Research Article

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ELBASVIR AND GRAZOPREVIR BY RP-HPLC

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INTRODUCTION

Elbasvir[1] and Grazoprevir[2] 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 of the method was done in accordance with USP and ICH guideline[3] for the assay of active ingredient.

HPLC METHOD DEVELOPMENT TRAILS

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 Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.03ml of Elbasvir and 3.0ml of Grazoprevir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the samples into WATERS HPLC, Alliance 2695 separation module. (Software: Empower 2,996 PDA detectors) by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer pH 3.9 in proportion 55:45 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Zorbax C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used: Waters HPLC with auto sampler and PDA Detector 996 model.

Temperature	:	35ºC
Column	:	Zorbax C18 (4.6×150mm, 5µ)
Mobile phase	:	Methanol: Phosphate Buffer pH 3.9 (55:45v/v)
Flow rate	:	1ml/min
Wavelength	:	255nm
Injection volume	:	10 µl
Run time	:	8 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Phosphate buffer pH 3.9

Accurately weighed 6.8 grams of KH2PO4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.9.

Preparation of mobile phase

Accurately measured 550 ml (55%) of Methanol and 450ml of Buffer (45%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

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 Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.03ml of Elbasvir and 3.0ml of Grazoprevir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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.

Preparation of Sample Solution
Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Elbasvir and Grazoprevir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Further pipette 0.03ml of Elbasvir and 3.0ml of Grazoprevir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY
Linearity performed in the range of 1µg/ml-5µg/ml for Elbasvir and100µg/ml-500µg/ml for Grazoprevir.

PRECISION
REPEATABILITY
Preparation of Elbasvir and Grazoprevir Product Solution for Precision:
Prepare 1mg/ml of Elbasvir and Grazoprevir working standard (Stock solution).Further pipette 0.03ml of Elbasvir and 3.0ml of Grazoprevir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents and injected for five times and measured the area for all five injections in HPLC.

INTERMEDIATE PRECISION
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions by injecting for six times and measured the area for all six injections in HPLC.

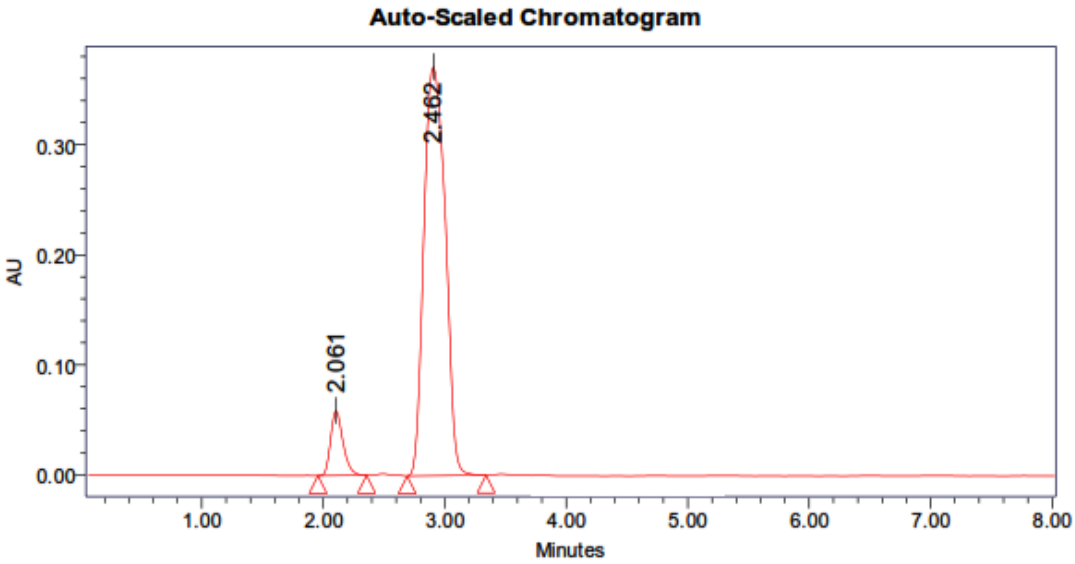
Accuracy
Injected the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculated the Amount found and Amount added for Elbasvir and Grazoprevir and calculated the individual 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.
Prepare 1mg/ml of Elbasvir and Grazoprevir working standard (Stock solution). Further pipette 0.03ml of Elbasvir and 3.0ml of Grazoprevir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

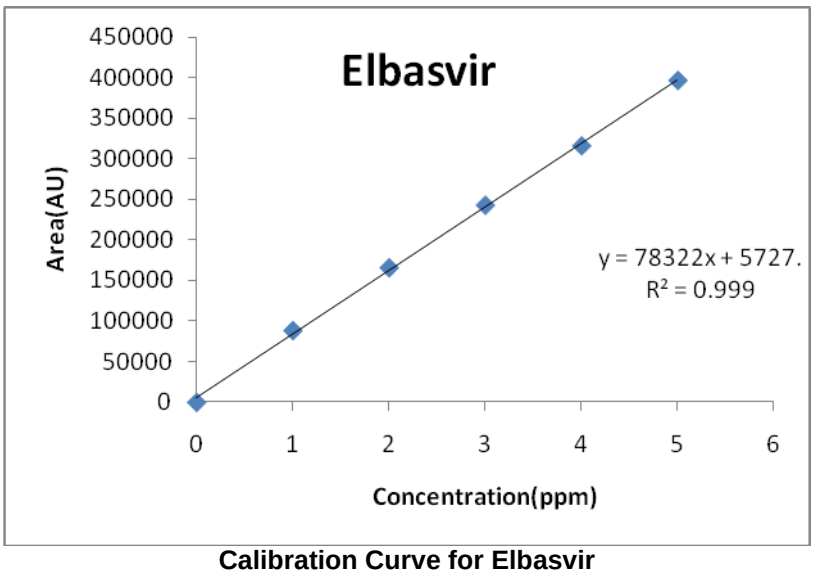
Effect of Variation of flow conditions
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition
The sample was analyzed by variation of mobile phase i.e. Methanol: Buffer was taken in the ratio and 50:50, 60:40 instead (55:45), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

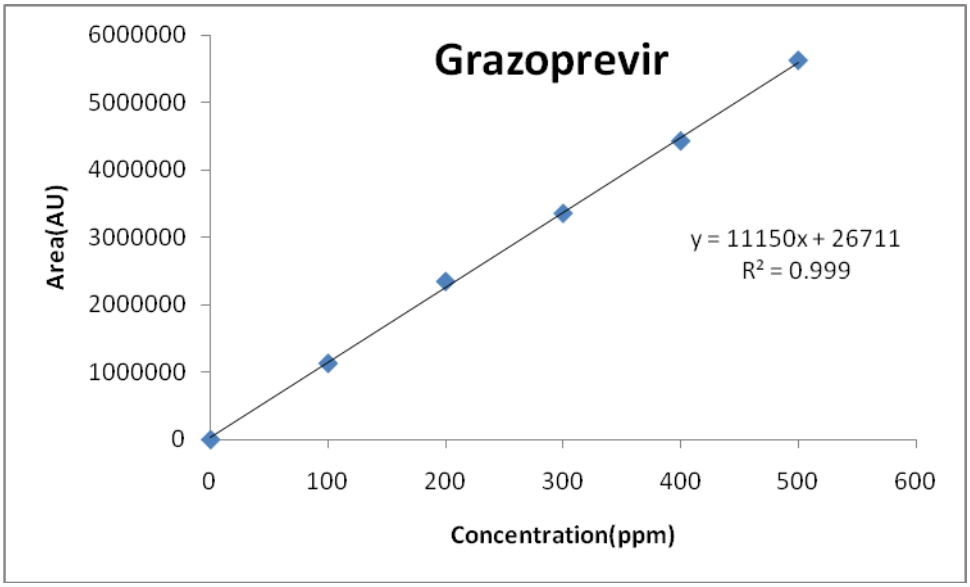
CONCLUSION
In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Elbasvir and Grazoprevir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Methanol: Phosphate Buffer pH 3.9 (55:45v/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.



Optimized Chromatogram



Calibration Curve for Elbasvir



Calibration Curve for Grazoprevir

Table 1: peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Elbasvir	2.061	247392	58952	1.2	7243
2	Grazoprevir	2.462	3530866	371748	1.1	3389

Table 2: Results of system suitability for Elbasvir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Elbasvir	2.048	246713	73455	11318	1.1
2	Elbasvir	2.074	245617	78152	7105	1.2
3	Elbasvir	2.071	245830	78146	8974	1.2
4	Elbasvir	2.069	240552	78242	7087	1.2
5	Elbasvir	2.070	245725	77705	5124	1.2
Mean			244887.4			
Std. Dev			2462.26			
% RSD			1.005466			

Table 3: Results of system suitability for Grazoprevir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Grazoprevir	2.446	3363754	636862	8484	1.1
2	Grazoprevir	2.490	3326434	641486	7889	1.0
3	Grazoprevir	2.489	3345949	638081	7846	0.9
4	Grazoprevir	2.488	3336621	617725	6772	0.9
5	Grazoprevir	2.490	3355244	631710	6884	0.9
Mean			3345600			
Std. Dev			14753.43			
% RSD			0.44098			

Table 4: Results of repeatability for Elbasvir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Elbasvir	2.065	249684	12079	5343	1.0
2	Elbasvir	2.064	249696	12068	5473	1.2
3	Elbasvir	2.064	246325	11949	5473	1.1
4	Elbasvir	2.065	249816	11811	5389	1.1
5	Elbasvir	2.067	249892	11735	5180	1.0
Mean			249082.6			
Std. Dev			1543.964			
% RSD			0.61986			

Table 5: Results of repeatability for Grazoprevir

S. No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Grazoprevir	2.486	3233700	59095	6654	1.2
2	Grazoprevir	2.484	3241323	57552	6524	1.3
3	Grazoprevir	2.482	3245927	57213	6440	1.3
4	Grazoprevir	2.483	3245927	57096	6411	1.4
5	Grazoprevir	2.483	3222194	54363	6260	1.4
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

Table 6: Results of Intermediate precision for Elbasvir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Elbasvir	2.066	242721	11323	5272	1.21
2	Elbasvir	2.066	240155	11564	5168	1.16
3	Elbasvir	2.066	240945	11887	5310	1.14
4	Elbasvir	2.065	240385	11938	5275	1.19
5	Elbasvir	2.069	249920	11652	5078	1.10
6	Elbasvir	2.067	240820	11750	5225	1.17
Mean			243991			
Std. Dev			4641.97			
% RSD			1.5			

Table 7: Results of Intermediate precision for Grazoprevir

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Grazoprevir	2.477	3325309	54143	6149	1.25
2	Grazoprevir	2.478	3323780	53740	6127	1.21
3	Grazoprevir	2.483	3328190	54791	6607	1.28
4	Grazoprevir	2.486	3329035	55098	6769	1.28
5	Grazoprevir	2.489	3325968	52379	6709	1.30
6	Grazoprevir	2.483	3327725	54779	6756	1.36
Mean			3326668			
Std. Dev			1985.641			
% RSD			0.059689			

Table 8: The accuracy results for Elbasvir

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	124675.7	15	15.1	101%	100.4%
100%	242006.3	30	30.1	100.5%	
150%	357449	45	44.9	99.7%	

Table 9: The accuracy results for Grazoprevir

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	1696259	18.75	18.71	99.8%	99.2%
100%	3351661	37.5	37.2	99.4%	
150%	4975094	56.25	55.47	98.6%	

Table 10: RESULTS FOR ROBUSTNESS-ELBASVIR

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	247392	2.061	7243	1.2
Less Flow rate of 0.9 mL/min	69214	2.267	4713	1.3
More Flow rate of 1.1 mL/min	388838	1.864	4740	1.2
Less organic phase	445628	2.165	4709	1.2
More organic phase	69404	1.967	5590	1.4

Table 11: RESULTS FOR ROBUSTNESS-GRAZOPREVIR

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3530866	2.462	3389	1.1
Less Flow rate of 0.9 mL/min	527373	2.690	5275	1.0
More Flow rate of 1.1 mL/min	4363129	2.284	5611	1.0
Less organic phase	3965572	2.590	5550	1.0
More organic phase	527708	2.390	6273	1.0

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