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# VALIDATED SIMULTANEOUS HPLC METHOD FOR THE ESTIMATION OF GRAZOPREVIR AND ELBASVIR

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**ABSTRACT:**

The objective of the research is to create a rapid, uncomplicated, accurate, and cost-effective RP-HPLC technique for quantifying the quantities of Grazoprevir and Elbasvir concurrently in both pharmaceutical products and bulk materials. This approach has successfully accomplished the separation of Grazoprevir and Elbasvir in significant quantities. The separation was conducted with a Waters C18 (250 x 4.6 mm x 5 µ particle size) analytical column, with detection at a wavelength of 260 nm. The mobile phase included a blend of Methanol and Phosphate Buffer pH 4.0 at a volumetric ratio of 60:40. The separation was conducted using isocratic elution mode at a flow rate of 1.0 ml/min. Grazoprevir had a retention time of 2.400 minutes, whereas Elbasvir showed a retention length of 3.016 minutes. The quantification of Grazoprevir and Elbasvir was performed by utilizing PDA detection at a wavelength of 260 nm, employing a linear calibration curve. For accurate quantification, the concentration ranges of 20-100 µg/ml (with a correlation value of 0.9999) and 10-50 µg/ml (with a correlation coefficient of 0.999) were used. The suggested approach is well-suited for use in quality-control labs for the quantitative examination of medicines, both when employed

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separately or in combination, particularly in bulk quantities. This approach is distinguished by its effectiveness, while nevertheless guaranteeing a high degree of accuracy and precision.

**Keywords:** Grazoprevir, Elbasvir, Simultaneous and RP-HPLC.

**Introduction:**

Elbasvir is chemically known as 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]carbamate. In January 2016, the FDA authorized this medication for the treatment of hepatitis C. Zepatier, a medication created by Merck, underwent Phase III studies. It is used in conjunction with the NS3/4A protease inhibitor grazoprevir, either with or without ribavirin. Elbasvir is a powerful and specific inhibitor of the NS5A replication complex of the hepatitis C virus [1-5].

Grazoprevir is chemically designated as s (1R, 18R, 20R, 24S, 27S)- N- {(1R, 2S)-1-[(cyclopropylsulfonyl) carbamyl]- 2-vinyl(cyclopropyl)- 7-methoxy-24-(2- methyl-2-propanyl)-22,25-dioxo-2, 21-dioxa-4, 11, 23, 26-tetraazapentacyclo [24.2.1.03.12.05.1.0.0.18. 20] nonacosa-3,5,7,9,11-pentaene-27-carboxamide [5]. This medication has received approval [6] for the treatment of hepatitis C. Grazoprevir is a second-generation inhibitor of the NS3/4A protease targets of the hepatitis C virus. It has significant efficacy against many HCV genotype variations, including those that demonstrate resistance to the majority of now used antiviral drugs. Elbasvir and Grazoprevir have been analyzed in the scientific literature both as separate components and in conjunction with other substances.

The literature survey shows that there are few methods for the determination of Grazoprevir and Elbasvir individually in tablet dosage form by using various analytical instruments like UV-Vis spectrophotometer [7], HPLC [8-11], RP-UPLC [12, 13] and LC-MS/MS [14].

**Materials and Methods:**

All chemicals and reagents were HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

**Instrumentation:**

The analysis was carried out with the assistance of the Waters-2695 (Modal Alliance) High Performance liquid chromatography, the Mettler Toledo analytical balance, the PDA Detector with a Standard cell, the Empower 2 data processing system, the lab India pH meter, and the Sonicator.

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Waters C18 column, which has dimensions of 250 x 4.6 mm and a particle size of 5 µ, is the analytical column that is used with an isocratic approach, the flow rate that is being used is one milliliter per minute.

**Preparation of mobile phase:**

To prepare the mobile phase, a mixture of potassium dihydrogen orthophosphate and methanol was mixed in a ratio of 60:40. The pH of the buffer was adjusted to 4.0, and the mixture was filtered through a 0.45µ membrane.

**Preparation of standard stock solution:**

Ten milligrams of grazoprevir (API) and five milligrams of elbasvir (API) were each put into a separate volumetric flask that was ten milliliters in capacity and had been well cleaned and dried. After adding three quarters of the diluents to each of these flasks, sonicate the mixture for ten minutes, and then finally add the remaining diluent to get it up to the desired level. It has been determined that the concentrations of Grazoprevir and Elbasvir are 1000 µg/ml and 500 µg/ml, respectively.

**Preparation of Standard Working Solutions:**

Using a pipette, transfer one milliliter of each stock solution into a volumetric flask that has been well cleaned and dried. Finally, use diluent to bring the volume up to the desired level. A total of 100 µg/ml of Grazoprevir and 50 µg/ml of Elbasvir were found to be the quantities that were obtained.

**Preparation of Sample Stock Solutions:**

A sample of 10 tablets is chosen at random and their weights are measured. The average weight of each tablet is then computed. Subsequently, all the pills are crushed into a fine powder. The weight corresponding to 1 tablet was transferred into a 100ml volumetric flask, followed by the addition of 60ml of diluent. The mixture was then subjected to sonication for a duration of 25 minutes, and lastly, the flask was filled up to the mark with diluent. The whole material was filtered using a 0.45 µ filter paper. The concentration of Grazoprevir is 1000 µg/ml while the concentration of Elbasvir is 500 µg/ml. Extract 1 milliliter of the filtered sample stock solution using a pipette, then transfer it into a volumetric flask with a capacity of 10 milliliters. Fill the flask up to the designated mark using a diluent. The concentrations obtained were 100 µg/ml of Grazoprevir and 50 µg/ml of Elbasvir.

**Procedure:**

Five injections of 20 µl each of active GPR and EBR standard solutions were performed. Chromatograms were obtained and peak responses were evaluated. The system's suitability was calculated by evaluating its parameters. The quantification of GPR and EBR in the sample was achieved by the analysis of the peak responses.

**Chromatographic conditions:**

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High Performance Liquid Chromatography equipped with PDA detector.

For Grazoprevir and Elbasvir (isocratic)

**Column** : Waters C18 (250 x 4.6 mm x 5 µ particle size) analytical column

**Wavelength** : 260 nm

**Injection Volume** : 20µl

**Column Temperature**: Ambient

**Flow rate** : 1.0 ml/min

The GPR peak, seen at 2.400 minutes, had an area of 854623, accompanied by a tailing factor of 1.12. According to the data shown in Figure 1 and Table 1, the EBR peak was seen at a retention time of 3.016 minutes. The peak had an area of 425678, a tailing factor of 1.05, and a resolution of 2.52. This experiment was considered ideal since it yielded favorable results and had a shorter time of retention. The retention time for GPR is around 2.400 minutes, whereas the retention time for EBR is 3.016 minutes.

**Table 1: System suitability parameters**

| **No.** | **ame of the eak** | **etention ime (Mins)** | **eak Area** | **ailing Factor** | **esolution** | **ate Count** |
|---|---|---|---|---|---|---|
| 1 | razoprevir | 400 | 54623 | 12 | - | 342 |
| 2 | lbasvir | 016 | 25678 | 05 | 52 | 561 |

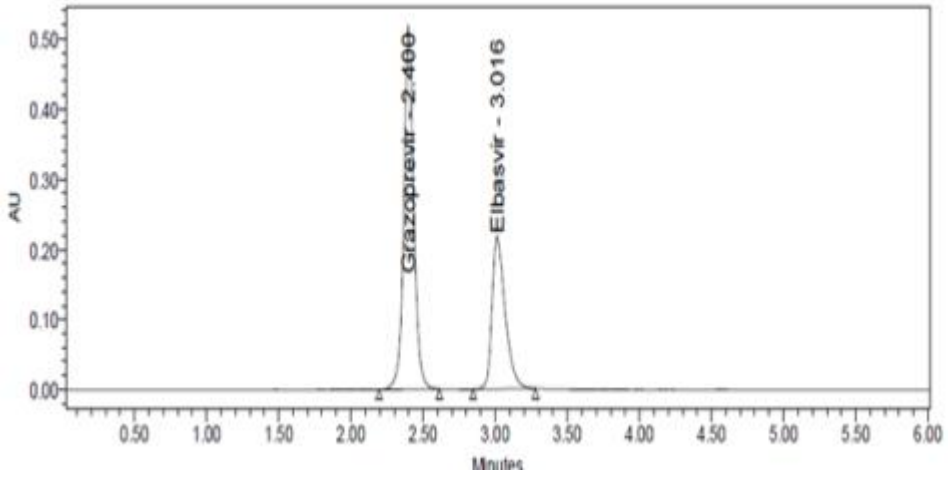


Fig.No. 01 : Typical Chromatogram of Grazoprevir and Elbasvir

**Method Validation:**

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The present study examined many parameters to establish the validity of the HPLC methodology for quantifying GPR and EBR in accordance with the specified procedure, hence demonstrating its suitability for the intended use. The implementation of all validation criteria was done in compliance with the standards set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

**Linearity and Range:**

The concentrations of GPR and EBR that showed a linear relationship with peak area were (20 - 100µg/ml), (10 - 50µg/ml).Results are shown in (Fig.2 & 3), (Table 2 & 3), and the linearity of the calibration curve is confirmed by the high value of the correlation coefficient of the regression equation.

**Table 2: Linearity data of GPR**

| S.No. | Concentration (µg/ml) | Peak Area |
|---|---|---|
| **1** | 0 | 0 |
| **2** | 20 | 854623 |
| **3** | 40 | 1729856 |
| **4** | 60 | 2586457 |
| **5** | 80 | 3415624 |
| **6** | 100 | 4273118 |
| **ope** | | **42722** |
| **tercept** | | **7194.4** |
| **egression** | | **0.9999** |

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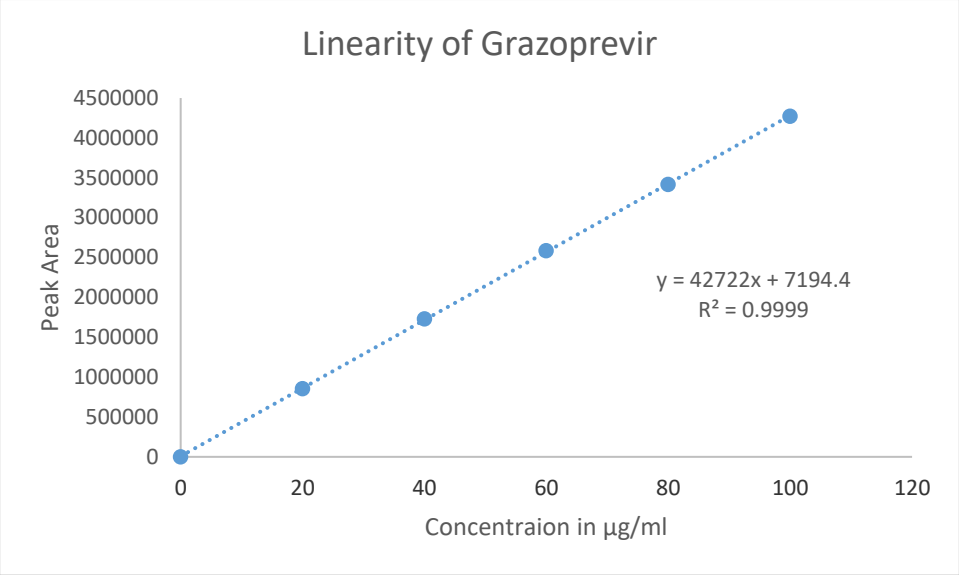


Fig.No. 02 : Linearity ofGrazoprevir

**Table 3: Linearity data of EBR**

| S.No. | Concentration (µg/ml) | Peak Area |
|---|---|---|
| **1** | 0 | 0 |
| **2** | 10 | 425678 |
| **3** | 20 | 854526 |
| **4** | 30 | 1279564 |
| **5** | 40 | 1781254 |
| **6** | 50 | 2144568 |
| **ope** | | **43470** |
| **ntercept** | | **-5823.9** |
| **egression** | | **0.999** |

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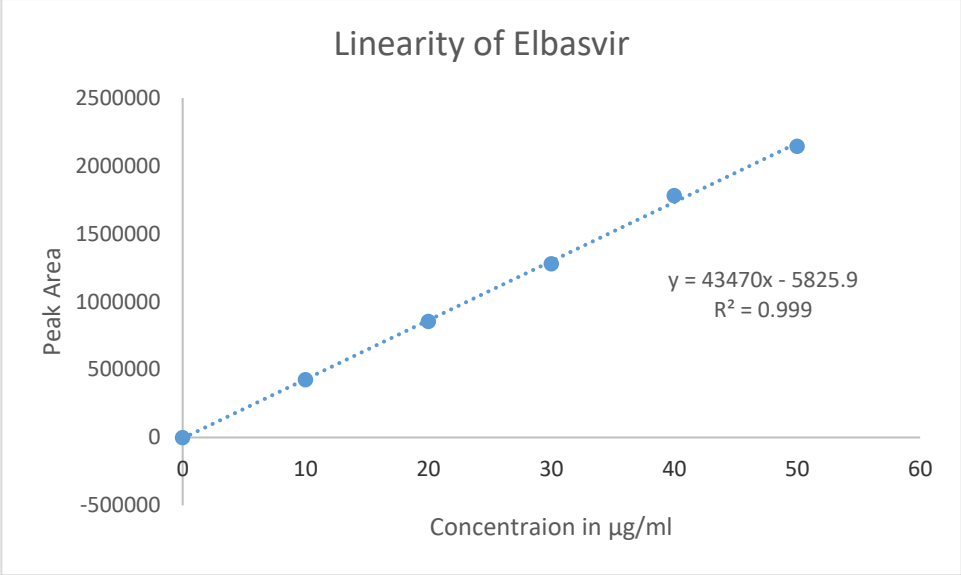


**Fig.No. 03 : Linearity of Elbasvir**

**Accuracy and Precision:**

The accuracy of recovery was assessed by adding more Standard medication to a previously tested test solution at three distinct concentration levels. By achieving a relative standard deviation (RSD) of less than 2%, we have determined that the recommended approach is very accurate in simultaneously estimating both GPR and EBR. The recovery rates for GPR and EBR were found to be 100.45% and 99.86% respectively. The Method's reliability is shown by its great repeatability and low RSD readings. The tables numbered 4 and 5.

**Table 4: Precision data of GPR and EBR**

| Injection Number | GPR | | | | EBR | | | |
|---|---|---|---|---|---|---|---|---|
| | Retention Time | Peak Area | Plate Count | Peak Symmetry | Retention Time | Peak Area | Plate Count | Peak Symmetry |
| **1** | 2.4 | 2155381 | 6945 | 1.12 | 3.016 | 1066303 | 9567 | 1.15 |
| **2** | 2.42 | 2168547 | 6952 | 1.1 | 3.01 | 1065648 | 9896 | 1.14 |
| **3** | 2.39 | 2156345 | 6452 | 1.13 | 3.04 | 1065489 | 9567 | 1.02 |
| **4** | 2.4 | 2156987 | 6358 | 1.14 | 3.05 | 1062389 | 9785 | 1.15 |
| **5** | 2.38 | 2153645 | 6349 | 1.16 | 3.021 | 1068945 | 9658 | 1.16 |
| **6** | 2.39 | 2158456 | 6894 | 1.14 | 3.04 | 1065843 | 9845 | 1.14 |
| **Average** | 2.40 | 2158227 | | | 3.03 | 1065770 | | |
| **Standard Deviation** | 0.01 | 5305.26 | | | 0.02 | 2092.61 | | |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **% RSD** | 0.5701 | 0.2458 | | | 0.5272 | 0.1963 | | |

**Table 5: Accuracy data of GPR and EBR**

| **Level** | **Grazoprevir** | | | | **Elbasvir** | | | |
|---|---|---|---|---|---|---|---|---|
| | **mount und g/ml)** | **mount dded ng/ml )** | **ecovery %)** | **lean (%)** | **mount und g/ml)** | **mount dded(mg/m** | **ecovery %)** | **lean (%)** |
| **evel-1 0%)** | 25.15 | 25 | 100.6 | 101.72 | 12.6 | 12.5 | 100.8 | 100.27 |
| | 25.56 | 25 | 102.24 | | 12.55 | 12.5 | 100.4 | |
| | 25.58 | 25 | 102.32 | | 12.45 | 12.5 | 99.6 | |
| **evel-2 00%)** | 50.12 | 50 | 100.24 | 100.39 | 25.12 | 25 | 100.48 | 100.49 |
| | 50.24 | 50 | 100.48 | | 25.02 | 25 | 100.08 | |
| | 50.23 | 50 | 100.46 | | 25.23 | 25 | 100.92 | |
| **evel-3 50%)** | 75.45 | 75 | 100.60 | 100.50 | 37.15 | 37.5 | 99.07 | 99.24 |
| | 75.12 | 75 | 100.16 | | 37.23 | 37.5 | 99.28 | |
| | 75.56 | 75 | 100.75 | | 37.26 | 37.5 | 99.36 | |
| **lean** | | | 100.45 | | | | 99.86 | |
| **D** | | | 0.22 | | | | 0.74 | |
| **RSD** | | | 0.2180 | | | | 0.7456 | |

**Robustness:**

The results of the robustness analysis are shown in Table no.6. Both components exhibited comparable tailing factors, elution orders, resolutions, relative standard deviations, and recoveries. The analysis revealed that the relative standard deviation (RSD) of the peak sites was much below 2.0%.

**Table 6: Robustness data of GPR andEBR**

| **Condition** | **Grazoprevir** | Elbasvir |
|---|---|---|

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| | % RSD | Tailing Factor | % Recovery | % RSD | Tailing Factor | % Recovery |
|---|---|---|---|---|---|---|
| **Change in Flow rate** | | | | | | |
| ormal Condition .0 ml per minute) | 0.15 | 1.12 | 100.23 | 0.15 | 1.05 | 100.12 |
| ow rate(1.2ml per minute) | 0.45 | 1.05 | 99.78 | 0.16 | 1.45 | 99.82 |
| ow rate(0.8 ml per minute) | 0.78 | 1.45 | 100.12 | 0.21 | 1.11 | 99.85 |
| **Change in minor component in the mobile phase** | | | | | | |
| ormal Condition Aethanol: Phosphate Buffer H 4.0 (60:40v/v)) | 0.41 | 1.12 | 99.98 | 0.78 | 1.04 | 100.26 |
| Aethanol: Phosphate Buffer H 4.0 (50:50v/v)) | 0.10 | 1.08 | 100.25 | 0.62 | 1.16 | 99.89 |
| Aethanol: Phosphate Buffer H 4.0 (40:60v/v)) | 0.25 | 1.14 | 100.78 | 0.78 | 1.09 | 100.23 |
| **Change in Wave Length** | | | | | | |
| ormal:Wave Length 260 nm | 0.21 | 1.11 | 100.12 | 0.11 | 1.05 | 100.45 |
| Vave Length 265 nm | 0.16 | 1.05 | 99.58 | 0.56 | 1.08 | 100.15 |
| Vave Length 255 nm | 0.45 | 1.06 | 100.05 | 0.89 | 1.12 | 100.02 |
| **Change in pH** | | | | | | |
| ormal:pH 4.0 | 0.45 | 1.04 | 100.45 | 0.52 | 1.05 | 100.25 |
| H 4.5 | 0.26 | 1.06 | 98.56 | 0.42 | 1.04 | 100.45 |
| H 3.5 | 0.89 | 1.09 | 98.36 | 0.36 | 1.09 | 99.45 |

**Ruggedness:**

Grazoprevir and Elbasvir had respective mean peak areas of 2157049and 1064428 with an RSD of 0.1963 and 0.2399 %, respectively and results are tabulated in Table 7.

**Table 7: Ruggedness data of GPR and EBR**

| Injection Number | GPR | EBR |
|---|---|---|
| | Peak Area | |
| 1 | 2155381 | 1062895 |
| 2 | 2158785 | 1065487 |

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| | | |
|---|---|---|
| 3 | 2156389 | 1064523 |
| 4 | 2159853 | 1068956 |
| 5 | 2156897 | 1062359 |
| 6 | 2154987 | 1062345 |
| Average | 2157049 | 1064428 |
| Standard Deviation | 2092.61 | 2553.55 |
| % RSD | 0.1963 | 0.2399 |

**SUMMARY:**

A novel and validated RP-HPLC method has been devised to determine GPR and EBR in bulk and pharmaceutical samples. Based on the results of the literature review, which showed a lack of techniques for measuring GPR and EBR in large amounts, there is an immediate need for a direct, cost-effective, and accurate solution to tackle this problem.

The concentrations of GPR and EBR were quantified by infusing a blend of Methanol and Phosphate Buffer pH 4.0, with a volumetric ratio of 60:40, onto an analytical column of Waters C18 (250 x 4.6 mm x 5 µ particle size). The flow rate was adjusted to 1.0 milliliters per minute, while the injection volume was 20 microliters. The GPR peak had a retention time of 2.400 minutes, whereas the EBR peak showed a retention time of 3.016 minutes.

Following its improvement, the method was verified in accordance with ICH guidelines to assess its compatibility with the system, linearity, sensitivity parameters, precision, accuracy, and robustness. All validation parameters yielded results within acceptable thresholds. The experiments had RSD values below 2. The range of recoveries varied between 98% and 102%.

**CONCLUSION:**

The suggested RP-HPLC technology provides a time-efficient and uncomplicated method that is straightforward, quick, accurate, particular, robust, and cost-effective. Hence, it is a preferred technique for the concurrent analysis of Grazoprevir and Elbasvir. The adopted approach underwent comprehensive verification in conformity with ICH requirements in all aspects.

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