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Original Article

# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ELBASVIR BY USING 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, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Phosphate Buffer pH 3.9 (55:45v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 255nm. The retention time of the Elbasvirwas 2.061 ±0.02min. The method produce linear responses in the concentration range of 1-5µg/ml of Elbasvir. 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.*

*Keywords: Elbasvir, RP-HPLC, validation.*

## INTRODUCTION

Elbasvir is chemically methyl N-[(2S)-1-[(2S)-2-{5-[(9S)-14-{2-[(2S)-1-[(2S)-2-[(methoxycarbonyl)amino]-3-methyl butanoyl]pyrrolidin-2-yl]-1H-imidazol-5-yl}-9-phenyl-8-oxa-10-azatetracyclo[8.7.0.0^{2,7}.0^{11,16}]heptadeca-1(17),2(7),3,5,11(16),12,14-heptaen-5-yl]-1H-imidazol-2-yl}pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate. 1 It is a drug approved by the FDA in January 20162 for the treatment of hepatitis C. It was developed by Merck and completed Phase III trials, used in combination with the NS3/4A protease inhibitor grazoprevir under the trade name Zepatier, either with or without ribavirin.[3]

## MATERIALS AND METHODS

Elbasvir Provided by Sura labs, Water and Methanol for HPL from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck and Phosphate buffer from Sura labs.

## HPLC METHOD DEVELOPMENT:

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## TRAILS

### Preparation of standard solution:

Accurately weigh and transfer 10 mg of Elbasvir 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 from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

### 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.

### 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. ZorbaxC18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

**VALIDATION**

**PREPARATION OF BUFFER AND MOBILE PHASE:**

**Preparation of Phosphate buffer pH 3.9:**

Accurately weighed 6.8 grams of $KH_2PO4$ 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 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 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 ofElbasvirsample 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 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 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 DRUGSOLUTIONS FOR LINEARITY:**

Accurately weigh and transfer 10 mg of Elbasvirworking 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).

**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 Product Solution for Precision:**

Accurately weigh and transfer 10 mg of Elbasvirworking 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 Elbasvirfrom the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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.

**Procedure:**

**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:**

**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 Elbasvirand 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 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 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.

**RESULTS AND DISCUSSION**

**Optimized Chromatogram (Standard)**

**Mobile phase:** Methanol: Phosphate Buffer pH 3.9 (55:45v/v)

**Column:** Zorbax C18 (4.6×150mm, 5.0 µm)

**Flow rate:** 1 ml/min

**Wavelength**: 255 nm

**Column temp**: 35ºC

**Injection Volume**: 10 µl

**Run time**: 8minutes

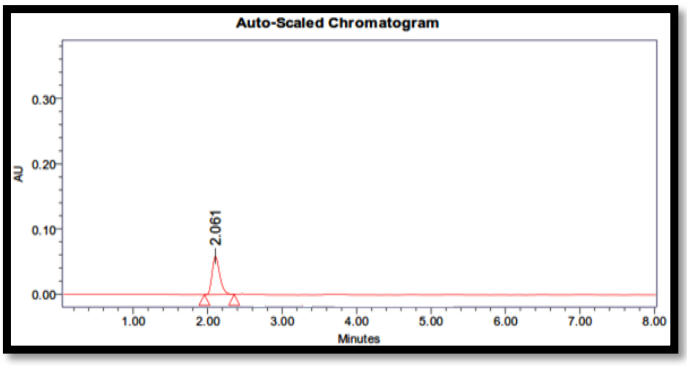


**Fig 1: Optimized Chromatogram**

**Table 1: - peak results for optimized**

| S. No | Peak name | Rt | Area | Height | USP Tailing | USP plate count |
|---|---|---|---|---|---|---|
| 1 | Elbasvir | 2.061 | 247392 | 58952 | 1.2 | 7243 |

**Optimized Chromatogram (Sample)**

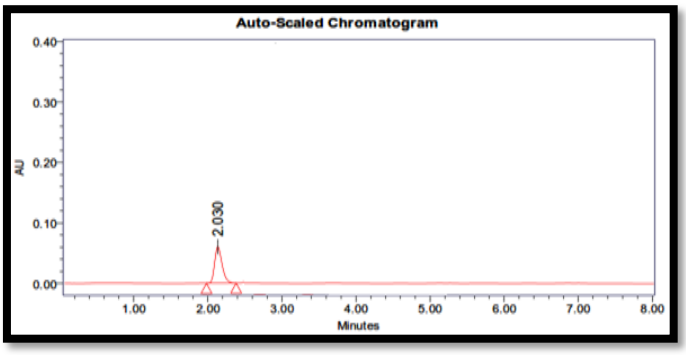


**Figure 2: Optimized Chromatogram (Sample)**

**Table 2: Optimized Chromatogram (Sample)**

| S. No | Peak name | Rt | Area | Height | USP Tailing | USP plate count |
|---|---|---|---|---|---|---|
| 1 | Elbasvir | 2.030 | 240019 | 60878 | 1.2 | 7246 |

**SPECIFICITY**

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that

 

may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Elbasvir in drug product.

**Assay (Standard):**

**Table 3: 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 | | | |

**Assay (Sample):**

**Table5: Peak results for Assay sample**

| S.No | Name | Rt | Area | Height | USP Tailing | USP plate count |
|---|---|---|---|---|---|---|
| **1** | Elbasvir | 2.068 | 244102 | 89282 | 1.2 | 5949 |
| **2** | Elbasvir | 2.070 | 240052 | 88021 | 1.2 | 5861 |
| **3** | Elbasvir | 2.067 | 243230 | 88882 | 1.2 | 5879 |

**% ASSAY =**

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

The % purity of Elbasvir in pharmaceutical dosage form was found to be 100.2 %.

**LINEARITY**

**Table 6: Chromatographic Data for Linearity Study Elbasvir**

| Concentration Level (%) | Concentration µg/ml | Average Peak Area |
|---|---|---|
| **33.3** | 1 | 88442 |
| **66.6** | 2 | 165724 |
| **100** | 3 | 242754 |
| **133.3** | 4 | 315906 |
| **166.6** | 5 | 396371 |

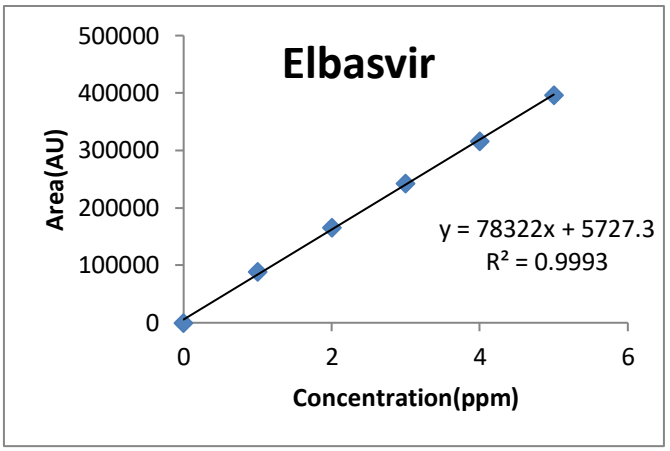


**Figure 3: calibration graph for Elbasvir**

**REPEATABILITY**

**Table 8: 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 | | | |

**Acceptance criteria:**

- % RSD for sample should be NMT 2

**Intermediate precision:**

**Table 10: 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 | | | |

**Acceptance criteria:**

• % RSD of six different sample solutions should not more than 2

**Table 12: Results of Intermediate precision Day 2 for Elbasvir**

| S no | Name | Rt | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| **1** | Elbasvir | 2.067 | 249499 | 11594 | 5240 | 1.2 |
| **2** | Elbasvir | 2.069 | 240991 | 11357 | 5130 | 1.2 |
| **3** | Elbasvir | 2.068 | 240431 | 11878 | 5136 | 1.2 |
| **4** | Elbasvir | 2.069 | 241330 | 11748 | 5267 | 1.2 |
| **5** | Elbasvir | 2.067 | 240519 | 11830 | 5222 | 1.2 |
| **6** | Elbasvir | 2.067 | 240470 | 11475 | 5982 | 1.2 |
| **Mean** | | | 242206.7 | | | |
| **Std. Dev** | | | 3590.034 | | | |
| **% RSD** | | | 1.48222 | | | |

**Acceptance criteria:**

• % RSD of six different sample solutions should not more than 2

**ACCURACY:**

**Table 14: 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% | |

**Acceptance Criteria:**

• The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

**LIMIT OF DETECTION**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

**LOD= 3.3 × σ / s**

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

**Result:**

**Elbasvir:**

=3.3 × 1760.8/78322

=0.07µg/ml

**LIMIT OF QUANTITATION**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

**Result:**

**Elbasvir:**

=10×1760.8/78322

= 0.2µg/ml

**Robustness**

**TABLE 16: RESULTS FOR ROBUSTNESSELBASVIR:**

| 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 |

**Acceptance criteria:**

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

**SUMMARY**

- The analytical method was developed by studying different parameters.

 

- First of all, maximum absorbance was found to be at 255nm 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 Zorbax C18 because it was giving good peak.
- 35ºC 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 Methanol: Phosphate Buffer pH 3.9 (55:45v/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 2.061, 2.462 ±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 was found to be accurate and well within range.
- The analytical method was found linearity over the range 1-5µg/ml of elbasvir of the target concentration.
- The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

**CONCLUSION**

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Elbasvirin bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Elbasvirwas freely soluble in ethanol, methanol and sparingly soluble in water.

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 inTablesfor 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 Elbasvirin bulk drug and in Pharmaceutical dosage forms.

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