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Development And Validation Of Analytical Method For Simultaneous Estimation Of Elbasvir And Grazoprevir By Rp-Hplc

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Abstract: A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Elbasvir and Grazoprevir, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Targetsil C18 (4.4 ×150mm, 5µm) column using a mixture of Acetone : 0.1% OPA (50:50v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 240nm. The retention time of the Elbasvir and Grazoprevir was 2.079, 4.045 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Elbasvir and 93.75-448.75µg/ml of Grazoprevir. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Index Terms **- Elbasvir, Grazoprevir, RP-HPLC, validation.**

I.Introduction

Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Elbasvir and Grazoprevir and single method is available for such estimation by RP-HPLC. In view of the need for a suitable RP-HPLC method for routine analysis of Elbasvir and Grazoprevir in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Elbasvir and Grazoprevir and extend it for their determination in formulation. Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.

II. Experimental work

Preparation of mobile phase:
Accurately measured 500 ml (50%) of Acetone, 500ml of 0.1% OPA (50%) were mixed and degassed in digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation: The Acetone was used as the diluent.
Preparation of standard solution:
Accurately weigh and transfer 10 mg of Elbasvir and Grazoprevir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Acetone and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Acetone. Further pipette 0.15ml of the Elbasvir and 2.81ml of the Grazoprevir stock solutions into a 10ml volumetric flask and dilute up to the mark with Acetone.
Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Shimadzu LC-10 AT VP with SPD-10A VP UV-Visible Detector
Temperature : Ambient
Column : Targetsil C18 (4.4 ×150mm, 5µm)
Mobile phase: Acetone : 0.1% OPA (50:50v/v)
Flow rate: 1ml/min
Wavelength: 240 nm
Injection volume: 10 µl
Run time : 8 min

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Elbasvir and 10mg of Grazoprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Acetone and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the Elbasvir and 2.81ml of the Grazoprevir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Elbasvir and 10mg of Grazoprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Acetone and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the Elbasvir and 2.81ml of the Grazoprevir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Weight 10 mg equivalent weight of Elbasvir and Grazoprevir sample into a 10mL clean dry volumetric flask and add about 7mL of Acetone and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.15ml of the Elbasvir and 2.81ml of the Grazoprevir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure: Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

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

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Elbasvir and 10mg of Grazoprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (5ppm of Elbasvir & 93.75ppm of Grazoprevir):

Pipette out 0.05ml of Elbasvir and 0.93ml of Grazoprevir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10ppm of Elbasvir & 187.5ppm of Grazoprevir):

Pipette out 0.1ml of Elbasvir and 1.87ml of Grazoprevir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15ppm of Elbasvir & 281.25ppm of Grazoprevir):

Pipette out 0.15ml of Elbasvir and 2.81ml of Grazoprevir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20ppm of Elbasvir & 375ppm of Grazoprevir):

Pipette out 0.2ml of Elbasvir and 3.75ml of Grazoprevir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25ppm of Elbasvir & 448.75ppm of Grazoprevir):

Pipette out 0.25ml of Elbasvir and 4.48ml of Grazoprevir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

REPEATABILITY

Preparation of Elbasvir and Grazoprevir Product Solution for Precision:

Accurately weigh and transfer 10 mg of Elbasvir and 10mg of Grazoprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the Elbasvir and 2.81ml of the Grazoprevir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:

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

DAY 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

DAY 2: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ACCURACY:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Elbasvir and 10mg of Grazoprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.075ml of the Elbasvir and 1.4ml of the Grazoprevir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Elbasvir and 10mg of Grazoprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the Elbasvir and 2.81ml of the Grazoprevir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Elbasvir and 10mg of Grazoprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.225ml of the Elbasvir and 4.2ml of the Grazoprevir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure: Inject the Three replicate injections of individual concentrations (50%, 100%, 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.

ROBUSTNESS:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Elbasvir and 10mg of Grazoprevir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the Elbasvir and

2.81ml of the Grazoprevir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Acetone : 0.1% OPA was taken in the ratio and 45:55, 55:45 instead (50:50), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

III RESULTS AND DISCUSSION

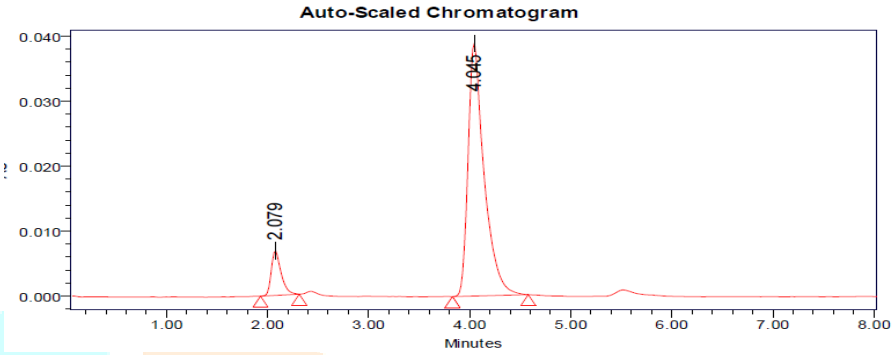

Fig : Optimized Chromatogram (Standard)

Table : Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Elbasvir	2.079	44148	4841	1.33	4251
2	Grazoprevir	4.045	429049	38885	1.59	5224

Table : Peak results for Assay sample of Elbasvir

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Elbasvir	2.078	44484	4918	1.34	5217	1
2	Elbasvir	2.079	44148	4841	1.33	5251	2
3	Elbasvir	2.077	44088	4851	1.37	7127	3

Table : Peak results for Assay sample of Grazoprevir

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Grazoprevir	4.050	430575	39127	1.40	4197
2	Grazoprevir	4.045	429049	38885	1.59	4224
3	Grazoprevir	4.037	429543	38892	1.58	8203

Table : Chromatographic data for linearity study for Elbasvir :

Concentration Level (%)	Concentration(µg/ml)	Average Peak Area
33.3	5	15045
44.4	10	31009
100	15	44144
133.3	20	40549
144.4	25	74842

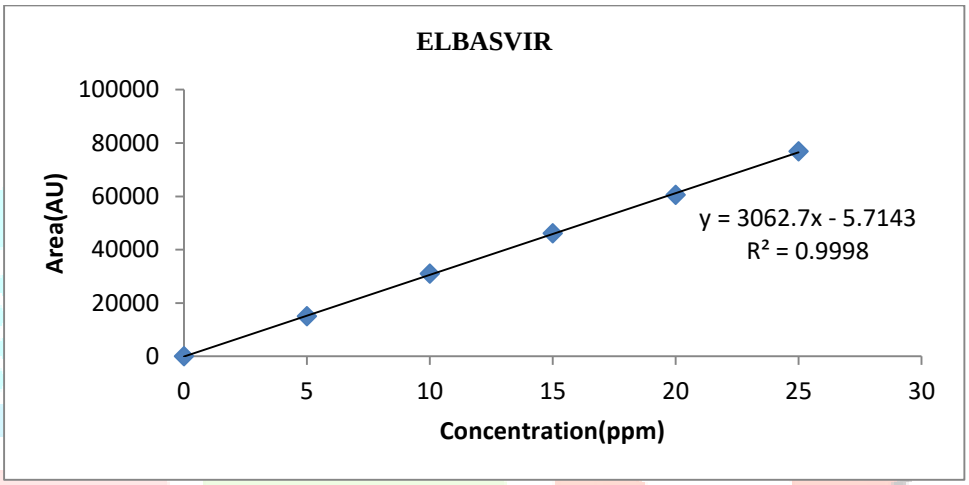

Fig.: Chromatogram showing linearity plot

Table : chromatographic data for linearity Grazoprevir:

Concentration Level (%)	Concentration(µg/ml)	Average Peak Area
33.3	93.75	131289
44.4	187.5	284775
100	281.25	427559
133.3	375	555841
144.4	448.75	712514

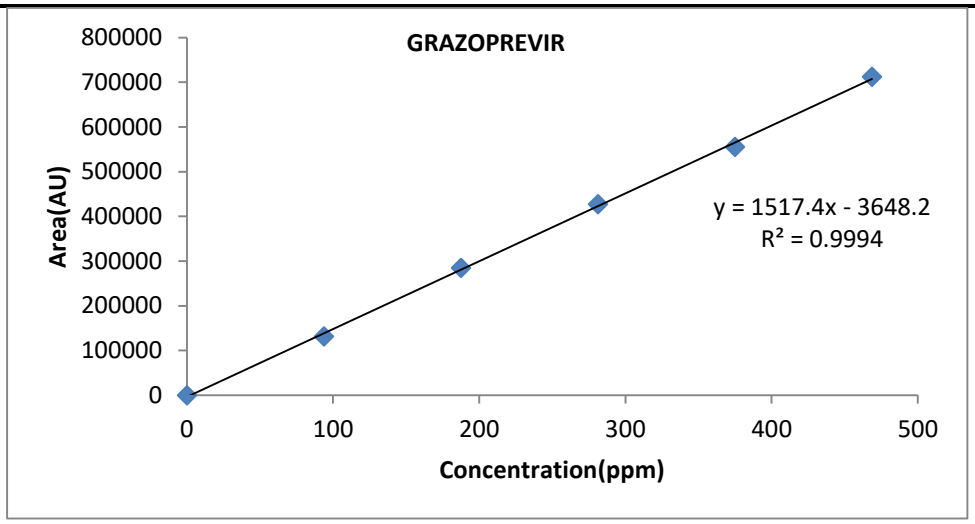

Fig.: Chromatogram showing linearity level

Table : Results of repeatability for Elbasvir :

S. No	Peak name	RT	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Elbasvir	2.077	44054	4784	4208	1.32
2	Elbasvir	2.074	44803	4847	4088	1.34
3	Elbasvir	2.074	44150	4744	4152	1.34
4	Elbasvir	2.077	44054	4715	4184	1.32
5	Elbasvir	2.074	44247	4744	4045	1.33
Mean			44242			
Std.dev			312.7099			
%RSD			0.475954			

Table: Results of repeatability for Grazoprevir:

S. No	Peak name	RT	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Grazoprevir	4.031	427942	38434	5158	1.57
2	Grazoprevir	4.024	429423	38473	5092	1.58
3	Grazoprevir	4.019	427824	38244	5071	1.58
4	Grazoprevir	4.014	427829	38310	5044	1.58
5	Grazoprevir	4.014	429559	38181	5034	1.58
Mean			428559.8			
Std.dev			943.2244			

%RSD			0.220092			

Table : Results of Intermediate precision day1 for Elbasvir

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Elbasvir	2.075	44204	4473	5117	1.33
2	Elbasvir	2.074	44300	4735	5043	1.34
3	Elbasvir	2.075	44259	4452	5087	1.28
4	Elbasvir	2.075	44223	4447	5134	1.31
5	Elbasvir	2.075	44205	4474	5151	1.32
6	Elbasvir	2.074	44189	4703	5157	1.33
Mean			44230			
Std. Dev.			41.88554			
% RSD			0.090403			

Table : Results of Intermediate precision day1 for Grazoprevir

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Grazoprevir	4.013	428922	38004	7038	1.58
2	Grazoprevir	4.011	428524	37935	7999	1.57
3	Grazoprevir	4.010	427239	37850	7003	1.57
4	Grazoprevir	4.008	427447	37780	7982	1.57
5	Grazoprevir	4.004	427824	37824	7983	1.57
6	Grazoprevi	4.004	427093	37970	7042	1.58
Mean			427878.5			
Std. Dev.			718.1952			
% RSD			0.14785			

Table: Results of Intermediate precision Day 2 for Elbasvir

S.No	Peak Name	RT	Area (µV*sec)	Height	USP Plate	USP Tailing
1	Elbasvir	2.074	44803	4847	5149	1.57
2	Elbasvir	2.074	44054	4715	5190	1.13
3	Elbasvir	2.077	44252	4452	4088	1.58
4	Elbasvir	2.075	44205	4474	5184	1.58
5	Elbasvir	2.075	44940	7249	5087	1.57
6	Elbasvir	2.072	44727	4983	5151	1.57
Mean			44497.17			
Std.			349.4739			
% RSD			0.794414			

Table: Results of Intermediate precision Day 2 for Grazoprevir

S.No	Peak Name	RT	Area	Height	USP Plate count	USP Tailing
1	Grazoprevir	4.024	429423	38473	4789	1.49
2	Grazoprevir	4.024	427829	38310	5772	1.34
3	Grazoprevir	4.014	427243	37850	5092	1.32
4	Grazoprevir	4.010	427824	37824	4044	1.28
5	Grazoprevir	4.004	421284	40752	4003	1.32
6	Grazoprevi	4.008	421832	40281	4983	1.33
Mean			425942.8			
Std. Dev.			3492.481			
% RSD			0.819988			

Table: The accuracy results for Elbasvir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	22938.33	7.5	7.3	99.88	100.144
100%	45424	15	14.7	98.89	
150%	70094.47	22.5	22.2	101	

Table: The accuracy results for Grazoprevir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	209357	140.4	140.2	99.7%	99%
100%	420497.7	281.25	281.1	99%	
150%	431550.7	421.8	421.4	99%	

LIMIT OF DETECTION FOR ELBASVIR AND GRAZOPREVIR

Elbasvir 0.54µg/ml **Grazoprevir** 17.2µg/ml

LIMIT OF QUANTITATION FOR ELBASVIR AND GRAZOPREVIR

Elbasvir 1.7µg/ml **Grazoprevir** 52.2µg/ml

Table : Results for Robustness -Elbasvir

Parameter used for sample	Peak	Retention Time	Theoretical plates	Tailing
Actual Flow rate of 0.9mL/min	44148	2.079	4251	1.33
Less Flow rate of 0.8mL/min	51177	2.29	5249	1.38
More Flow rate of 1.0mL/min	42190	1.890	5124	1.32
Less organic phase	42402	1.885	5124	1.19
More organic phase	42112	1.908	5854	1.34

Table : Results for Robustness-Grazoprevir

Parameter used for sample	Peak	Retention Time	Theoretical	Tailing
Actual Flow rate of 0.9mL/min	429049	4.045	5224	1.59
Less Flow rate of 0.8mL/min	472473	4.450	4328	1.58
More Flow rate of 1.0mL/min	392497	3.440	4217	1.54
Less organic phase	391379	4.251	4994	1.41
More organic phase	391703	3.239	4120	1.50

IV Summary

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 240 nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Targetsil C18 (4.4 ×150mm, 5µm) because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Acetone: 0.1% OPA (50:50v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 8min because analyze gave peak around 2.0, 4.0 ±0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range 5-25µg/ml of Elbasvir and 93.75-448.75 µg/ml of Grazoprevir of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

V Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Elbasvir and Grazoprevir in bulk drug and pharmaceutical dosage forms. Elbasvir and Grazoprevir was freely soluble in ethanol, Acetone and sparingly soluble in water. Acetone: 0.1% OPA (50:50v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.
The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Elbasvir and Grazoprevir in bulk drug and in pharmaceutical dosage forms.

VI References

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