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

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## 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 Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.399 mins and 3.907 mins. The % purity of Elbasvir and Grazoprevir was found to be 99.94% and 99.95% respectively. The system suitability parameters for Elbasvir and Grazoprevir such as theoretical plates and tailing factor were found to be 1.3, 4668.7 and 1.3, 6090.3 the resolution was found to be 2.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Elbasvir and Grazoprevir was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100.4% and 100.5%, %RSD for repeatability was 0.7 and 0.4, % RSD for intermediate precision was 0.18 and 0.39 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Elbasvir and Grazoprevir in API and Pharmaceutical dosage form.

**KEYWORDS:** Elbasvir, Grazoprevir, RP-HPLC, Validation.

## INTRODUCTION

Elbasvir is a direct acting antiviral medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV). HCV is a single-stranded RNA virus that is categorized into nine distinct genotypes, with genotype 1being the most common in the United States, and affecting 72% of all chronic HCV patients. Treatment options for chronic Hepatitis C have advanced significantly since 2011, with the development of Direct Acting Antivirals (DAAs) such as Elbasvir.

Elbasvir is an inhibitor of NS5A, a protein essential for viral replication and virion assembly. The barrier for develoment of resistance to NS5A inhibitors is lower than that of NS5B inhibitors, another class of DAAs.

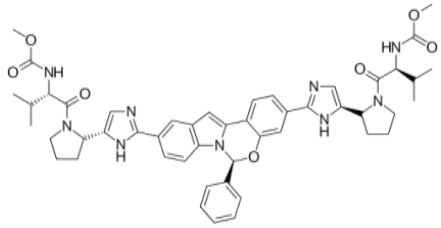

**Fig. no: 1: Elbasvir.**

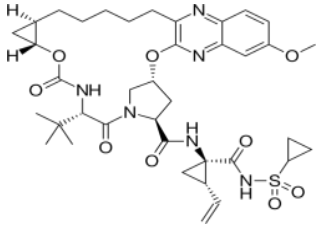

**Fig. no: 2: Grazoprevir.**

Grazoprevir is a second generation NS3/4a protease inhibitor used to inhibit viral HCV replication. NS3/4a protease is an integral part of viral replication and mediates the cleavage the virally encoded polyprotein to mature proteins (NS3, NS4A, NS4B, NS5A and NS5B). Grazoprevir inhibits the NS3/4 protease enzymes of HCV genotype 1a, 1B, and 4 with IC50values of 7pM, 4pM, and 62pM, respectively.

## EXPERIMENTAL WORK

**Table no. 1: List of Instruments.**

| S.No. | Instrument | Model No. | Software | Manufacturer'sname |
|---|---|---|---|---|
| 1 | HPLC Alliance | Waters2695 | Empower | Waters |
| 2 | UV double Beam | UV3000 | UVWin5 | LabIndia |
| 3 | Digital Weighing | BSA224SC | - | Satorius |
| 4 | pHmeter | AD102U | - | LabIndia |
| 5 | Ultrasonicator | SE60US | - | - |
| 6 | Suction pump | VE115N | - | - |

**Table no. 2: Chemicals.**

| S.No. | Chemicals | Manufacturer | Grade |
|---|---|---|---|
| 1 | Water | Merck | HPLC Grade |
| 2 | Methanol | Merck | HPLC Grade |
| 3 | Acetonitril | Merck | HPLC Grade |
| 4 | Potassium Dihydrogenorthophosphate | Merck | A.R |
| 5 | Elbasvir & GrazoprevirAPI | - | - |
| 6 | Eurepamf tablets | LocalPharmacy | - |

## METHOD DEVELOPMENT

### Selection of Detection of Wavelength

10 mg of Elbasvir and Grazoprevir was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overla y spectrum was used for selection of wavelength for Elbasvir and Grazoprevir. The isobestic point was taken as detection wavelength.

### Selection of Column

Column is selected based on solubility, polarity and chemical differences among Analytes [Column: Xterra C18 (4.6 x 250mm, 5µm, Make: Waters)]

### Selection of Mobile Phase

Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) has been selected as mobile phase. Buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved.

### Selection of Flow Rate

Flow rate selected was 1ml/min. Flow rate is selected based on Retention time, Column back pressure, Peak symmetry, Separation of impurities.

## PREPARATION AND PROCEDURES

### Preparation of Phosphate buffer: (PH: 4.6)

Weighed 6.8 grams of KH2PO4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

### Preparation of mobile phase

A mixture of pH 4.6 Phosphate buffer 550 mL (55%), 450 mL of ACN (45%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

### Diluant Preparation

Mobile phase is used as Diluant.

### Preparation of the individual Elbasvir standard preparation

10mg of Elbasvir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

### Preparation of the individual Grazoprevir standard preparation

10mg of Grazoprevir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

### Preparation of Sample Solution: (Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Grazoprevir and Elbasvir (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL

of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluant.

**Procedure**
20µL of the standard, sample are injected into the chromatographic system and the areas for Grazoprevir and Elbasvir peaks are measured and the %Assay are calculated by using the formulae.

**System Suitability**
Tailing factor for the peaks due to Grazoprevir and Elbasvir in Standard solution should not be more than 2.0. and Elbasvir peaks in Standard solution should not be less than 2000.

**ANALYTICAL METHOD VALIDATION**
**Accuracy**
**Preparation of standard solution (Elbasvir and Grazoprevir)**
Accurately weighed 10mg of Grazoprevir and 10mg of Elbasvir working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks.

About 7mL and 70ml of Diluents are added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 3ml and 0.3ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted upto the mark with diluents.

**Preparation of Sample solutions**
**For preparation of 50% solution (With respect to target Assay concentration):** Accurately 5mg of Grazoprevir and 5mg of Elbasvir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Grazoprevir and Elbasvir stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluant.

**For preparation of 100% solution (With respect to target Assay concentration):** Accurately 10mg of Grazoprevir and 10mg of Elbasvir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Grazoprevir and Elbasvir stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluant.

**For preparation of 150% solution (With respect to target Assay concentration):** Accurately 15mg of Grazoprevir and 15mg of Elbasvir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Grazoprevir and Elbasvir stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluant.

**Procedure**
The standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions were injected. The Amount found and Amount added for Grazoprevir & Elbasvir and the individual recovery and mean recovery values were calculated.

Acceptance criteria
Correlation coefficient should be not less than 0.999.

**PRECISION**
**Repeatability**
**Preparation of standard stock solution**
Accurately 10 mg of Grazoprevir and 10mg of Elbasvir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further it was pipette (3ml and 0.3ml) into a 10ml volumetric flask and diluted up to the mark with diluents.

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

**Acceptance criteria**
The % RSD for the area of five standard injections results should not be more than 2.

**Intermediate Precision (Ruggedness)**
To evaluate the intermediate precision (also known as ruggedness) of the Method, precision was performed on different days by using different make column of same dimensions.

**Preparation of standard stock solution**
Accurately 10 mg of Grazoprevir and 10mg of Elbasvir working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further this Stock was pipette (3ml and 0.3ml) into a 10ml volumetric flask and dilute up to the mark with diluents.

**Procedure**
The standard solution was injected for five times and the

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

**Acceptance criteria**
The % RSD for the area of five sample injections results should not be more than 2%.

**Specificity**
The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

**LOD**
LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

**LOQ**
LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines.

**Linearity**
**Preparation of stock solution**
Accurately 10 tablets were weighed & crushed in mortar and pestle and weight equivalent to 10 mg of Grazoprevir and Elbasvir (marketed formulation) sample were transferred into a 10mL clean dry volumetric flask and about 7mL of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

**Preparation of Level – I (100ppm of Grazoprevir & 1pm of Elbasvir)**
1ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluant.

**Preparation of Level – II (200ppm of Grazoprevir & 2ppm of Elbasvir)**
2ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluant.

**Preparation of Level – III (300ppm of Grazoprevir & 3ppm of Elbasvir)**
3ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluant.

**Preparation of Level – IV (400ppm of Grazoprevir & 4ppm of Elbasvir):**
4ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluant.

**Preparation of Level – V (500ppm of Grazoprevir & 5ppm of Elbasvir)**
5ml of stock solution has taken in 10ml of volumetric flask and diluted up to the mark with diluent.

**Procedure**
Each level was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated.

**Acceptance criteria**
Correlation coefficient should be not less than 0.999.

**Range**
Based on precision, linearity and accuracy data it can be concluded that the assa y method is precise, linear and accurate in the range of 1µg-5µg and 100µg- 500µg of Elbasvir and Grazoprevir respectively.

**Robustness**
As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.8ml/min to 1.2 ml/min. Standard solution 3ppm of Elbasvir and 300ppm of Grazoprevir was prepared and analyzed using the varied flow rates along with method flow rate.
b) The organic composition in the mobile phase was varied from 65% to 75% standard solution 3 µg/ml of Elbasvir and 300 µg/ml of Grazoprevir were prepared and analyzed using the varied mobile phase composition along with the actual mobile phase composition in the method. System suitability:

5 mg of Elbasvir and 500 mg of Grazoprevir working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask and add about 20ml of diluant and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further 10 ml of Elbasvir and Grazoprevir was pipetted out from the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluant.

## RESULTS AND DISCUSSION

**Wavelength Detection**
The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Elbasvir and Grazoprevir was obtained and the isobestic point of Elbasvir and Grazoprevir showed absorbance's maxima at 255nm.

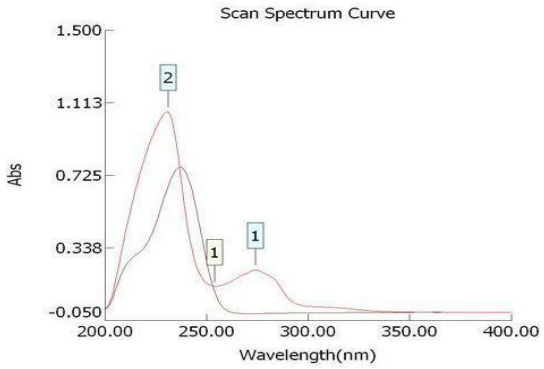


**Fig. 3: Overlay spectrum of Elbasvir and Grazoprevir.**

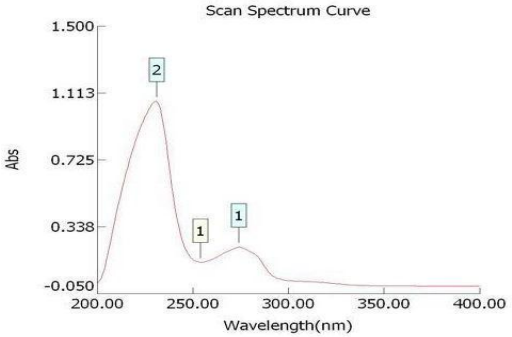


**Fig. 4: Spectrum of Elbasvir.**

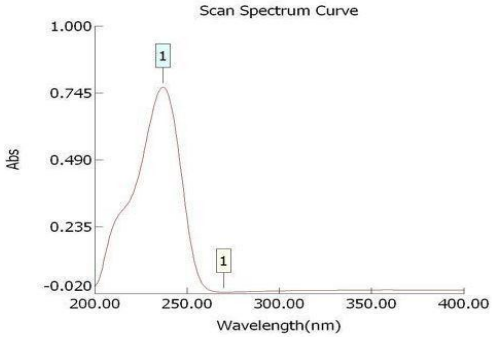


**Fig. 5: Spectrum of Grazoprevir.**

**METHOD DEVELOPMENT**

Chromatographic conditions:

Column : Xterra C18 5µm (4.6*250mm)

Mobile phase ratio : Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v)

Detection wavelength : 255nm

Flow rate : 1ml/min

Injection volume : 20µl

Column temperature : Ambient

**Table no 3: Details of optimized Trail.**

| S.No | Peak name | $R_t$ | Area | Height | USP Plate count | USP Tailin | USP Resolutio |
|---|---|---|---|---|---|---|---|
| 1 | Grazoprevir | 2.39 | 946124 | 155429 | 5105 | 1.3 | 8.1 |
| 2 | Elbasvir | 3.90 | 111541 | 13239 | 3788 | 1.4 | |

**Observation**

The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

**Table No. 4: Details of Elbasvir (Sample).**

| | NAME | RT | AREA |
|---|---|---|---|
| 1 | ELBASVIR | 3.527 | 828933 |
| 2 | ELBASVIR | 3.528 | 810493 |
| MEAN | | | 819713 |
| STD DEVIATION | | | 13039.2 |
| %RSD | | | 1.59 |

**Table No. 5: Details of Grazoprevir (Sample).**

| | NAME | RT | AREA |
|---|---|---|---|
| 1 | GRAZOPREVIR | 3.527 | 828933 |
| 2 | GRAZOPREVIR | 3.528 | 810493 |
| MEAN | | | 819713 |
| STD DEVIATION | | | 13039.2 |
| %RSD | | | 1.59 |

**Table 6: Details of Elbasvir (Standard).**

| | NAME | RT | AREA | USB PLATE COUNT | USP TAILING | USP RESOLUTION |
|---|---|---|---|---|---|---|
| 1 | Elbasvir | 3.525 | 810802 | 3527.8 | 1.0 | 2.4 |
| 2 | Elbasvir | 3.528 | 808709 | 3566.2 | 1.0 | 2.3 |
| MEAN | | | 809796 | 3547.0 | 1.0 | |
| STD DEVIATION | | | 1422.2 | | | |
| % RSD | | | 0.18 | | | |

**Table 7: Details of Grazoprevir (Standard).**

| | NAME | RT | AREA | USB PLATE COUNT | USP TAILING | USP RESOLUTION |
|---|---|---|---|---|---|---|
| 1 | Grazoprevir | 2.984 | 681469 | 3115.4 | | 1.1 |
| 2 | Grazoprevir | 2.989 | 683696 | 3209.7 | | 1.1 |
| MEAN | | | 682582 | 3162.5 | | 1.1 |
| STD DEVIATION | | | 1575.2 | | | |
| % RSD | | | | | | |

**VALIDATION RESULTS**

**ACCURACY**

**Table 8: Accuracy results of Grazoprevir.**

| %Concentration (at specification Level) | Area | Amount added(mg) | Amount found(mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 544711 | 5 | 5.10 | 101.8% | 100.5% |
| 100% | 675935 | 10 | 9.99 | 99.9% | |
| 150% | 812764 | 15 | 14.9 | 99.1% | |

**Table 9: Accuracy results of Elbasvir.**

| %Concentration (at specification level) | Area | Amount Added(mg) | Amount Found(mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 644765 | 5 | 5.0 | 101.3% | 100.0% |
| 100% | 803722 | 10 | 9.94 | 99.4% | |
| 150% | 962917 | 15 | 14.8 | 99.2% | |

**PRECISION**

**i) Repeatability**

**ii) Intermediate precision (Ruggedness) Repeatability**

**Table 10: Repeatability results of Grazoprevir.**

| | NAME | RT | AREA |
|---|---|---|---|
| 1 | Grazoprevir | 3.019 | 691143 |
| 2 | Grazoprevir | 3.011 | 685431 |
| 3 | Grazoprevir | 3.044 | 683543 |
| 4 | Grazoprevir | 2.997 | 683564 |
| 5 | Grazoprevir | 2.994 | 683532 |
| MEAN | | | 685443 |
| STD DEVIATION | | | 3289.7 |
| %RSD | | | 0.48 |

**Table 10: Repeatability results of Elbasvir.**

| | NAME | RT | AREA |
|---|---|---|---|
| 1 | Elbasvir | 3.557 | 819305 |
| 2 | Elbasvir | 3.547 | 807157 |
| 3 | Elbasvir | 3.544 | 804070 |
| 4 | Elbasvir | 3.537 | 808474 |
| 5 | Elbasvir | 3.534 | 804505 |
| MEAN | | | 808702 |
| STD DEVIATION | | | 6203.7 |
| %RSD | | | 0.77 |

**Intermediate precision/Ruggedness**

**Table 11: NAME: Elbasvir.**

| | NAME | RT | Area |
|---|---|---|---|
| 1 | Elbasvir | 3.524 | 813507 |
| 2 | Elbasvir | 3.533 | 817673 |
| 3 | Elbasvir | 3.533 | 815189 |
| 4 | Elbasvir | 3.517 | 815816 |
| 5 | Elbasvir | 3.530 | 815356 |
| Mean | | | 815508 |
| Std.Dev. | | | 1492.7 |
| RSD% | | | 0.18 |

**Table 12: NAME: Grazoprevir.**

| | NAME | RT | Area |
|---|---|---|---|
| 1 | Grazoprevir | 3.001 | 673725 |
| 2 | Grazoprevir | 3.009 | 672535 |
| 3 | Grazoprevir | 3.010 | 676216 |
| 4 | Grazoprevir | 2.997 | 679037 |
| 5 | Grazoprevir | 3.007 | 677101 |
| Mean | | | 675723 |
| Std.Dev. | | | 2611.5 |
| RSD% | | | 0.39 |

**SPECIFICITY**

**Table 13: Details of Elbasvir.**

| | NAME | RT | AREA | USP PLATE COUNT | USP TAILING | USP RESOLUTION |
|---|---|---|---|---|---|---|
| **1** | Elbasvir | **3.525** | **810802** | **3527.8** | **1.0** | **2.4** |
| **2** | Elbasvir | **3.528** | **808790** | **3566.2** | **1.0** | **2.3** |
| **MEAN** | | | **809796** | **3547.0** | **1.0** | |
| **STD DEVIATION** | | | **1422.2** | | | |
| **%RSD** | | | **0.18** | | | |

**Table 14: Details of Grazoprevir.**

| | NAME | RT | AREA | USP PLATE COUNT | USP TAILING |
|---|---|---|---|---|---|
| **1** | Grazoprevir | **2.984** | **681469** | **3115.4** | **1.1** |
| **2** | Grazoprevir | **2.989** | **683696** | **3209.7** | **1.1** |
| **MEAN** | | | **682582** | **3162.5** | **1.1** |
| **STD DEVIATION** | | | **1575.2** | | |
| **%RSD** | | | **0.23** | | |

**DETECTION OF LIMIT**

The LOD was performed for Elbasvir and Grazoprevir was found to be 2.95and 3.04 respectively.

**QUANTITATION LIMIT**

The LOQ was performed for Elbasvir and Grazoprevir was found to be 9.87and 10 respectively**.**

**LINEARITY**

**Table 15: Linearity Results.**

| | NAME | RT | AREA | HEIGHT(µV) |
|---|---|---|---|---|
| 1 | Elbasvir | 2.996 | 226418 | 26134 |
| 2 | Grazoprevir | 3.519 | 277182 | 28872 |
| 3 | Elbasvir | 3.003 | 432920 | 50127 |
| 4 | Grazoprevir | 3.528 | 521695 | 54273 |
| 5 | Elbasvir | 3.005 | 677256 | 78323 |
| 6 | Grazoprevir | 3.529 | 808274 | 83849 |
| 7 | Elbasvir | 2.998 | 869825 | 100093 |

| | | | | |
|---|---|---|---|---|
| 8 | Grazoprevir | 3.522 | 1033875 | 106297 |
| 9 | Elbasvir | 2.987 | 1095759 | 125962 |
| 10 | Grazoprevir | 3.510 | 125804 | 132354 |

**Linearity results of Grazoprevir**
**Table 16.**

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 100ppm | 226418 |
| 2 | II | 200ppm | 432920 |
| 3 | III | 300ppm | 677256 |
| 4 | IV | 400ppm | 869825 |
| 5 | V | 500ppm | 1095759 |
| Correlation Coefficient | | | 0.999 |

**Linearity results of Elbasvir**
**Table 17.**

| S.No | Linearity | Concentratio | Area |
|---|---|---|---|
| 1 | I | 1ppm | 277182 |
| 2 | II | 2ppm | 521695 |
| 3 | III | 3ppm | 808274 |
| 4 | IV | 4ppm | 1033875 |
| 5 | V | 5ppm | 1285804 |
| Correlation Coefficient | | | 0.999 |

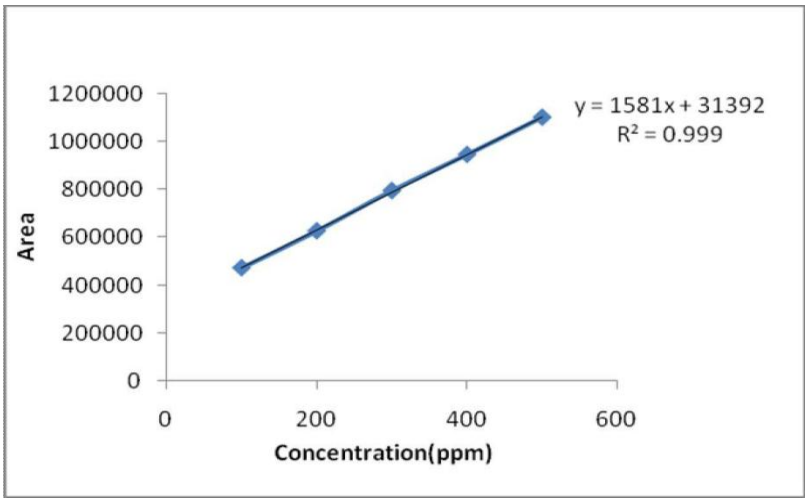


**Figure 6: Calibration curve of Grazoprevir.**

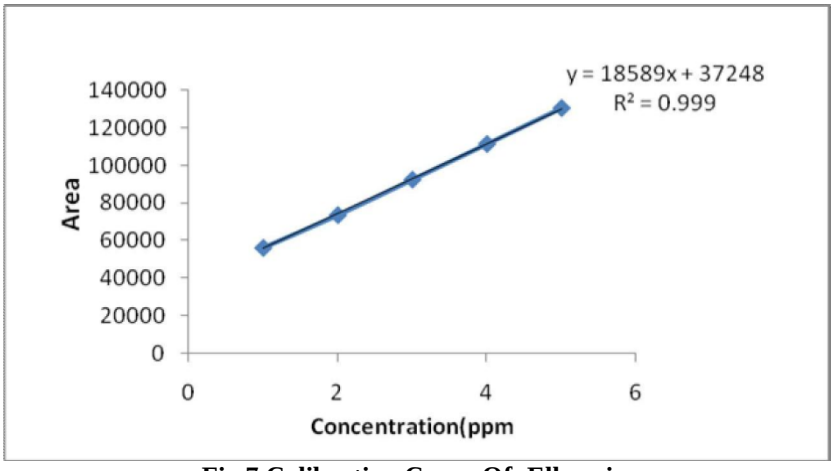


**Fig.7 Calibration Curve Of Elbasvir.**

**ROBUSTNESS**

**A) Flow Rate**

**Table 18: Details of Robustness more flow.**

| | NAME | Retention Time (min) | Area (µV*Sec) | Height (µV) | USP PLATE COUNT | USP TAILING | USP RESOLUTION |
|---|---|---|---|---|---|---|---|
| 1 | Elbasvir | 2.747 | 623847 | 75117 | 2503.3 | 0.9 | |
| 2 | Grazopre | 3.220 | 756748 | 80446 | 2685.4 | 0.9 | 1.9 |

**Table 19: Details of Robutness less flow.**

| | NAME | Retention Time (min) | Area (µV*Sec) | Height (µV) | USP PLATE COUNT | USP TAILING | USP RESOLUTION |
|---|---|---|---|---|---|---|---|
| 1 | Elbasvir | 3.276 | 740721 | 73704 | 2690.4 | 0.9 | |
| 2 | Grazopre | 3.847 | 903225 | 78896 | 2716.2 | 0.9 | 1.9 |

**System suitability results for Grazoprevir**

**Table 20: System suitability results For Grazoprevir (Flow rate).**

| S.No | Flow Rate (ml/min) | System suitability results | |
|---|---|---|---|
| | | USP Plate count | USP Tailing |
| 1 | 0.8 | 2690 | 0.9 |
| 2 | 1.0 | 3115 | 1.1 |
| 3 | 1.2 | 2503 | 0.9 |

**System suitability results for Elbasvir**

**Table 21: System suitability results for Elbasvir (Flow rate).**

| S.No | Flow Rate(ml/min) | System suitability results | |
|---|---|---|---|
| | | USP Plate count | USP Tailing |
| 1 | 0.8 | 2716 | 0.9 |
| 2 | 1.0 | 3527 | 1.0 |
| 3 | 1.2 | 2685 | 0.9 |

**B) Mobile Phase**

**Table 22: Details of Robustness more org.**

| | NAME | RETENTION TIME(Min) | AREA (µV*Sec) | HEIGHT (µV) | USP PLATE COUNT | USP TAILING | USP RESOLUTION |
|---|---|---|---|---|---|---|---|
| 1 | Elbasvir | 2.743 | 623812 | 75134 | 2707.1 | 1.1 | |
| 2 | Grazopr | 3.221 | 756795 | 80412 | 3001.8 | 1.0 | 1.9 |

**Table 23: Details of Robustness les organic.**

| | NAME | RETENTION TIME(Min) | AREA (µV*Sec) | HEIGHT (µV) | USP PLATE COUNT | USP TAILING | USP RESOLUTION |
|---|---|---|---|---|---|---|---|
| 1 | Elbasvir | 3.275 | 740841 | 73795 | 2818.9 | 1.1 | |
| 2 | Grazopre | 3.846 | 903365 | 98845 | 3107.7 | 1.0 | 1.9 |

**System suitability results for Grazoprevir**

**Table 24: System suitability results for Grazoprevir (Mobile phase).**

| S.No. | Changein Organic Composition in the Mobile Phase | System suitability results | |
|---|---|---|---|
| | | USP Plate count | USP Tailing |
| 1 | 10% Less | 2818 | 1.1 |
| 2 | Actual | 3125 | 1.1 |
| 3 | 10% More | 2707 | 1.1 |

**System suitability results for Elbasvir**

**Table 25: System suitability results for Elbasvir (Mobile phase).**

| S.No | Changein Organic Composition in the Mobile Phase | System suitability results | |
|---|---|---|---|
| | | USP Plate count | USP Tailing |
| 1 | 10% Less | 3107 | 1.0 |
| 2 | Actual | 3526 | 1.0 |
| 3 | 10% More | 3001 | 1.0 |

## SUMMARY AND CONCLUSION

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 Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.

The retention times were found to be 2.399mins and 3.907mins. The % purity of Elbasvir and Grazoprevir was found to be 99.94% and 99.95% respectively. The system suitability parameters for Elbasvir and Grazoprevir such as theoretical plates and tailing factor were found to be 1.3, 4668.7 and 1.3, 6090.3 the resolution was found to be 2.4.

The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Elbasvir and Grazoprevir was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100.4% and 100.5%, %RSD for repeatability was0.7 and 0.4, % RSD for intermediate precision was 0.18 and 0.39 respectively.

The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Elbasvir and Grazoprevir in API and Pharmaceutical dosage form.

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