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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ELBASVIR AND GRAZOPREVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, Accurate and precise method was developed for the simultaneous estimation of Elbasvir and Grazoprevir in Tablet dosage form. Chromatogram was run through Inertsil ODS C_{18} (250 x 4.6 mm, 5µ) Column. Mobile phase containing Methanol: Acetonitrile taken in the ratio 35: 65(pH 4.6 adjusted with OPA) was pumped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 259 nm. Retention time of Elbasvir and Grazoprevir and were found to be 2.499 min and 2.998 min. respectively. %RSD of the Elbasvir and Grazoprevir were and found to be 0.5 and 0.8 respectively. % Recovery was obtained in the range of 98-102% for Grazoprevir and Elbasvir respectively. LOD, LOQ values obtained from regression equations of Grazoprevir and Elbasvir were 0.21, 0.78 and 0.09, 0.24 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: Elbasvir, Grazoprevir, RP-HPLC.

INTRODUCTION

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

Elbasvir[2] 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.

Grazoprevir[3] is a second generation NS3/4a protease inhibitor approved for the treatment of hepatitis C virus (HCV) in combination with Elbasvir as the fixed-dose combination product Zepatier (FDA). By inhibiting protease activity, Grazoprevir prevents the formation of structural and non-structural proteins required for replication and assembly.

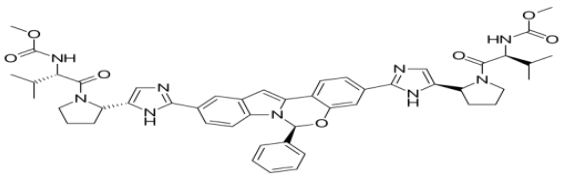

Fig 1: Chemical Structure of Elbasvir.

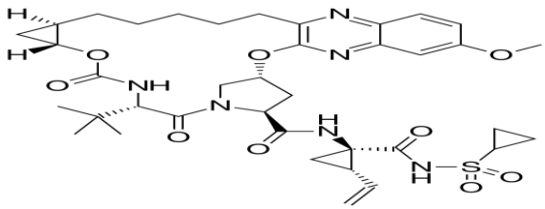

Fig 2: Chemical Structure of Grazoprevir.

MATERIALS AND METHODS

Chemical and Reagents

Elbasvir and Grazoprevir were kindly gifted by Nutech Biosciences Pvt ltd certified to contain 99.94% and 99.96% 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 20µL. The column used was Inertsil ODStandard discovery C_{18}(250x4.6mm)5µ at ambient temperature. Different mobile phases were tested in order to find the best conditions for separating both the drugs simultaneously.

Optimized Chromatographic conditions

The mobile phase having Methanol: Acetonitrile (35:65) v/v was selected because it was found that it ideally resolve the peaks. The retention time was found to be 2.499 min and 2.998 minutes for Elbasvir and Grazoprevir respectively. Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200 nm to 400 nm. Both the components showed reasonably good response at 259 nm.

Preparation of Mobile Phase

Accurately measured 350 ml (35%) of Methanol and 650 ml of Acetonitrile (65%) 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 Preparation

Accurately Weighed and transferred 10 mg of Elbasvir and 20 mg of Grazoprevir 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 with diluent.

Sample Preparation

10 tablets were weighed and weight equivalent to 10 mg of Elbasvir and 20 mg of Grazoprevir was powdered and then 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 pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Recovery studies

To check the accuracy of sample by the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 50, 100 and 150% level. From the total amount of drug found, the percentage recovery was calculated.

RESULTS AND DISCUSSION

HPLC Method Validation

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

Specificity: The system suitability for 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.

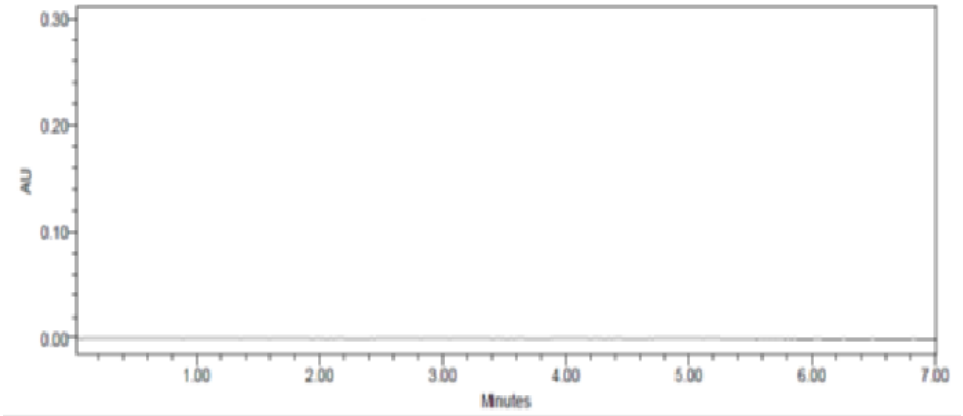

Fig 3: Blank Chromatogram.

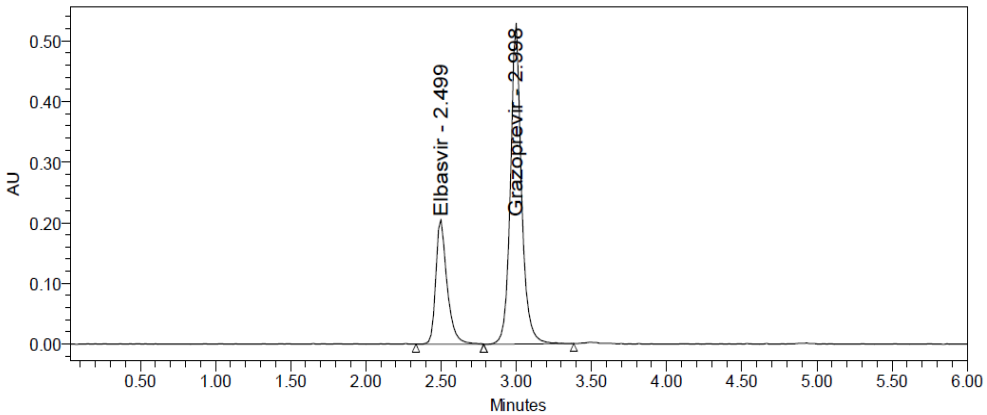

Fig 4: Standard Chromatogram of Elbasvir and Grazoprevir.

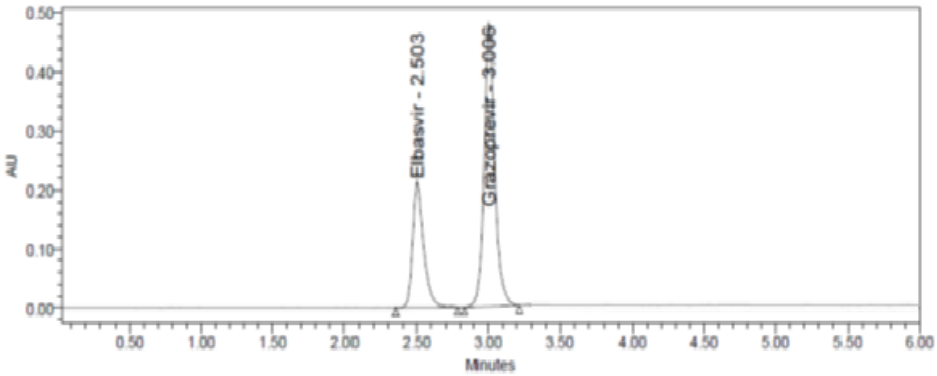

Fig 5: Sample Chromatogram of Elbasvir and Grazoprevir.

Linearity

The linearity of the proposed RP-HPLC method for determination of Elbasvir and Grazoprevir was evaluated by analysing a series of different concentrations of standard drug.

In this study five concentrations were chosen, ranging between 12.5-75 µg/ml of Elbasvir and 25-150 µg/ml of Grazoprevir. Each concentration was repeated three times. The linearityof the calibration graphs was validated by the high value of correlation coefficient, slope andthe intercept value.

Table 1: Linearity Table of Elbasvir and Grazoprevir.

Grazoprevir		Elbasvir	
Conc. (µg/mL)	**Peak area**	**Conc. (µg/mL)**	**Peak area**
25	668123	12.5	293765
50	1247856	25	557492
75	1944623	37.5	798576
100	2491298	50	1111612
125	3230897	62.5	1395289
150	3789046	75	1647281

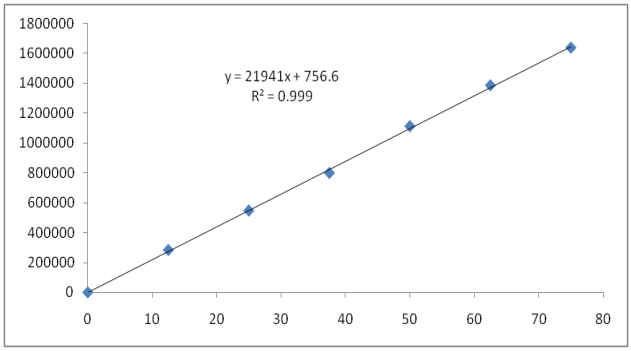

Fig 6: Calibration curve of Elbasvir.

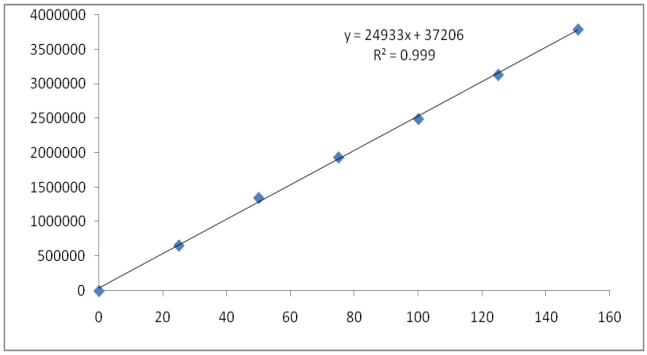

Fig 7: Calibration curve of Grazoprevir.

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 RP- 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 %RSD values for Elbasvir and Grazoprevir were found to be less than 2 indicate that the developed method was precise.

Table 2: Precision Data for Elbasvir and Grazoprevir.

S. No	Area of Elbasvir	Area of Grazoprevir
1.	1139123	2472672
2.	1141891	2457939
3.	1138987	2471417
4.	1137123	2484924
5.	1145642	2462281
6.	1152531	2445152
Mean	1142533	2465712
S.D	5713.4	13779.0
%RSD	0.5	0.8

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 Elbasvir and Grazoprevir using standards at 50%, 100% and 150%. Each sample was analysed in triplicate foreach 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.

Table 3: Sensitivity Tables of Elbasvir and Grazoprevir.

Molecule	LOD	LOQ
Grazoprevir	0.21	0.78
Elbasvir	0.07	0.17

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 4: Robustness data for Elbasvir and Grazoprevir.

S.No	Condition	%RSD of Elbasvir	%RSD of Grazoprevir
1	Flow rate (-) 1.1ml/min	0.4	0.5
2	Flow rate (+) 1.3ml/min	0.6	1.1
3	Mobile phase (-) 60B:40A	0.4	1.8
4	Mobile phase (+) 50B:50A	0.4	0.3
5	Temperature (-) 25°C	0.5	0.2
6	Temperature (+) 35°C	0.4	0.4

CONCLUSION

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

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