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Stability indicating RP-HPLC method development and validation for the simultaneous estimation of *Grazoprevir* and *Elbasvir* in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, Accurate and precise method was developed for the simultaneous estimation of the Grazoprevir and Elbasvir in Tablet dosage form. Chromatogram was run through Kromosil C18 (250 x 4.6 mm), 5m. Mobile phase containing Buffer: Acetonitrile taken in the ratio 45:55 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was Di Potassium Hydrogen ortho Phosphate. Temperature was maintained at 30°C. Optimized wavelength selected was 215 nm. Retention time of Elbasvir and Grazoprevir and were found to be 2.503 min and 3.004. %RSD of the Elbasvir and Grazoprevir were and found to be 0.3 and 0.4 respectively. %Recovery was obtained as 98.17% and 99.83% for Grazoprevir and Elbasvir respectively. LOD, LOQ values obtained from regression equations of Grazoprevir and Elbasvir were 0.24, 0.73 and 0.06, 0.19 respectively. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Grazoprevir; Elbasvir; RP-HPLC

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INTRODUCTION

Development of simple and reproducible analytical methods for estimation of multicomponent drugs is very important part of quality control and for social awareness which is established in present work [1]

Grazoprevir[3] is a second generation protease inhibitor approved for the treatment of hepatitis C virus (HCV) in combination with Elbasvir as the fixed-dose combination product Zepatier (FDA). Use of this medication is indicated, with our without ribavirin, for the treatment of adults with HCV genotypes is an integral part of viral replication as it is responsible for cleaving the long polypeptide produced following translation of the viral genome[2]. By inhibiting protease activity, Grazoprevir prevents the formation of structural and non-structural proteins required for replication and assembly.

Elbasvir[4] is an inhibitor of the Hepatitis C Virus (HCV) Non-Structural protein 5A (NS5A). Although NS5A has no known enzymatic function, it has been shown to have multiple functions at various stages of the life cycle, including viral replication, virion assembly, and use within multi-protein binding complexes. Combining Elbasvir with other drugs that target other points of the viral life cycle and with non-overlapping resistance profiles results in increased potency and an improved barrier to resistance[2].

MATERIALS AND METHODS

Chemical and Reagents: Grazoprevir and Elbasvir were kindly gifted by Nutech Labs Pvt Ltd, Hyderabad

certified to contain 99.9% and 99.6% purity respectively. The drugs were used without further purification. All the solvents used in analysis were of HPLC grade.

HPLC method

Instrument: LC system used consists of Waters HPLC having Empower Software with 2695 separation module having PDA detector with universal loop injector of injection capacity 20 µL. The column used was Kromosil C18 Column, 5µ (250× 4.6 mm) at ambient temperature. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously.

Optimised Chromatographic conditions

The mobile phase having DiPotassium hydrogen phosphate buffer (pH 4.0) and Acetonitrile in the ratio of 45:55 v/v was selected because it was found that it ideally resolve the peaks with retention time (RT) 3.004 min and 2.503 min for Grazoprevir and Elbasvir respectively. Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200nm to 350nm. Both the components showed reasonably good response at 215 nm.

Preparation of Phosphate Buffer: Accurately weigh 1.732g of DiPotassium hydrogen ortho phosphate was taken in a 500ml volumetric flask, dissolved and diluted to 500ml with HPLC water and the volume was adjusted to pH 4.0 with Ortho Phosphoric Acid.

Preparation of Mobile Phase: Accurately measured 450 ml (45%) of above buffer and 550 ml of Acetonitrile (HPLC Grade) (55%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: Water and Acetonitrile in the ratio 50:50

Standard Solution Preparation

Accurately Weighed and transferred 10mg of Grazoprevir and 5mg of Elbasvir working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, Sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above stock solution was taken into a 10ml volumetric flask and made up to 10ml.

Procedure: Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Grazoprevir and Elbasvir peaks and calculate the %Assay by using the formulae.

Pharmaceutical Sample Solution (from Formulation)

Sample Solution Preparation: 1tablet was weighed, powdered and then was transferred into a 100mL volumetric flask, 50mL of diluent added and Sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1 ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

RESULTS AND DISCUSSION

Preparation of Calibration Curves by HPLC: In a series of 10 ml volumetric flask several dilutions of Grazoprevir (25-150 µg/ml) and Elbasvir (12.5-75 µg/ml) were prepared using mobile phase as solvent. Each solution was injected into HPLC system and the chromatograms were recorded. The peak areas of both drugs were calculated and the respective calibration curves were plotted against ratio of area under curve and concentration of drug.

The equations of the regression lines obtained are

For Grazoprevir: $R^2 = 0.999$ for Elbasvir: $R^2 = 0.999$

HPLC Method Validation: As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, Specificity, precision, limit of detection, limit of quantitation.

Linearity

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of Grazoprevir and Elbasvir are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of Grazoprevir, and 12.5ppm, 25ppm, 37.5ppm, 50ppm, 62.5ppm, 75ppm of Elbasvir.

Precision

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Precision was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for each title ingredients. The repeatability (within-day in triplicates) and intermediate precision (for 2 days) were carried out at five concentration levels for each compound. Triplicate injections were made and the obtained results within and between the days of trials were in acceptable range. The value of %RSD for Grazoprevir and Elbasvir were found to be less than 2 indicates that the developed method is precise.

Accuracy

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its linearity range. Accuracy was performed in three different levels, each level in triplicate for Grazoprevir and Elbasvir using standards at 50%, 100% and 150%. Each sample was analysed in

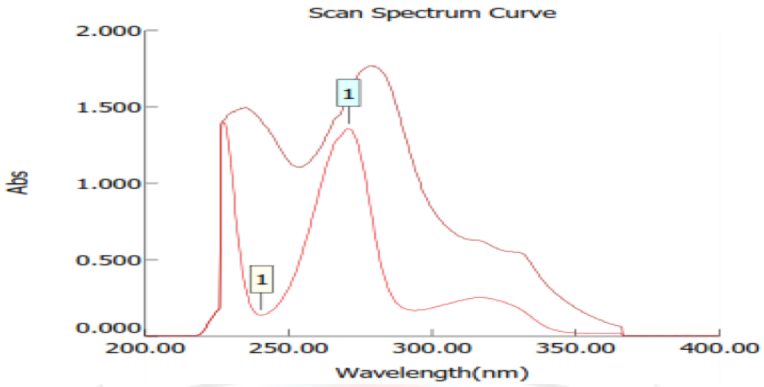

Figure 1: Overlain UV Spectrum of Grazoprevir and Elbasvir

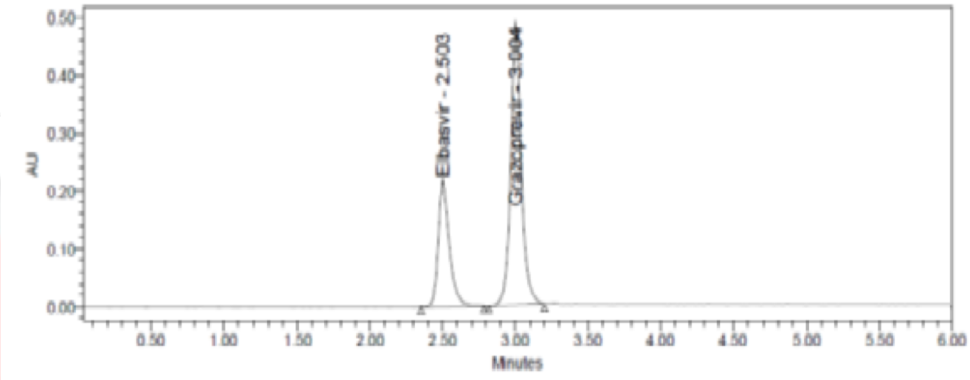

Figure 2: Chromatogram of Standard solution 1. Elb (Rt 2.503), 2.Gra (Rt 3.004) at 215 nm

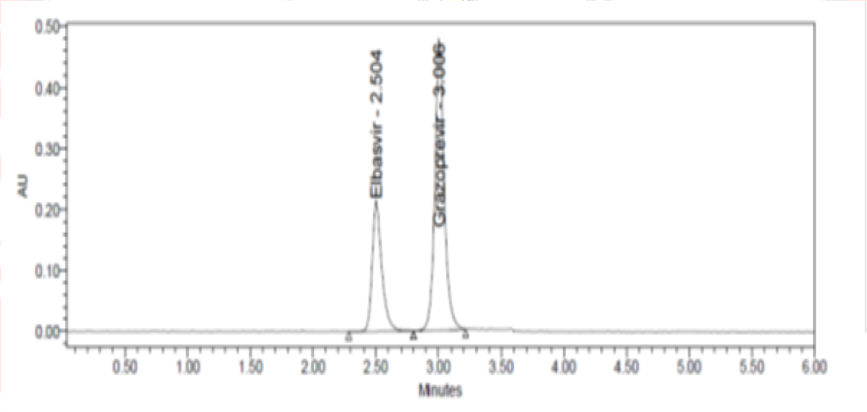

Figure 3: Chromatogram of Sample solution

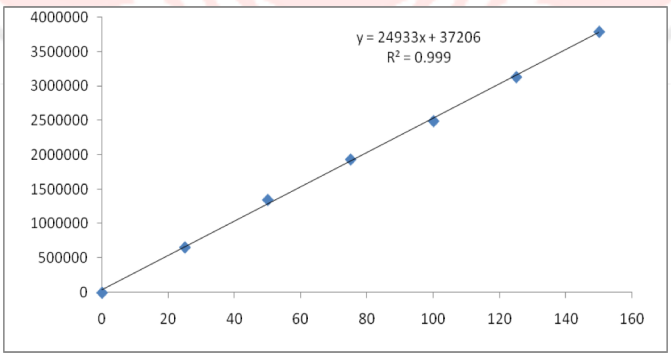

Figure 4: Linerity curve of Grazoprevir

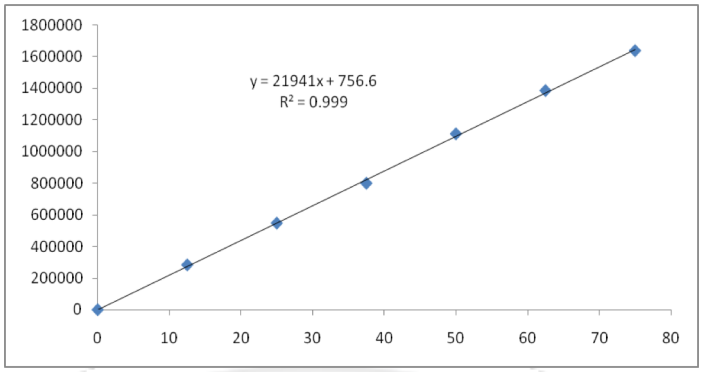

Figure 5: Linearity curve of Elbasvir

Table 1: Precision Results for Grazoprevir and Elbasvir

S. No	Area of Grazoprevir	Area of Elbasvir
1.	2492363	1192724
2.	2497047	1187341
3.	2496265	1181393
4.	2490241	1192596
5.	2491112	1189902
6.	2511739	1195604
Mean	2496461	1189927
S.D	7973.6	5033.7
%RSD	0.3	0.4

Table 2: The accuracy results for Grazoprevir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	50	49.27554	98.55	99.17%
	50	49.36205	98.72	
	50	50.16528	100.33	
100%	100	99.54017	99.54	
	100	100.117	100.12	
	100	100.5057	100.51	
150%	150	147.3853	98.26	
	150	147.3525	98.24	
	150	147.3391	98.23	

Table 3: The accuracy results for Elbasvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	25	24.7406	98.96	99.83%
	25	25.17932	100.72	
	25	25.16172	100.65	
100%	50	50.38295	100.77	
	50	50.3068	100.61	
	50	50.3577	100.72	
150%	75	74.36386	99.15	
	75	73.69611	98.26	
	75	73.9683	98.62	

Table 4: LOD and LOQ

Drug	LOD	LOQ
Grazoprevir	0.24	0.73
Elbasvir	0.06	0.19

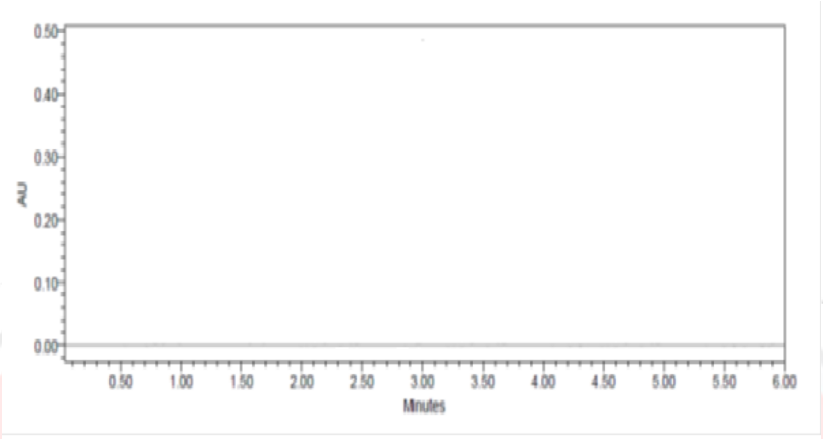

Figure 6: Specifity of Blank Chromatogram

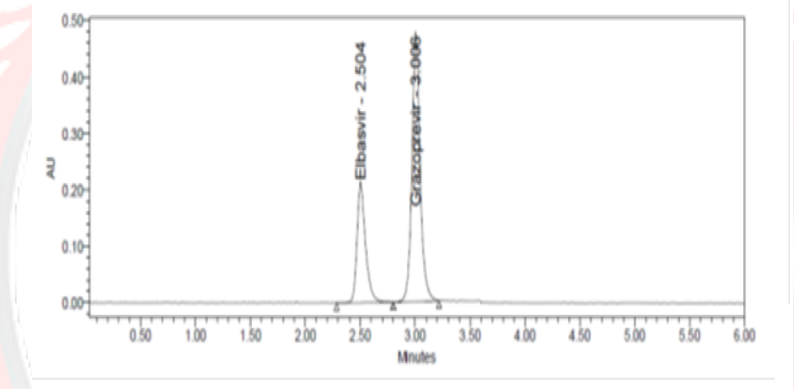

Figure 7: Specifity of Drug Chromatogram

Table 5: Degradation Data of Grazoprevir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.54	0.663	0.795
2	Alkali	2.75	0.807	0.988
3	Oxidation	1.70	0.663	0.895
4	Thermal	0.73	0.807	0.988
5	UV	0.90	0.746	0.955
6	Water	0.73	0.601	0.860

Table 6: Degradation Data of Elbasvir

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.80	1.123	1.315
2	Alkali	2.48	0.964	1.319
3	Oxidation	1.57	1.123	1.315
4	Thermal	0.56	0.964	1.319
5	UV	0.56	0.984	1.314
6	Water	0.53	0.964	1.319

triplicate for each level. The mean recoveries were found in the range of 98 – 102%, by which we can say the method was accurate.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

Specifity

Volume of 20 µL of working placebo sample solution was injected into the chromatograph and the chromatogram was recorded and presented below.

Degradation studies

Oxidation: To 2 ml of stock solution of Grazoprevir and Elbasvir, 2 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 100µg/ml&50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 2 ml of stock s solution Grazoprevir and Elbasvir, 2ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c .The resultant solution was diluted to obtain 100µg/ml&50µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 2 ml of stock solution Grazoprevir and Elbasvir, 2 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 100µg/ml&50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml&50µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 1000µg/ml& &500µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/ml 50µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under the neutral conditions was studied by refluxing the drug in water for 1 hour at 60°C temperature. For HPLC study, the resultant solution was diluted to 100µg/ml& &50µg/ml solution and 10 µl were injected into the system and chromatograms were recorded to access the stability of sample.

CONCLUSION

The methods described for simultaneous estimation of Grazoprevir and Elbasvir are found to be simple, sensitive, accurate, precise, rapid and economical. Hence method could be successfully employed for routine analysis of Grazoprevir and Elbasvir in their combined dosage form.

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