumetric flask and about 10ml of methanol was added. Then it was sonicated to dissolve the drug completely and made volume up to the mark with methanol. From that, 10 µg /ml of Elbasvir solution was made by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Grazoprevir

Accurately 10mg of Grazoprevir was weighed and transferred in to a clean dry 100ml volumetric flask and about 10ml of methanol was added. Then it was sonicated to dissolve the drug completely and made volume up to the mark with methanol. From that, 10 µg /ml of Grazoprevir solution was made by diluting 1ml to 10ml with methanol.

Preparation of tablet sample solution:

Accurately 10 tablets (each tablet contains Grazoprevir 100mg and Elbasvir 50mg) were weighed and transferred into a clean dry mortar and crushed with clean pestle to get uniformity. Tablet stock solutions of Grazoprevir and Elbasvir were prepared by dissolving weight equivalent to 10 mg of GRAZOPREVIR and 60 mg of ELBASVIR were taken into 100ml volmetric flask and around 10ml of mobile phase was added to dissolve the drugs aided by sonication for 5 min. Then the solution was filtered through 0.45µ syringe filter, Further dilutions were prepared by adding 1 ml of stock solution to 10 ml of mobile phase to get 10µg/ml of Grazoprevir and 60µg/ml of Elbasvir.

Procedure:

20µl of the prepared standard and sample solutions were injected into the chromatographic system and the areas of Elbasvir and Grazoprevir peaks were measured.

ANALYTICAL METHOD VALIDATION[10-12]

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analysed sample solution at three different levels 100%, 120%, 140%. Each concentration was measured in triplicate and the percentage recovery and percentage mean recovery were calculated for both the drugs as depicted in Table 1.

Precision (Repeatability)

Procedure:

The standard solutions of Elbasvir and Grazoprevir were injected repeatedly for six times and the areas for all six injections in HPLC were measured and %RSD was calculated and depicted in Table 2.

Specificity

Specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

LOD and LOQ

LOD and LOQ values were calculated by diluting known concentrations of of Elbasvir and Grazoprevir till the normal response was approximately 3 to 10 times the standard deviation (SD) of response (peak area) for the three replicate determinations.

Linearity

Linearity was calculated by preparing aliquots of standards to obtain final concentrations of 36-84 µg/ml Elbasvir and 6-14µg/ml Grazoprevir. Around 20 µl of the standard solutions were injecting into the column and peak areas were recorded. Calibration plots were constructed with average peak areas *versus* concentrations and regression equations were figured for both the drugs.

Robustness:

To demonstrate the robustness of the method, both the drug solutions were injected at variable conditions like varied flow rate and wavelength.

a) Standard solution of Elbasvir Grazoprevir were analyzed at varied flow rates 0.8ml/min and 1.2 ml/min.

b) Standard solution of Elbasvir Grazoprevir were analyzed at varied wavelengths 258nm and 262nm.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

III. Results And Discussion

The objective was to develop a unique HPLC method for the simultaneous detection and quantitation of Elbasvir and Grazoprevir. The isobestic point was measured by preparing 10µg/ml of individual and mixed standards and the solution was scanned in U.V region from 200-400nm. The intersection spectrum of Elbasvir and Grazoprevir was obtained and the isobestic point was observed at 260 nm. Many trails were performed with various column and several mobile phases compositions under gradient and isocratic conditions to optimize appropriate conditions for the detection and quantification of Elbasvir and Grazoprevir.

Although the use of buffers is advocated and often used in RP chromatographic separations, it would be prudent to avoid buffers in view of the harsh pH conditions and inevitable accumulation of salt in the HPLC system, column and column frits. Hence it is most desirable that a simple HPLC mobile phase and pH conditions that provide satisfactory resolution of sample components for the purposes of estimation at lowest possible concentrations.This study results indicate a good resolution between the compounds with appropriate peak shapes and retention times were achieved on a Inertsil ODS, C18 (150x4.6 ID) 5µm column using a linear gradient of mobile phase consisting of methanol: water (80:20) at a flow rate of 1 ml/min under ambient column temperature.

A demonstrative chromatogram showing resolution between Elbasvir and Grazoprevir is shown in Fig. 1.

The LOD and LOQ values for Elbasvir and Grazoprevir were found to be 0.81 µg/ml, 2.46µg/ml and 0.53µg/ml, 1.633 µg/ml respectively. The LOD for Elbasvir and Grazoprevir were found to be 0.81 µg/ml and 0.53µg/ml respectively. The LOQ for Elbasvir and Grazoprevir were found to be 2.46µg/ml and 1.633µg/ml respectively**.** The results for validation and system suitability test parameters are summarized in Table 4. Results for robustness evaluation for both the drugs are presented in Table 3. Insignificant differences in peak areas and less variability in retention times were observed.

A validated HPLC method for the simultaneous quantification of Elbasvir and Grazoprevir has been established as per ICH guidelines. It has shown that the developed method achieved accuracy, reproducibility, repeatability, linearity, precision, and selectivity, which prove the reliability of the method. The method enabled accurate, sensitive, and reproducible quantification of tablet formulation in routine analysis. The result shows that the method could find practical application as a quality control tool for the simultaneous estimation of two drugs from their combined dosage form in a quality control laboratory.

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Table 1: Recovery results for Elbasvir and Grazoprevir

Recovery level	**Amount taken**		**Amount recovered**	
	Elbasvir Taken(mcg/ml)	Grazoprevir Taken(mcg/ml)	Elbasvir (mcg/ml)	Grazoprevir (mcg/ml)
100%	60	10	31.78	8.7
	60	10		
	60	10		
120%	72	12	40.04	10.02
	72	12		
	72	12		
140%	84	14	79.76	13.03
	84	14		
	84	14		
Average % Recovery			98.33%	102.45%

Table 2: Results for Method precision of Elbasvir and Grazoprevir

ELBASVIR		
Injection Number	Retention Time	Area
1	2.443	5710.568
2	2.417	5683.849
3	2.423	5662.646
4	2.423	5679.338
5	2.447	5659.977
6	2.423	5645.244
avg	2.429333	5673.604
stdev	0.01242	22.86586
%RSD	0.005113	0.00403

GRAZOPREVIR		
Injection Number	Retention Time	Area
1	4.293	991.742
2	4.257	954.143
3	4.270	948.278
4	4.263	955.360
5	4.293	951.175
6	4.267	968.288
avg	4.273833	961.4977
stdev	0.015471	16.33345
%RSD	0.00362	0.016988

Table 3: Robustness of Elbasvir and Grazoprevir

Parameter	**ELBASVIR**		**GRAZOPREVIR**	
	Retention time(min)	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow Rate				
0.8 ml/min	3.040	1.429	5.343	1.261
1.2 ml/min	2.043	1.400	3.593	1.200
Wavelength				
258nm	2.450	1.379	4.293	1.263
262nm	2.420	1.448	4.270	1.225

Table 4: Summary of validation and system suitability parameters

Parameter (Units)	Elbasvir	Grazoprevir
Linearity range (µg/ml)	36-84 µg/ml	6-14 µg/ml
Correlation coefficient	0.999	0.999
LOD (µg/ml)	0.81 µg/ml	0.53 µg/ml
LOQ (µg/ml)	2.46 µg/ml	1.63 µg/ml
Recovery (%)	98.33 %	102.45%
Retention time±allowable time (min.)	2.44±0.20	4.27±0.20
% Purity	100.3%	101.1%
Theoretical Plates	2278	4109
Tailing Factor (asymmetry factor)	1.42	1.25

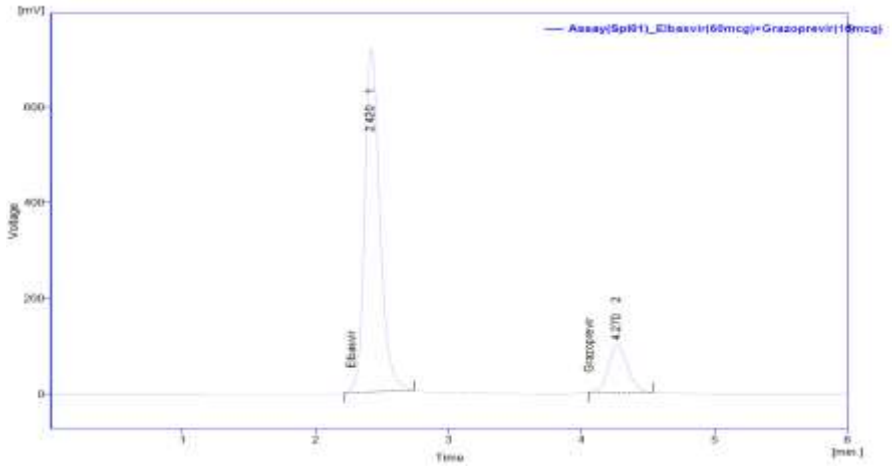

Fig. 1. Chromatogram of Elbasvir and Grazoprevir using methanol: water (80:20%v/v)

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