ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

RP-HPLC ANALYTICAL METHOD VALIDATION FOR ELBASVIR AND GRAZOPREVIR IN BULK AND ITS TABLETS

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ARTICLE INFO

Key words:
Elbasvir, grazoprevir, ICH guidelines, anti-viral drug, accuracy, precision

Access this article online
Website:
https://www.jgtps.com/
Quick Response Code:

ABSTRACT

An innovative, precise, accurate, fast, and cost-effective RP-HPLC method was developed, optimized, and validated to estimate elbasvir (ELB) and grazoprevir (GRA) in bulk and pharmaceutical dose forms. The drug was estimated using a Phenomenex Gemini C_{18} (4.6 mm x 150 mm, 5.0 µm) particle size column. The separation was carried out using methanol and tri ethylamine buffer in the ratio of 32:68 v/v and flow rate was set to 1.0 ml/min. 248 nm was discovered to be the detecting wavelength. With retention time (R_t) of 3.297 min for elbasvir and 5.405 min for grazoprevir, the linearity ranges were found to be 30-70 µg/ml for elbasvir and 10-50 µg/ml for grazoprevir respectively. For both medications, the correlation coefficient values were 0.999. Elbasvir and grazoprevir percentage recoveries were determined to be 100.1873% and 100% respectively.

INTRODUCTION:

Elbasvir is a direct-acting anti-viral drug is prescribed in combination therapy to treat chronic Hepatitis C, an infectious liver circumstance laid on by HCV infection.[1-3] The structure is shown in Figure 1.

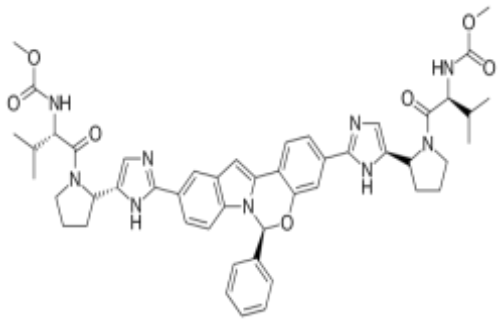

Figure 1: Structure of elbasvir

Grazoprevir 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).[4, 5] The structure is depicted in Figure 2.

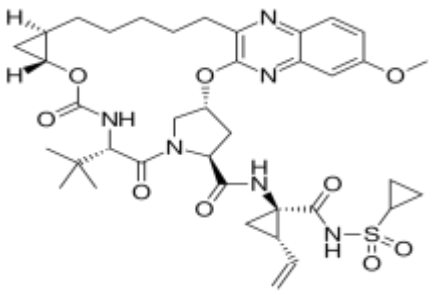

Figure 2: Structure of grazoprevir

The aim of the present research is to develop a simple, accurate, and precise RP-HPLC method development for determining the quantity of elbasvir and grazoprevir in both its bulk and tablet dosage form.

MATERIALS AND METHODS

Instrumentation: Chromatographic analysis was done using waters HPLC with auto sampler and PDA Detector 996 model, Phenomenex Gemini C_{18} (4.6mm×150mm, 5.0 µm) particle size column.

Chemicals: All the chemicals and reagents procured and used were of AR/HPLC grade. Pure standards of Elbasvir, Grazoprevir gift samples obtained from Sura Labs, Hyderabad. HPLC grade methanol, acetonitrile as well as AR grade. Tablets (Zepatier - Elbasvir/Grazoprevir - 50/100 mg) was purchased from Apollo Pharmacy, Hyderabad.

Method development[6-8]

Preparation of elbasvir and grazoprevir working standard: Weigh accurately about 10mg of Elbasvir and Grazoprevir transfer into separate 10ml volumetric flask dissolve with diluent and make up the volume with the diluent (stock solution). Pipette out about 0.5ml of the above elbasvir and 0.3ml of the grazoprevir stock solution into a 10ml volumetric flask and dilute up to mark with methanol.

Preparation of elbasvir and grazoprevir sample solution[9,10]: Take average weight of 10 tablets and crush in mortar and pestle weigh equivalent to 10 mg of elbasvir and grazoprevir transfer into separate 10ml volumetric flask dissolve with diluent and make up the volume with the diluent (stock solution). Pipette out about 0.5ml of the above elbasvir and 0.3ml of the grazoprevir stock solution into a 10ml volumetric flask and dilute up to mark with methanol. The combined marketed dosage form of elbasvir and grazoprevir brand name is Zepatier (50/100mg) manufactured by Merck. Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%\text{Assay} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Chromatographic conditions[11]: Using waters HPLC with sampler and PDA Detector 996 model used for chromatographic conditions for Elbasvir and Grazoprevir are TEA buffer (pH 4.8) combined with methanol (32:68 v/v) produced the best peak shape, Phenomenx Gemini C_{18} column (4.6×150 mm, 5 µm). The flow rate was set to 1 ml/min. Detection wave length is 248 nm and sample injection volume was 20µl. The column oven's temperature was set at 38 ^{0}C. Total analysis run time is 7 min.

Analytical method validation[12-14]:

Linearity: For elbasvir and grazoprevir linearity studies calibration standards were prepared, and five duplicate assessments were conducted over a period to obtain the linearity range of 30, 40, 50, 60, 70 and 10, 20, 30, 40 and 50 µg/ml respectively. The calibration curve was plotted with area of peaks and concentration of the drug. The % RSD was calculated.

Preparation of solution: Weigh accurately about 10mg of elbasvir and grazoprevir transfer into separate 10ml volumetric flask dissolve with diluent and make up the volume with the diluent (stock solution).

Level – I (30ppm of elbasvir and 10ppm of grazoprevir) : Pipette out 0.3ml of the elbasvir and 0.1ml of the grazoprevir from the above stock solutions in to a 10ml of volumetric flask and dilute the solution.

Level – II (40ppm of elbasvir and 20ppm of grazoprevir): Pipette out 0.4ml of the elbasvir and 0.2ml of the grazoprevir from the above stock solutions in to a 10ml of volumetric flask and dilute the solution.

Level – III (50ppm of elbasvir and 30ppm of grazoprevir) Pipette out 0.5ml of the

elbasvir and 0.3ml of the grazoprevir from the above stock solutions in to a 10ml of volumetric flask and dilute the solution.

Level – IV (60ppm of elbasvir and 40ppm of grazoprevir) Pipette out 0.6ml of the elbasvir and 0.4ml of the grazoprevir from the above stock solutions in to a 10ml of volumetric flask and dilute the solution.

Level – V (70ppm of elbasvir and 50ppm of grazoprevir) Pipette out 0.7ml of the elbasvir and 0.5ml of the grazoprevir from the above stock solutions in to a 10ml of volumetric flask and dilute the solution.

Accuracy: To determine the accuracy of the developed method, an elbasvir and grazoprevir recovery studies was performed. The method's accuracy was assessed by using the usual addition method to calculate elbasvir and grazoprevir recoveries. Pre-quantified sample solution (10µg/ml) was mixed with a known volume of elbasvir and grazoprevir standard solutions (50%, 100%, and 150%). A calibration curve was used to determine the amount of elbasvir and grazoprevir.

Precision: For several samplings of a homogeneous sample, the precision of the analytical method was determined. Repeatability and intermediate precision measurements of peak area and peak symmetry parameters were used to demonstrate the reproducibility of the method. Single concentration levels were used to test the intermediate precision (for two days) and repeatability (within a day in triplicates). Six injections were performed, and the findings were expressed as a percentage of RSD both within and between trial days.

Robustness: Deliberate modifications to the procedure, such as changing the flow rate and detection wavelength, were used to examine the method's robustness.

Limit of detection (LOD) and Limit of quantification (LOQ): Signal-to-noise ratio is the basis for the method used in the calculation of LOD and LOQ, as per ICH guidelines. A signal to noise ratio of 3:1 and 10:1 was used for calculating LOD and LOQ.

RESULTS AND DISCUSSION

The assay of tablet dosage form results is depicted in Table 1, and the system suitability parameters of elbasvir and grazoprevir were shown in Table 2. Figure 3 shows the chromatographic condition of elbasvir and grazoprevir.

Accuracy- Three replicate injections of individual concentrations (50%, 100% and 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the amount found and amount added for elbasvir and grazoprevir and calculate the individual recovery and mean recovery values. The results are tabulated in Table 4

Precision- The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Intermediate precision: To evaluate the intermediate precision (also known as ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Repeatability- Five replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD. Tables 5 & 6 display the results.

Robustness- The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for elbasvir and grazoprevir. The method is robust only in less flow condition and the method is robust even by change in the mobile phase ±5%.

Table 1: Assay of tablet dosage form

Drug	Label claim	Amount found	% Assay
Elbasvir	50 mg	49.2	98.4
Grazoprevir	100 mg	99.8	99.8

Table 2: System suitability parameters of elbasvir and grazoprevir

S. No.	Name	RT	Area	Height	USP tailing	USP plate count	Resolution
1	Elbasvir	3.222	865898	43659	1.26	7985	
2	Grazoprevir	5.453	5789	3785	1.38	6659	7.0

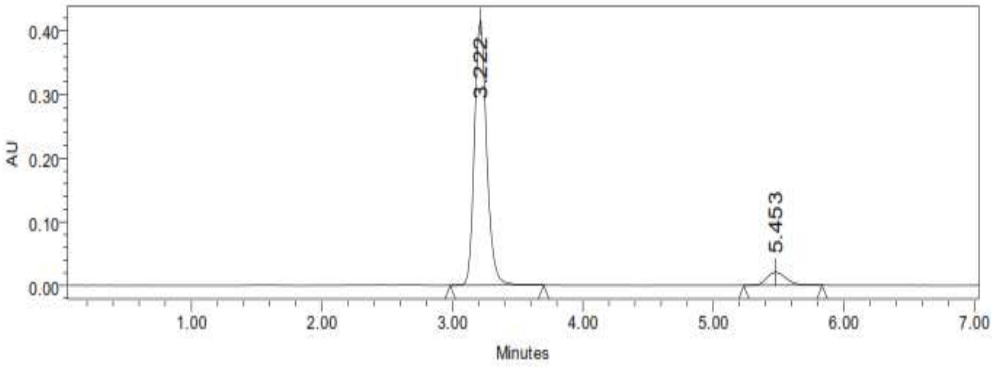

Figure 3: Optimized chromatogram of elbasvir and grazoprevir

Linearity- The linearity findings are displayed in Table 3, Figure 4 and 5.

Table 3: Linearity of elbasvir and grazoprevir

Linearity of elbasvir		Linearity of grazoprevir	
Conc. (µg/ml)	**Peak area**	**Conc.(µg/ml)**	**Peak area**
30	545894	10	2038
40	725985	20	3859
50	897856	30	5698
60	1068594	40	7489
70	1245698	50	9218
Linearity parameters of calibration curve (Y = mx+c)			
Slope (m)	17769	183.6	
Intercept (c)	6945	125.4	
R^2	0.999	0.999	

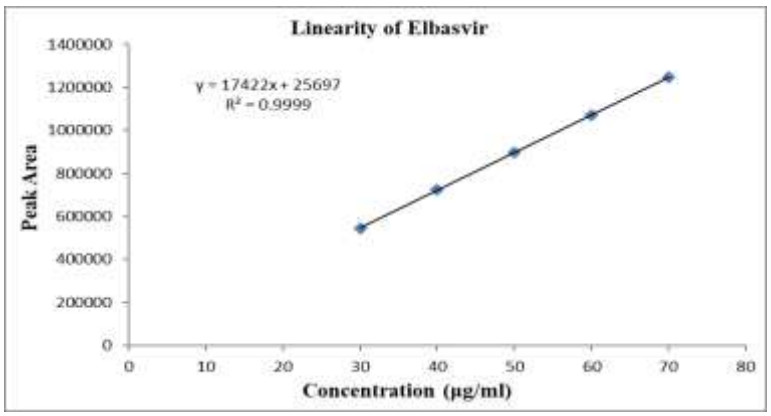

Figure 4: Linearity graph of elbasvir

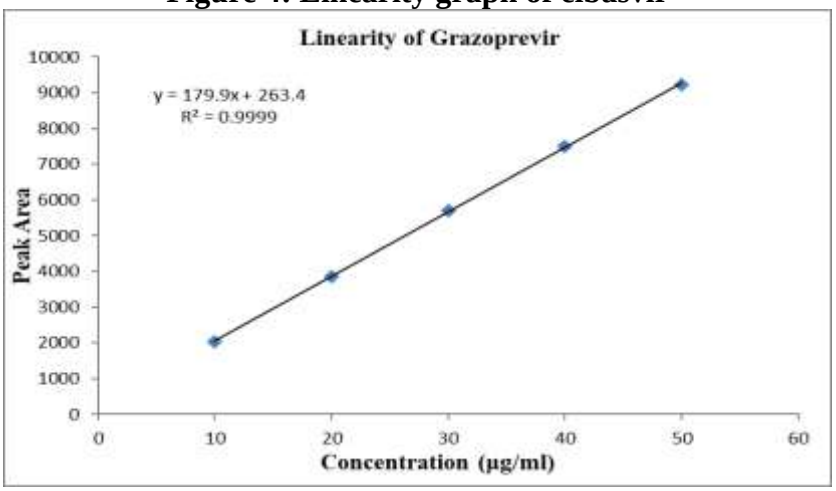

Figure 5: Linearity graph of grazoprevir

Table 4: Recovery studies of elbasvir and grazoprevir

% of Level	Area	Amount added (ppm)	Amount found (ppm)	% Recovery	% Mean recovery
Recovery studies of elbasvir					
50	451144.3	25	24.998	99.992	**100.19%**
100	897248.3	50	50.104	100.208	
150	1344562	75	75.278	100.362	
Recovery studies of grazoprevir					
50	2895	15	15.084	100.560	**100.75%**
100	5685	30	30.282	100.940	
150	8449	45	45.335	100.744	

Table 5: Repeatability of elbasvir

S. No.	Drug name	Retention time	Peak area (µV*sec)	Height	USP plate count	USP tailing
1	Elbasvir	3.213	859856	42659	7859	1.24
2	Elbasvir	3.253	857985	42598	7869	1.24
3	Elbasvir	3.297	856984	42587	7846	1.25
4	Elbasvir	3.215	856987	42569	7819	1.25
5	Elbasvir	3.254	859878	42894	7856	1.24
Mean			**858338**			
Std. dev			**1454.222**			
% RSD			**0.169423**			

Table 6: Repeatability of grazoprevir

S. No.	Drug name	Retention time	Peak area (µV*sec)	Height	USP plate count	USP tailing
1	Grazoprevir	5.441	5697	3659	6592	1.36
2	Grazoprevir	5.442	5689	3648	6539	1.36
3	Grazoprevir	5.409	5698	3692	6584	1.37
4	Grazoprevir	5.520	5639	3648	6579	1.36
5	Grazoprevir	5.424	5688	3689	6549	1.36
Mean			**5682.2**			
Std. dev			**24.57031**			
% RSD			**0.432408**			

Table 7: Robustness of elbasvir

Parameter used for sample analysis	Peak area	Retention time	Theoretical plates	Tailing factor
Actual flow rate of 1.0 mL/min	859856	3.297	7896	1.24
Less flow rate of 0.9 mL/min	915847	3.639	7251	1.20
More flow rate of 1.1 mL/min	842564	2.859	7415	1.21
Less organic phase (about 5% decrease in organic phase)	825498	3.460	7365	1.23
More organic phase (about 5% Increase in organic phase)	814578	3.022	7258	1.22

Table 8: Robustness of grazoprevir

Parameter used for sample analysis	Peak area	Retention time	Theoretical plates	Tailing factor
Actual flow rate of 1.0 mL/min	5698	5.405	6582	1.36
Less flow rate of 0.9 mL/min	6452	6.250	6785	1.32
More flow rate of 1.1 mL/min	5254	4.863	6365	1.34
Less organic phase (about 5% decrease in organic phase)	5487	6.196	6254	1.38
More organic phase (about 5% Increase in organic phase)	5369	5.010	6298	1.33

Table 9: LOD and LOQ

Drug	LOD	LOQ
Elbasvir	2.6 µg/ml	7.8 µg/ml
Grazoprevir	3.4 µg/ml	10.2 µg/ml

The standard sample of elbasvir and grazoprevir were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count. Robustness results are illustrated in Table 7 & 8.

LOD and LOQ- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected and quantitated. The LOD and LOQ values of elbasvir and grazoprevir are 2.6µg/ml, 3.4µg/ml, 7.8µg/ml and 10.2µg/ml

respectively. The values are depicted in Table 9.

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of elbasvir and grazoprevir in pharmaceutical dosage forms. The results are accordance with ICH guidelines. Hence, this method can easily and conveniently adopt for routine quality control analysis of elbasvir and grazoprevir in pure and its pharmaceutical dosage forms. Based on the experimental inquiries, one can infer that the suggested method is viable for the regular analysis of elbasvir and grazoprevir.

Conflict of interest: The authors declare that there are no conflicts of interest.

REFERENCES:

1. Madana GN Sridhar C. Stability indicating validated RP-UPLC method for simultaneous determination of Elbasvir and Grazoprevir in bulk and pharmaceutical dosage form. International Journal Pharmaceutical Sciences Review and Research. 2017; 44(2):43-48.
2. Rockstroh JK, Nelson M, Katlama C, Lalezari J, Mallolas J and Bloch M. Efficacy and safety of grazoprevir (MK5172) and elbasvir (MK- 8742) in patients with hepatitis C virus and HIV. Lancet HIV. 2015; 2(8):319-327.
3. Potluri H, Battula SR, Yeturu S. Picogram level quantification of Grazoprevir and Elbasvir with deuterated internal standards in human plasma samples by LC-ESIMS/MS. *Indian Journal of Pharmaceutical Education and Research*. 2016; 50(4):612-618.
4. Pallapati S, Tirukkovalluri SR, Kallam KR. High Performance Liquid Chromatographic determination of antiviral drugs, Grazoprevir and Elbasvir Simultaneously in bulk and tablets. Indo American Jour of Pharmaceutical Research. 2017; 7(08):120-126.
5. Neelaveni K, Gangu Sreelatha, Gayatri Devi B. Derivative UV-Spectrophotometric Estimation of Tenofovir alafenamide fumarate in Bulk and solid Dosage Form. International Journal of Pharmaceutical research and Applications. 2023; 8(4):1611-1616.
6. Sumalatha Nallagundla. Analytical Method Development and Validation of Elbasvir and Grazoprevir in Bulk and Tablet Formulations by RP-HPLC. International Journal of Pharmaceutical Science Invention. 2017; 6(8):01-05.
7. Akiful Haque M, Shanthi Priya D, Mohanty D, Bakshi V, Boggula N. Analytical method development and validation of amlodipine in human plasma using liquid chromatography-mass spectrometry/mass spectrometry. Asian Journal of Pharmaceutical and Clinical Research. 2018; 11(7):393-397.
8. Sekaran CB, Bind VH, Damayanthi MR, Sireesha A. Development and validation of UV Spectrophotometric method for the determination of rivaroxaban. Der Pharma Chemica. 2013; 5(4):1-5.
9. Naresh Bonagani, Manda Rammohan, V. Nikitha, G. Prashanth, N. Santhosh, D. Praveen Reddy. A Validated RP-HPLC Method for Aspirin and Omeprazole in its Pure and Capsule Dosage Form. *International Journal of*

Pharmaceutical Biological and Chemical Sciences. 2017; 6(4):01-14.
10. Banothu Bhadru, V. Venkata Rao and Narender Boggula. Simultaneous estimation of Levodopa and Benserazide by using Reverse Phase High Performance Liquid Chromatography in API and marketed formulation. *International Journal of Recent Advances in Multidisciplinary Research*. 2023; 10(10):9020-9024.
11. Swapna B, Kiran G, Vasudha B, Kumar JR. Stability indicating RP-HPLC method for simultaneous estimation of betamethasone dipropionate and calcipotriene in bulk and pharmaceutical dosage form. Biointerface Research in Applied Chemistry. 2018; 8(1):3089-94.
12. Srinidhi M, Basha MM, Kumar VR, Kumar JR. Stability indicating RP-HPLC method development and validation for the estimation of sumatriptan in bulk and pharmaceutical dosage form. Journal of Applied Pharmaceutical Science. 2016; 6(6):020-5.
13. Ananda Kumar Chettupalli, Vivek Kunduru, Narender Boggula, Vasudha Bakshi. Development and Validation of Capecitabine Tablet (Pharmaceutical Dosage Form) by using RP-HPLC Method. Indo Am. J. P. Sci. 2017; 4(03):550-557.
14. Narender Boggula, Banothu Bhadru, Naveen Pathakala, Himabindu Peddapalli, Sunand Katta. Method Development and Validation of RP-HPLC Method for the Estimation of Tolvaptan in Bulk and its Tablet Dosage Form. *Eur. Chem. Bull*. 2023; 12(10):5158-5171.