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Development and validation of stability indicating RP-HPLC method for the estimation of Elbasvir and Grazoprevir in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Elbasvir and Grazoprevir in tablet dosage form. Chromatogram was run through Denali C18 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1% OPA (2.8ph): Acetonitrile taken in the ratio 600:30 was pumped through column at a flow rate of 0.8 ml/min. Buffer used in this method was 0.1% OPA. Temperature was maintained at 30°C. Optimized wavelength selected was 260 nm. Retention time of Elbasvir and Grazoprevir were found to be 2.143 min and 2.694 min respectively. The drug was stressed under alkaline, oxidative, thermal, photolytic degradation were analysed. The developed method was validated as per ICH guidelines The Accuracy, Linearity, Precision, and Robustness were within the acceptance limits .Hence this HPLC method was a stability indicating method can be used for routine stability analysis of the Elbasvir and Grazoprevir in Pharmaceutical dosage forms.

Keywords: Elbasvir, Grazoprevir, RP-HPLC.

INTRODUCTION

ELBASVIR

The 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] Elbasvir is a Hepatitis C Virus NS5A Inhibitor. The mechanism of action of elbasvir is as a Breast Cancer Resistance Protein Inhibitor [1]

GRAZOPREVIR

Grazoprevir **(**1R,18R,20R,24S,27S)-24-tert-butyl-N-[(1R,2S)-1-[(cyclopropanesulfonyl)-C-hydroxycarbonimidoyl]-2-ethenylcyclopropyl]-22-hydroxy-7-methoxy-25-oxo-2,21-dioxa-4,11,23,26-tetraazapentacyclo[24.2.1.0^{3,12}.0^{5} ,^{10} .0^{18} ,^{20}]nonacos

a-3(12),4,6,8,10,22-hexaene-27-carboximidic acid. Grazoprevir anhydrous is a Hepatitis C Virus NS3/4A Protease Inhibitor. The mechanism of action of grazoprevir anhydrous is as a HCV NS3/4A Protease Inhibitor, and Breast Cancer Resistance Protein Inhibitor, and Cytochrome P450 3A Inhibitor [2]. Elbasvir is a complex organic heterotetracyclic compound that is a hepatitis C virus nonstructural protein 5A inhibitor used in combination with grazoprevir (under the brand name Zepatier) for treatment of chronic HCV genotypes 1 or 4 infection in adults. It has a role as an antiviral drug, a hepatoprotective agent and a hepatitis C virus nonstructural protein 5A inhibitor. It is a L-valine derivative, a member of imidazoles, a carbamate ester, a N-acylpyrrolidine, an organic heterotetracyclic compound and a ring assembly [3].

The literature survey indicates that Elbasvir and Grazoprevir estimation were carried by various HPLC analytical techniques [7-9] 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.

MATERIALS AND METHODS

Chemicals and Reagents

Acetonitrile (HPLC grade), orthophosphoric acid (HPLC grade), water (HPLC grade) were purchased from Mark (India) Ltd, Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of elbasvir and Grazoprevir as reference standards were procured from Spectrum Pharma labs, Hyderabad, India.

Instrumentation

In the present study Performed with Waters HPLC 2695 Photo diode array detector and Empower 2 software was used UV-Visible spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of elbasvir and Grazoprevir. Electronics Balance-Denver, P^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises

Determination of maximum absorbance:

elbasvir and Grazoprevir standard solution was scanned in the range of 200-400 nm against mobile phase as blank. Elbasvir and Grazoprevir shows maximum absorbance at 230nm.The wave length selected for the determination of elbasvir and Grazoprevir is 260nm.

Diluent

Based up on the solubility of the drugs, diluents was selected, Acetonitrile and Water taken the in the ratio of 50:50.

Preparation of Standard stock solutions

Accurately weighed 12.5mg of Elbasvir, 25mg of Grazoprevir and transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.

Preparation of Sample stock solutions

Tablet equivalent to 50mg Elbasvir and 100mg of Grazoprevir was transferred into a 100 ml volumetric flask, 20ml of diluents was added and sonicated for 25min, further the volume was made up with diluent and filtered by HPLC filters
1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent

Preparation of buffer

0.1% OPA Buffer

1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

To develop a stability indicating RP-HPLC method for estimation of Elbasvir and Grazoprevir in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 60% 0.1% OPA:40% Acetonitrile at a flow rate of 0.8ml/min, samples were analyzed at 260 nm detector wave

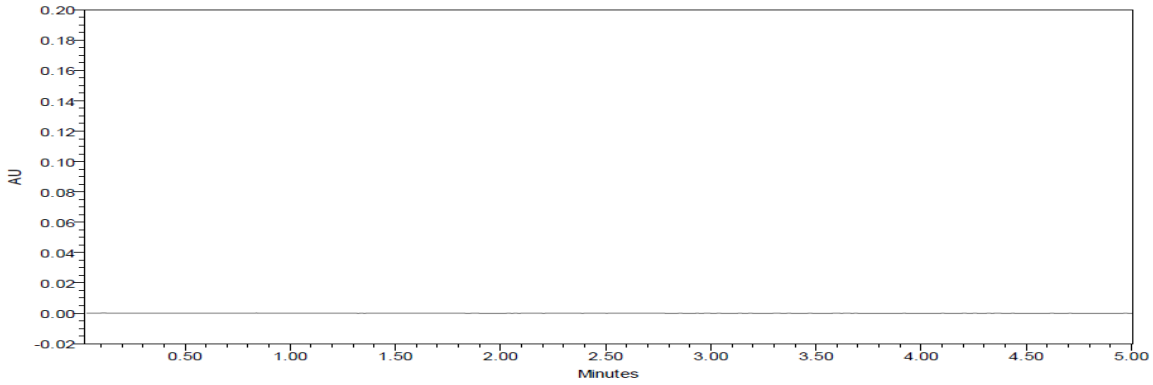
length and at an injection volume of 10 µL using Denali C18 (4.6 x 150mm, 5µm)with run time of 5 min. The proposed method Elbasvir and Grazoprevir was optimized to give sharp peak with good resolution and minimum tailing effect for the optimized chromatogram was obtained as shown in table no:1.

Analytical method validation

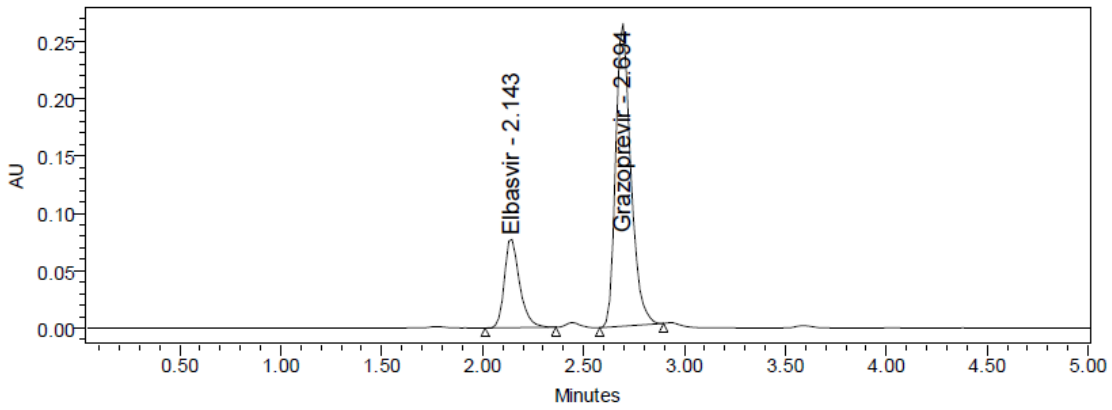
The analytical validation of Elbasvir and Grazoprevir by HPLC was carried out with respect to the following parameters.

Table 1: optimized chromatographic conditions

Parameter	Condition
Mobile phase	Acetonitrile:0.1% OPA(40:60)
Flow rate	1ml/min
Column	Denali C18 (4.6 x 150mm, 5µm)
Detector wave length	260nm
Column temperature	30°C
Injection volume	10µL
Run time	5 min
Diluent	Water and Acetonitrile in the ratio 50:50



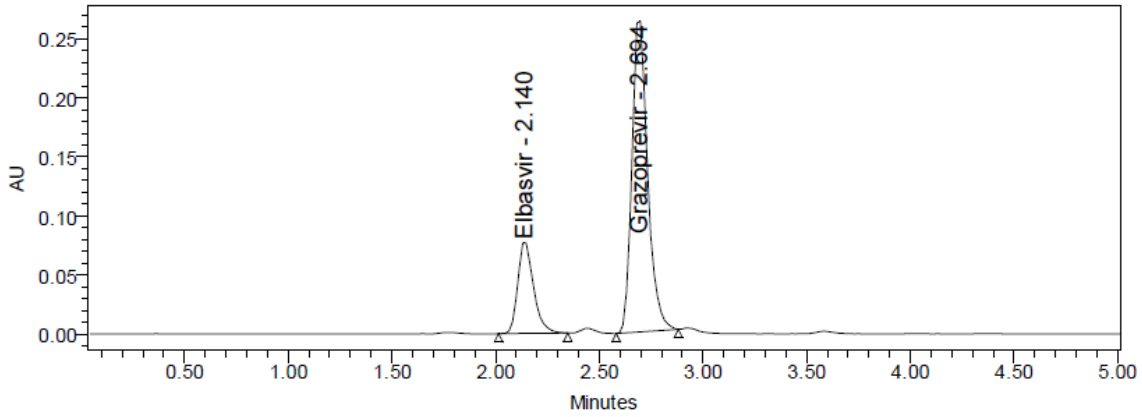
Blank chromatogram of Elbasvir and Grazoprevir



Standard chromatogram of Elbasvir and Grazoprevir

Table:2 System suitability parameters for Elbasvir and Grazoprevir

S no	Elbasvir			Grazoprevir.			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.139	3856	1.31	2.688	6225	1.27	3.9
2	2.140	3796	1.30	2.692	6571	1.26	3.9
3	2.143	4263	1.24	2.692	6770	1.24	3.9
4	2.144	4121	1.24	2.694	6622	1.22	3.9
5	2.144	3781	1.26	2.694	6625	1.25	3.9
6	2.144	4163	1.30	2.695	5882	1.31	3.8



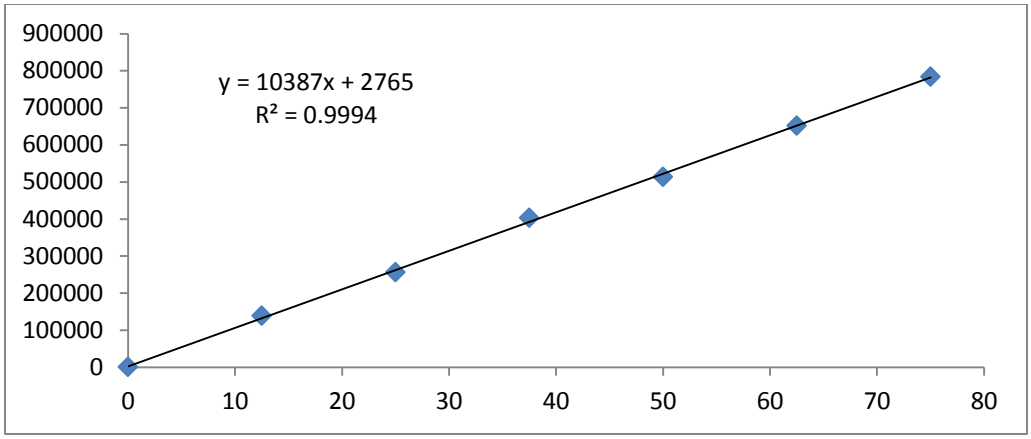
Formulation chromatogram

Linearity

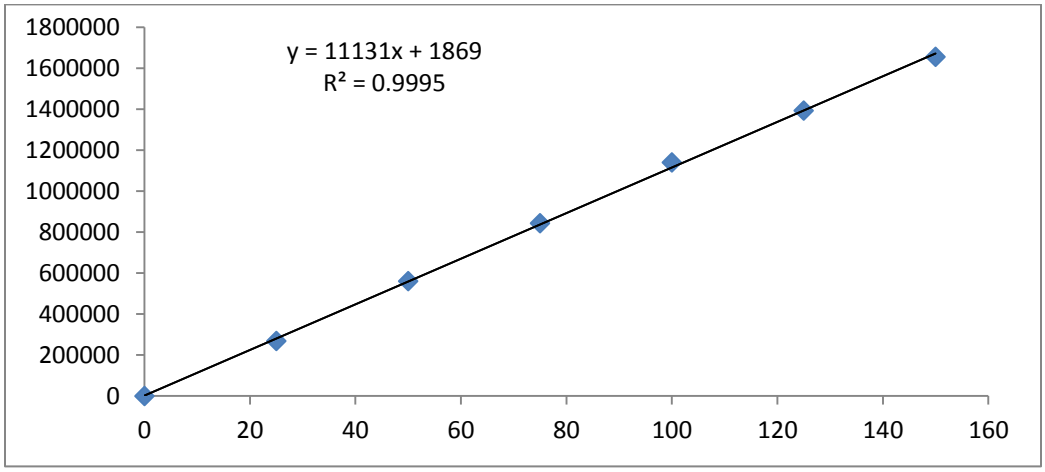
Linearity of Elbasvir and Grazoprevir were found by injecting six different concentrations of working standard solutions for Elbasvir (12.5-75µg/ml) and Grazoprevir (25-150µg/ml).Standard calibration curves were constructed by taking mean peak area on Y-axis and concentrations of drug on X-axis. The linearity equations obtained for Elbasvir was y = 10387x + 2765and of Grazoprevir was y = 11131x + 1869 Correlation coefficient obtained was 0.999 for the two drugs. The results were shown in table 3

Table 3 : Results for the linearity of Elbasvir and Grazoprevir

Elbasvir		Grazoprevir	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
12.5	138539	25	268291
25	255760	50	559373
37.5	402711	75	843065
50	513455	100	1139282
62.5	651578	125	1392129
75	783886	150	1654693



Calibration curve of Elbasvir



Calibration curve of Grazoprevir

Precision

The system precision was established by six repli-cate injections of the standard solution containing analytes of interest. The value of relative standard deviation of Elbasvir and Grazoprevir and was found to be as 0.7% and 0.9% within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analysis six times using the proposed method. The relative standard deviation of Elbasvir and Grazoprevir was found to be 0.6% and 0.3%within the limit, indicating the injection repeatability of the method.

Table:4 Precision data for of Elbasvir and Grazoprevir

S. No	Elbasvir	Grazoprevir	Elbasvir	Grazoprevir
	System precision		Method precision	
1.	520121	1133612	514729	1123965
2.	519099	1139205	518519	1122492
3.	517604	1112936	519996	1131079
4.	520994	1121940	518529	1130362
5.	516926	1133317	521493	1123922
6.	511187	1140085	514041	1124053

Mean	517655	1130183	517885	1125979
S.D	3512.4	10643.6	2933.5	3725.0
%RSD	0.7	0.9	0.6	0.3

Accuracy

To demonstrate the accuracy of the proposed method a standard addition method was used for analyzing the samples. For this purpose, known amounts of Elbasvir and Grazoprevir were supplemented to the working standard sample solution which was previously analyzed and then compared the obtained experimental values to the true values. Each solution was injected in six times and the percentage recovery was calculated. %Recovery was obtained as 99.33% and 99.35% for Elbasvir and Grazoprevir respectively.

Table 5: Accuracy data of Elbasvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	24.86	99.44	
	25	24.73	98.92	
	25	24.88	99.53	99.33%
100%	50	49.19	98.37	
	50	49.71	99.42	
	50	49.55	99.09	
150%	75	74.10	98.80	
	75	74.99	99.99	
	75	75.31	100.41	

Table 6: Accuracy data of Grazoprevir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	49.82	99.63	99.35%
	50	49.93	99.86	
	50	49.95	99.91	
100%	100	99.08	99.08	
	100	99.66	99.66	
	100	98.59	98.59	
150%	150	148.23	98.82	
	150	148.87	99.25	
	150	149.08	99.39	

Robustness

The different variations such as variation of pH of the buffer solution, flow rate, wavelength and mobile phase composition. The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay. This indicated the robustness of the method. The robustness study results are presented in table.

Table7: Robustness data for Elbasvir and Grazoprevir.

S.no	Condition	Change	%RSD of Elbasvir	%RSD of Grazoprevir .
1	Flow rate- 1	0.7ml/min	0.8	0.6
2	Flow rate- 2	0.9 ml/min	0.3	0.6
2	Mobile phase-1	65:35(%v/v)	0.2	0.3
3	Mobile phase-2	55:45(%v/v)	0.7	0.7
4	Temperature -1	25°C	0.4	0.5
5	Temperature -2	35°C	0.6	0.6

Limit of Detection and Quantification Limit

Determination of the Limit of Detection and Limit of Quantification was performed by standard deviation method. Standard with low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be readily detected

Table8 : Sensitivity table for Elbasvir and Grazoprevir

Molecule	LOD	LOQ
Elbasvir	0.14	0.43
Grazoprevir	0.38	1.17

Assay of formulation

We were prepared assay sample solution injected into the HPLC. Dr.reddys labs (Zepatier), bearing the labels claim Elbasvir 30mg, Grazoprevir 2mg. Assay was performed with the above formulation. Average % Assay for Elbasvir and Grazoprevir obtained was 99.64% and 99.23% respectively.

Table9: Assay Data of Elbasvir

S.no	% Assay
1	99.04
2	99.77
3	100.05
4	99.77
5	100.34
6	98.90
Avg	99.64
Std Dev	0.56
%RSD	0.6

Table10: Assay data of Grazoprevir

S. no	% Assay
1	99.05
2	98.92
3	99.68
4	99.62
5	99.05
6	99.06
Avg	99.23
Std Dev	0.3
%RSD	0.3

Degradation data

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Table11: Data of degradation studies

	Elbasvir			Grazoprevir		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	491783	94.62	5.38	1084146	95.54	4.46
Base	498371	95.89	4.11	1090104	96.07	3.93
Peroxide	499713	96.15	3.85	1105774	97.45	2.55
Thermal	504613	97.09	2.91	1109810	97.80	2.20
Uv	510736	98.27	1.73	1113549	98.13	1.87
Water	513003	98.27	1.73	1125852	99.22	0.78

CONCLUSION

The stability indicating RP HPLC method was developed and validated for the simultaneous determination of Elbasvir and Grazoprevir in bulk and its dosage form , The proposed method was validated in accordance with ICH guidelines by testing its parameters include linearity, accuracy, precision, robustness, LOD and LOQ. Stress induced studies proves the effectiveness of the proposed stability indicating method. So the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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