Available Online at

http://www.ijcpa.in

April-June 2017

DOI : http://dx.doi.org/10.21276/ijcpa

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-4 | Issue-3 | Article ID: 1307

A NEW VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF ELBASVIR AND GRAZOPREVIR IN ITS BULK AND PHARMACEUTICAL DOSAGE FORMS

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Received: 20 April 2017 / Revised: 4 May 2017 / Accepted: 13 May 2017 / Available online : 4 June 2017

ABSTRACT

A New method was established for simultaneous estimation of Elbasvir and Grazoprevir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Elbasvir and Grazoprevir by using Inertsil ODS column (4.6×250mm) 5µ, flow rate was 1ml/min, mobile phase ratio (40:60 v/v) Acetonitrile (CAN), phosphate buffer (KH2PO4) of pH 3 (pH adjusted with orthophosphoric acid), detection wavelength used by Waters HPLC Auto Sampler, Separation module 2695, UV detector 2489, Empower-software version-2. The retention times were found to be 2.841 mins and 4.337 mins. The % purity of Elbasvir and Grazoprevir were found to be 100.12 and 99.93 respectively. The present analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Elbasvir and Grazoprevir was found in the concentration range 100µg/ml-500µg/ml and 200µg/ml - 1000µg/ml and correlation coefficient (R^2) be 0.999 and 0.999, % recovery was found to be 100.11 and 100.38, %RSD for repeatability 0.5 and 0.1, % RSD for intermediate precision was 0.6 and 0.2 respectively. The precision study was precision, robustness and repeatability. It is a convenient, simple and quick method for the determination of Elbasvir and Grazoprevir in its bulk and pharmaceutical dosage forms.

Keywords *– Elbasvir, Grazoprevir, HPLC, Acetonitrile, Methanol.*

1. INTRODUCTION

Grazoprevir, a second generation NS3/4a protease inhibitor approved for the treatment of hepatitis C virus (HCV) with Elbasvir as the fixed-dose combination product, Zepatier (FDA). practice of this medication for adults is indicated, with or without ribavirin, for HCV genotypes 1a, 1b or 4. NS3/4a protease is an integral part of viral replication as it is responsible for cleaving the long polypeptide produced by translation of the viral genome. By inhibiting protease activity, Grazoprevir prevents the formation of structural and nonstructural proteins required for replication and assembly (E1, E2, NS2, NS3, NS4A, NS4B, NS5A and NS5B).

Elbasvir 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. Elbasvir is currently approved for use in combination with grazoprevir (as the combination product Zapatier) for the treatment of chronic hepatitis C genotypes 1 and 4. The structures of Elbasvir and Grazoprevir were shown in figures 1 and 2.

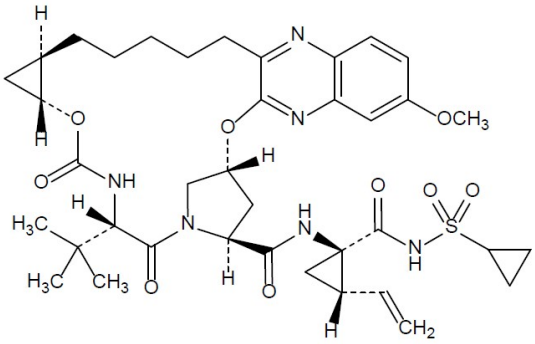

Fig. 1: Structure of Grazoprevir

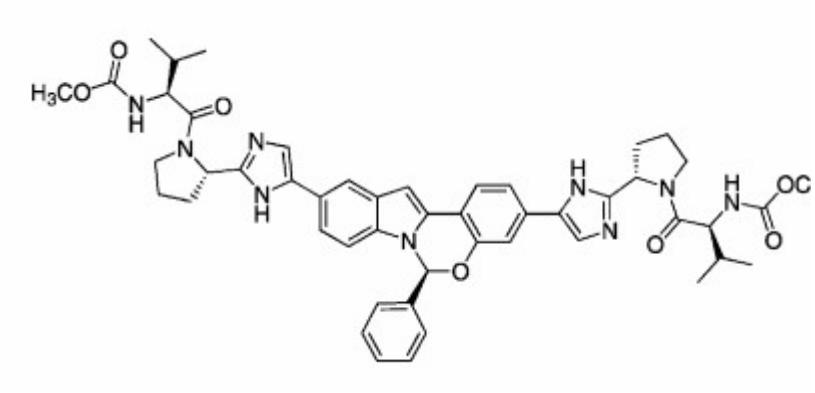

Fig. 2: Structure of Elbasvir

2. MATERIALS AND METHODS

2.1 Instrumentation

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. The analysis was carried out at 264 nm with an Inertsil ODS (4.6 x 250mm, 5µm) dimensions at ambient temperature(25^0c).

2.2 Chemicals and reagents

Grazoprevir and Elbasvir were supplied from Mylon laboratories, Hyderabad. KH_2PO_4 (AR) was supplied by Finer Chemicals Ltd., Mumbai, Orthophosphoric acid (OPA) (Merck), Acetonitrile (Molychem, HPLC grade) and Water for HPLC were employed in the present work.

2.3 Preparation of solutions

2.3.1 Preparation of buffer

3.4g of KH_2PO_4 was dissolved in 1000 ml of HPLC water. The P^H was adjusted to 3.0 with OPA. The final solution was filtered through 0.45 µm membrane filter and sonicate it for 10 mins.

2.3.2 Preparation of mobile phase

Accurately measured 400 ml (40%) of pH =3.0 buffer and 600 ml (60%) of Acetonitrile mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µm membrane filter under vacuum filtration. Figure 4 represents the Chromatograms of mobile phase (blank solution).

2.3.3 Diluent Preparation

The Mobile phase was used as the diluent.

2.3.4 Preparation of standard stock solution

10 mg of Elbasvir and 20 mg of Grazoprevir were accurately weighed and transferred into a 10 ml clean dry volumetric flask. Added about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further, 0.3 ml of the above prepared stock solution is pipetted into a 10ml volumetric flask and dilute up to the mark with diluent.

2.3.5 Preparation of Sample Solution

Accurately weigh the samples of 10 tablets. Tablets were crushed in mortar and pestle. Transferred equivalent to 10 mg of Elbasvir and 20 mg Grazoprevir sample into a 10 ml clean dry volumetric flask. Added about 7 mL of Diluent and sonicate it for 30 mins to dissolve it completely and volume was made to the mark with the same solvent. Then, the solution was Filtered using 0.45-micron Injection filter. Further, pipette 0.3 ml of Elbasvir and Grazoprevir from the above sample solution into a 10ml volumetric flask and dilute up to the mark with diluent. The standard solutions were prepared on daily basis from which stock solutions were prepared.

2.4 Procedure

20 µL of the standard, stock and sample solution were injected into the chromatographic system. The areas were measured for Elbasvir and Grazoprevir peaks are calculated. The %Assay by using the standard formula.

2.4.1 Method development and selection of wavelength

UV spectrum of 10 µg/ml Elbasvir and 10 µg/ml Grazoprevir in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. The UV Spectrum of Elbasvir and Grazoprevir is shown in the figure3. Form the UV spectrum, the analytical wavelength was selected as 264 nm. At this wavelength both the drugs showed good absorbance with no interference.

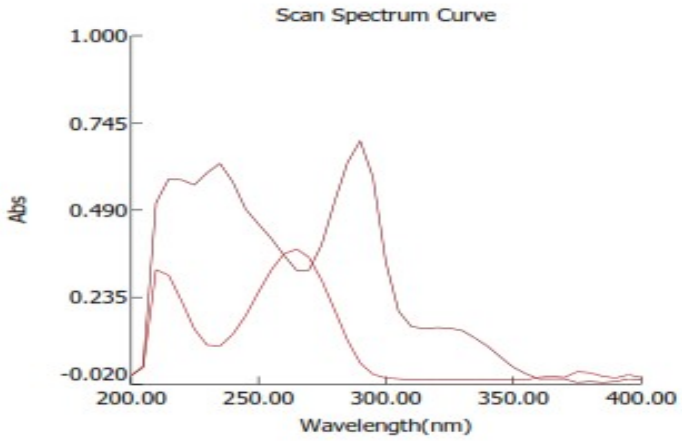

Fig. 3: UV Spectra of Grazoprevir and Elbasvire for Selection of Wavelength

2.4.2 Construction of calibration curve

Aliquots of different concentrations of standard solution were prepared and their chromatograms were recorded at the optimized chromatographic conditions. The mean peak areas at different concentration levels were calculated from the chromatograms. Then the linearity plot was constructed using the mean peak areas at their respective concentrations. (Fig. 8 and 9)

2.5 Method of validation

The developed method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness and system suitability parameters as described in ICH guidelines.

2.5.1 Linearity

From the stock solution, 100, 200, 300, 400, 500µg/ml solutions for Elbasvir and 200, 400, 600, 800, 1000µg/ml solutions for Grazoprevir were made and their chromatograms were recorded. From the recorded chromatograms, their respective mean peak areas were calculated and the linearity plot was constructed using the mean peak areas at their respective concentrations. The correlation coefficient was found to be 0.999. The linearity data of Grazoprevir and Elbasvire are shown in the Tables 1 & 2. The calibration plots, are given in the Fig. 8 and 9 [1].

3. RESULTS AND DISCUSSION

The present investigation reported by the authors are to develop a new validated method for the simultaneous estimation of Elbasvir and Grazoprevir by RP-HPLC method. Mobile phase contains the mixture of 40% PH 3.0 phosphate buffer and 60% of Acetonitrile. It is used as diluent in the present study. An Inertsil ODS column of 5µ (4.6X250mm) is employed for the simultaneous determination of Elbasvir and Grazoprevir by RP-HPLC method. A slow rate of 1ml or minute is used in this method. UV detection wavelength at 264mm and temperature of 25°C were maintained. Two sharp peaks were observed at 2.841mts and 4.337 minutes for Elbasvir and Grazoprevir respectively. The representative chromatograms of blank solution, Elbasvir and Grazoprevir shown in this figure.4 Chromatograms of assay of sample injection and standard of sample injection are shown in the Fig. 5 and 6 and assay results of purity in the table 1. The % purity of Elbasvir and Grazoprevir were found to be 100.12 and 99.93 respectively. As per the guidelines of ICH S/N ratio value shall be 3 for LOD solution and 10 for LOQ solution[2,3].

3.1 Linearity

Figures 7a to 7e represent the chromatograms showing different linearity levels with different concentrations of Elbasvir and Grazoprevir and results of given in the tables 2 & 3. Both Elbasvir and Grazoprevir obey Beer Lamberts Law in the range of concentrations of 90 µg /ml to 580 µg /ml and 200 µg /ml to 1100 µ g/ml respectively with regression equations Y= 149.21 X + 2219.8(correlation and coefficient) R^2= 0.99 for Elbasvir and Y= 173.88 X + 2487.2, R^2= 0.9994. for Elbasvir and Grazoprevir [4].

3.2 Precision

This validated method is more precise and the percentage of relative standardization (%RSD) and intermediate precision / Ruggedness were found to be 0.5 and 0.6 for Elbasvir and 0.1 and 0.2 for Grazoprevir. The results are given the in the tables 6 and 7 [5].

3.3 System suitability

The results for Elbasvir and Grazoprevir are given in the tables 889. It was performed to ensure that complete testing system was suitable for the intended application. The USP tailing factor for Elbasvir and Grazoprevir were 1.42 and 1.46 which is <2 and the USP plate found were 2935.56 and 4866.53 which is >2000 the results for actual flow of 1.0ml/min is considered from assay standard. Tablets for all shows system suitability results with change in the organic composition in the mobile phase for Elbasvir and Grazoprevir chromatograms of and Elbasvir and Grazoprevir are show in the figures 12 and 13.

3.4 Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Elbasvir and Grazoprevir. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. These results were given in the tables 4 and 5. The Mean % of recovery is 100.12 for Elbasvir and 100.38 for Grazoprevir (NLT 98% and NMT 102%) [6,7].

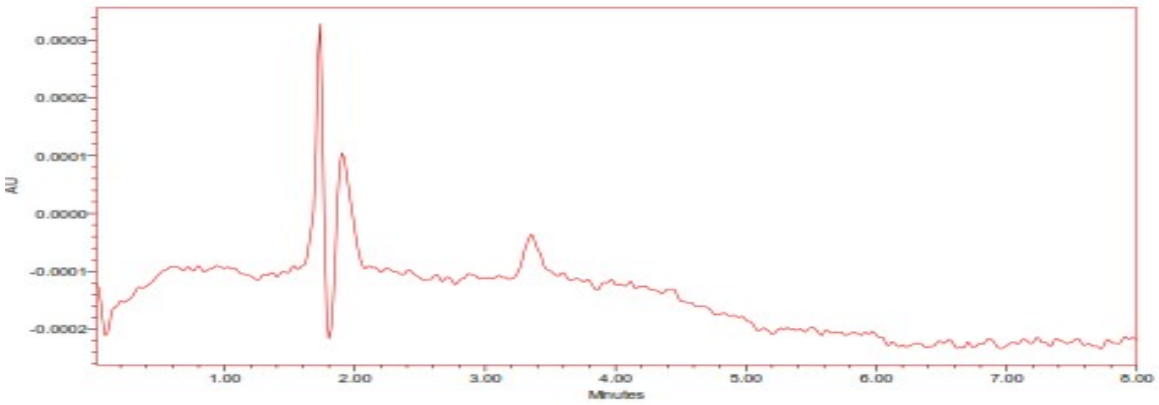

Fig. 4: Chromatogram showing blank Solution (mobile phase)

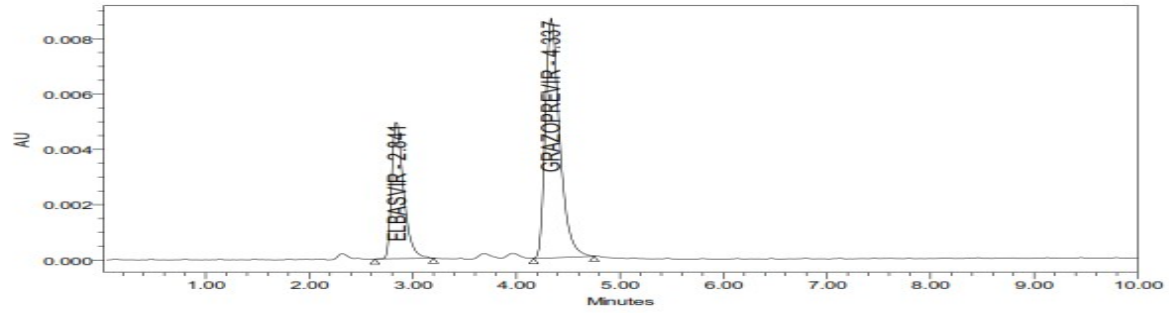

Fig. 5: Chromatogram showing assay of sample injection

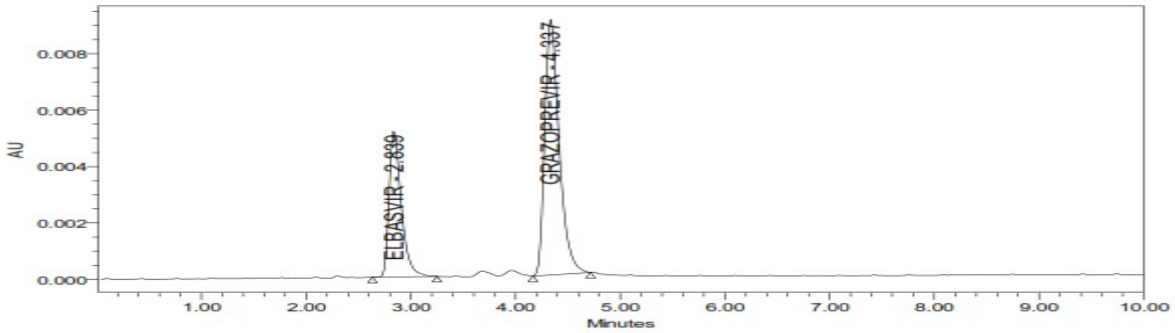

Fig. 6: Chromatogram showing standard of sample injection -

Table 1: Showing assay results

S. No	Name of compound	Amount taken(mg)	%purity
1	Elbasvir	100mg	100.12
2	Grazoprevir	200mg	99.93

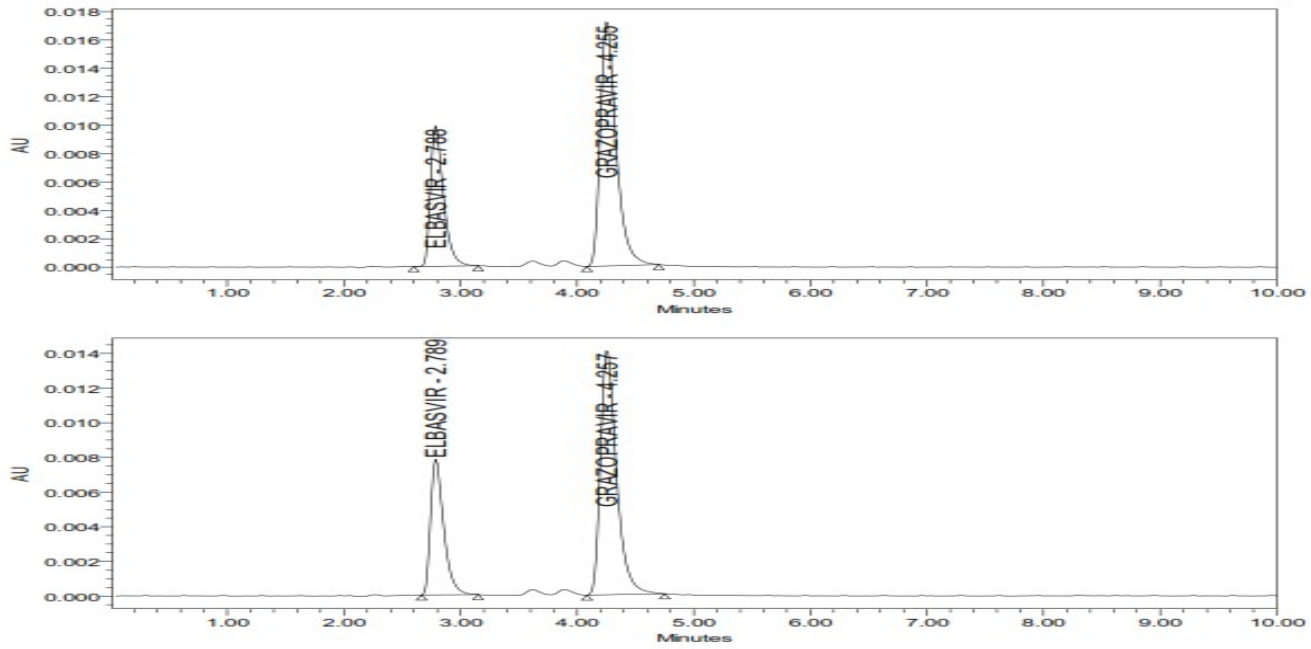

Fig. 7a,b: Level 1,2 Chromatograms showing Linearity of Elbasvir and Grazoprevir

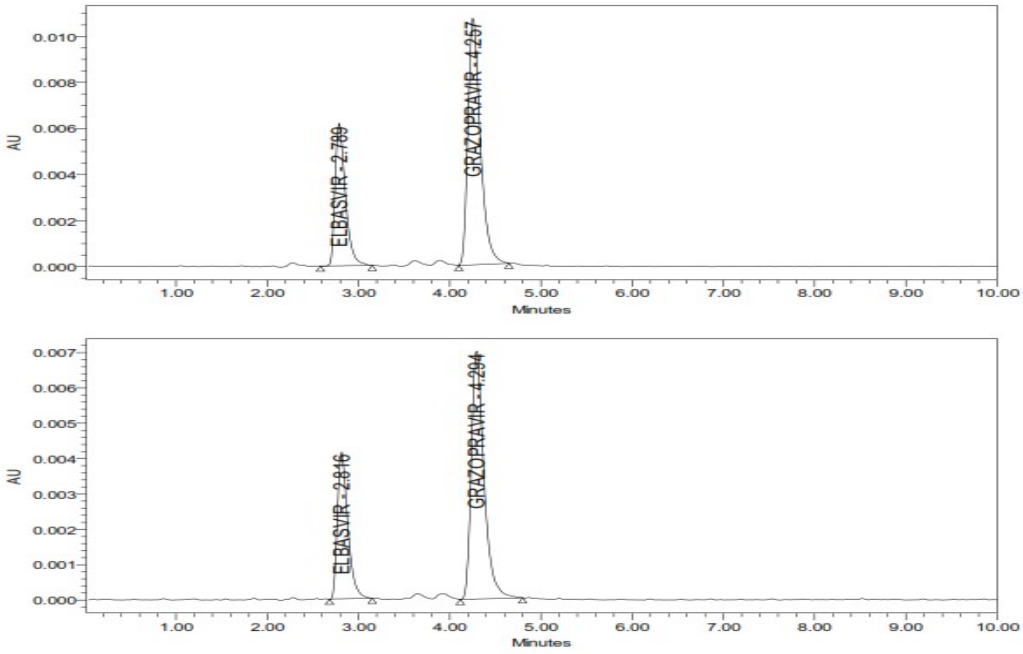

Fig. 7c,d: Level 3,4 Chromatograms showing Linearity of Elbasvir and Grazoprevir

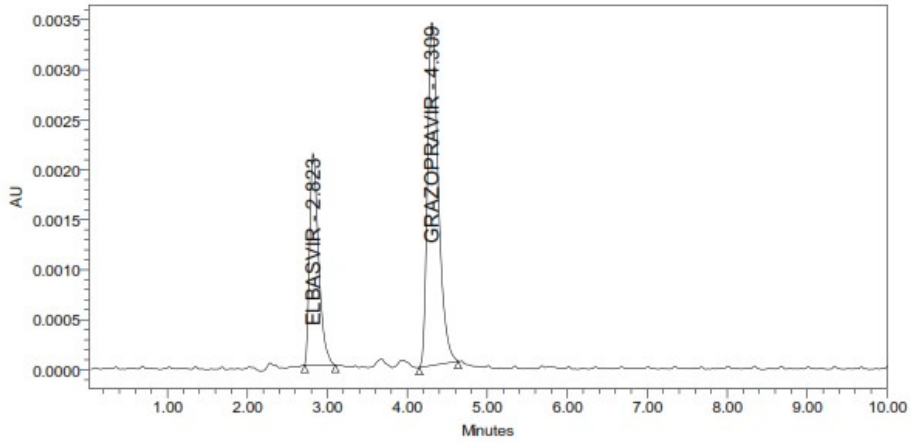

Fig.7e: Level 5 Chromatograms showing Linearity of Elbasvir and Grazoprevir

Table 2: Linearity results for Elbasvire

S. No	Linearity Level	Concentration (µm/ml)	Area
1	I	100	16472
2	II	200	32577
3	III	300	47931
4	IV	400	61145
5	V	500	76795
Correlation Coefficient			0.999

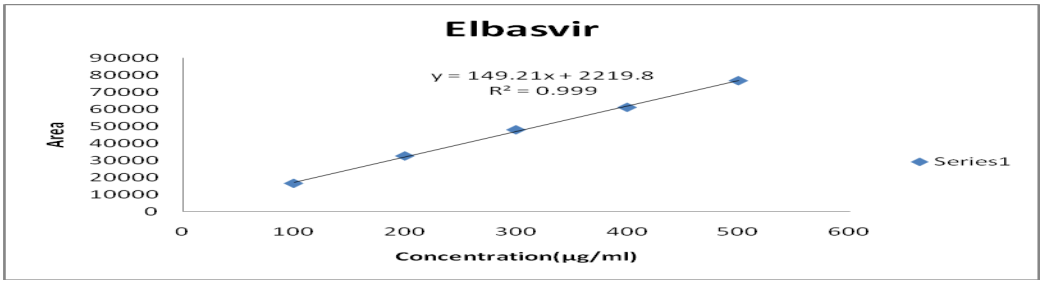

Fig. 8: Showing calibration graph for Elbasvire

Table 3: Linearity results for Grazoprevir

S. No	Linearity Level	Concentration(µm/ml)	Area
1	I	200	32441
2	II	400	67728
3	III	600	100630
4	IV	800	134448
5	V	1000	172463
Correlation Coefficient			0.999

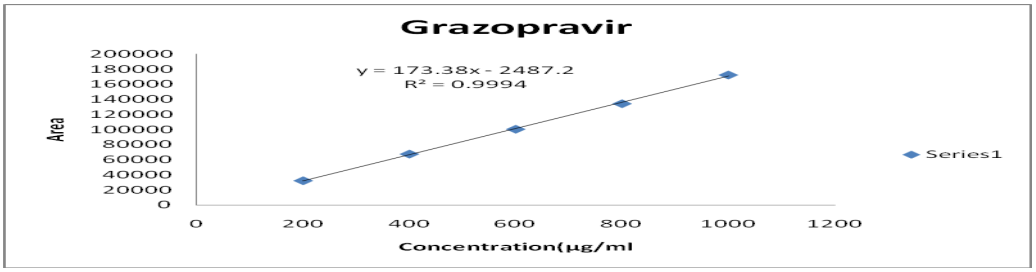

Fig. 9: Showing calibration graph for Grazoprevir

Table 4: Showing accuracy results for Elbasvir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	24492	50	49.95	99.89	100.11
100%	48488	100	99.70	99.70	
150%	73486	150	151.10	100.73	

Table 5: Showing accuracy results for Grazoprevir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	49702	100	100.52	100.52	100.38
100%	99405	200	201.04	100.52	
150%	148499	300	300.34	100.11	

Table 6: Showing% RSD results for Elbasvir and Grazoprevir

Injection	Area for Elbasvir	Area for Grazoprevir
Injection-1	48997	98709
Injection-2	48348	98962
Injection-3	48957	98700
Injection-4	48487	98687
Injection-5	48674	98901
Injection-6	48691	98960
Average	48692.3	98819.8
Standard Deviation	254.5	134.7
%RSD	0.5	0.1

Table 7: Showing results for intermediate precision of Elbasvir and Grazoprevir

Injection	Area for Elbasvir	Area for Grazoprevir
Injection-1	48673	98783
Injection-2	48720	98674
Injection-3	48793	98647
Injection-4	48657	98359
Injection-5	48082	98747
Injection-6	48956	98911
Average	48646.8	98686.8
Standard Deviation	297.4	185.7
% RSD	0.6	0.2

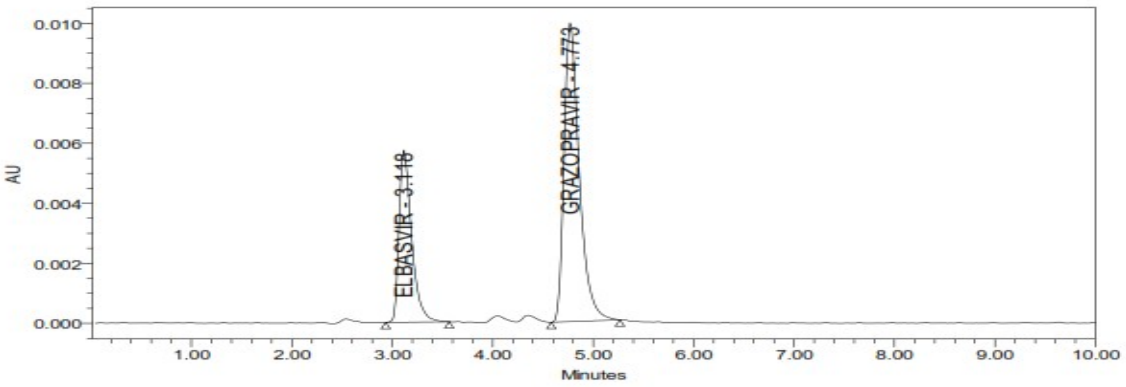

Fig. 10: Chromatogram showing less flow rate

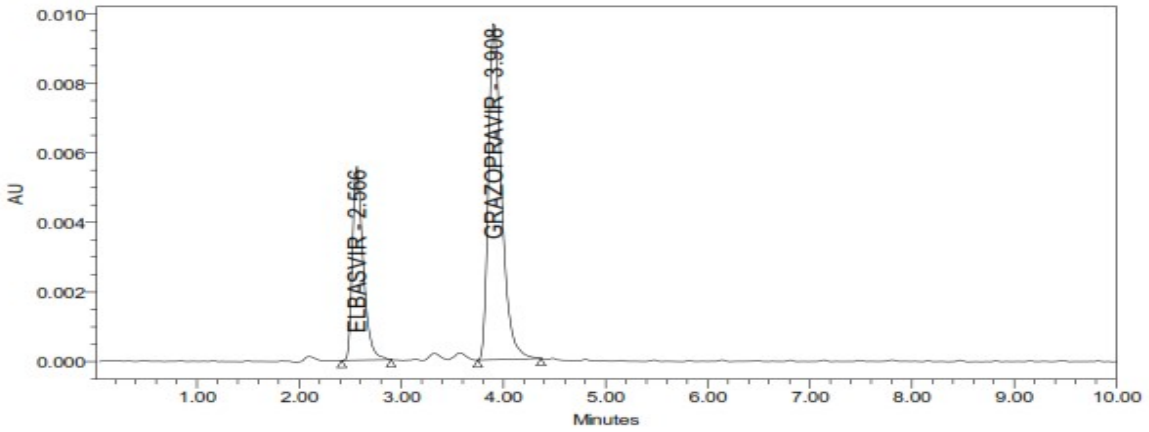

Fig. 11: Chromatogram showing more flow rate.

Table 8: System suitability results for Elbasvir:

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3013.80	1.4
2	1.0	2935.56	1.42
3	1.1	2845.18	1.43

Table 9: System suitability results for Grazoprevir:

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	0.9	4951.17	1.46	6.64
2	1.0	4800.53	1.46	6.50
3	1.1	4596.34	1.42	6.34

** Results for actual flow (1.0ml/min) have been considered from Assay standard.*

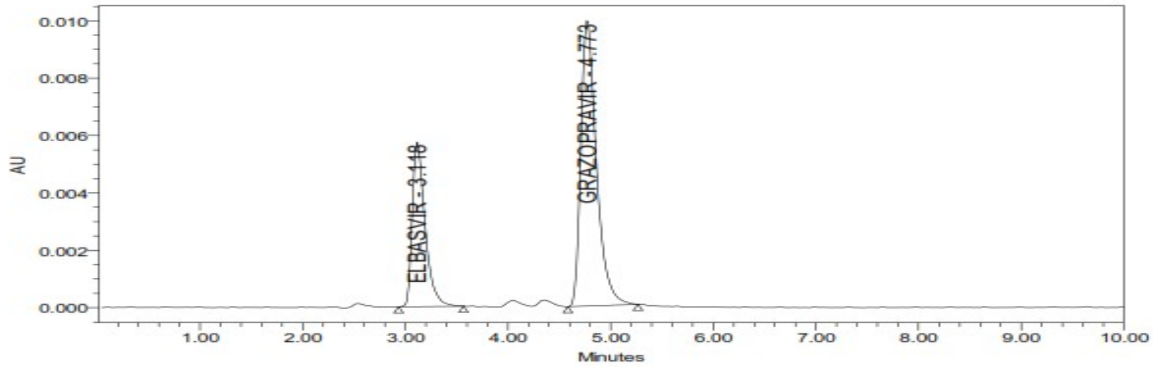

Fig. 12: Chromatogram showing less organic composition in the mobile phase

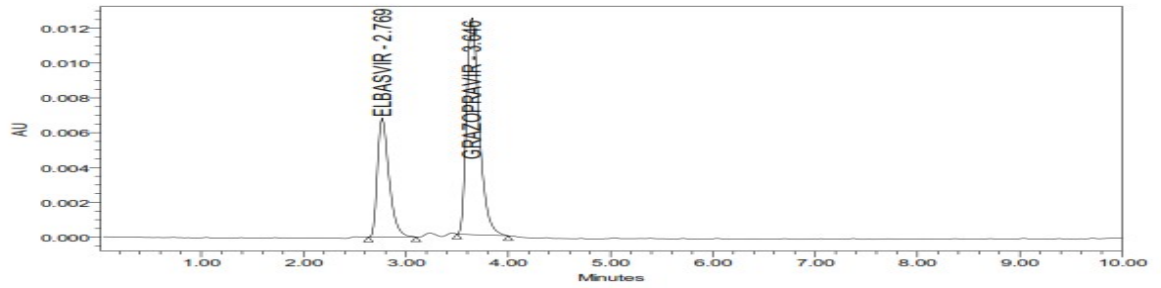

Fig. 13: Chromatogram showing more organic composition in the mobile phase

Table 10: Showing system suitability results for Elbasvir

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3013	1.1
2	*Actual	2935.56	1.42
3	10% more	2841.98	1.44

Table 11: Showing system suitability results for Grazoprevir

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	4751	1.45	6.64
2	*Actual	4800.53	1.46	6.50
3	10% more	4160.01	1.42	4.04

3.5 Detection limit

As per ICH guidelines S/N Ratio value shall be 3 for LOD solution.

As per ICH guidelines S/N Ratio value shall be 10 for LOQ solution.

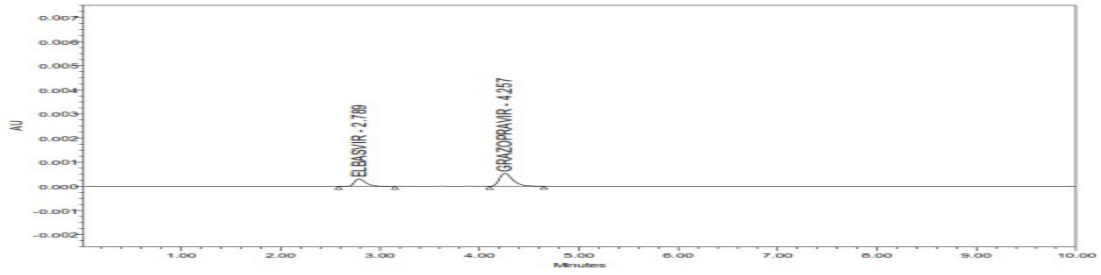

Fig. 14: Chromatogram showing LOD

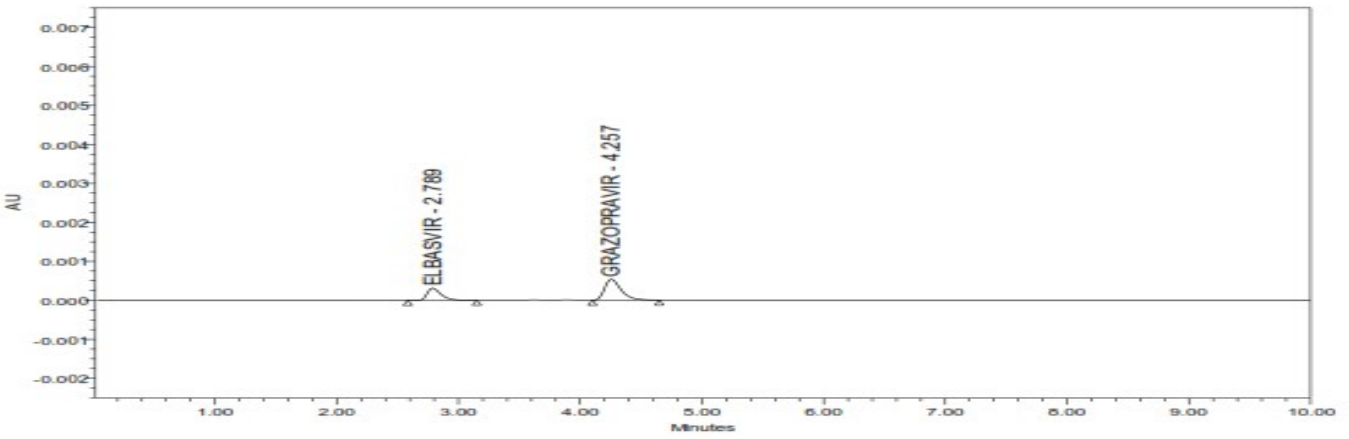

Fig. 15: Chromatogram showing LOQ

4. 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.

5. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

6. ACKNOWLEDGEMENT

Authors are thankful to the Pharma Train Lab, Kukatpally, for providing instrumental and analytical support. We extended our thanks to the Principal and Management for their timely help.

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