A robust stability indicating HPLC technique for evaluation of Pibrentasvir and Glecaprevir in tablet dosage form

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ABSTRACT

When liver cells get infected and vandalized, the condition is termed as Hepatitis. HCV therapy is performed with mixture of drugs. For the combined evaluation of Pibrentasvir and Glecaprevir in tablets, a rapid, selective and robust HPLC technique stability indicating was developed herein this work. Analysis was executed by Cosmicsil, with dimensions 250 mm by 4.6 mm column and mobile phase possessing KH2PO4 with 0.1M, 65 ml and 35 ml of methanol and 230 nm of PDA analysis. Elution times were found out as were 1.663 min and 2.249 min, for Pibrentasvir and Glecaprevir respectively with linear ranges 20µg/ml, 60 µg/ml and 50 µg/ml, 150 µg/ml, respectively having detection limits as 0.190 µg/ml and 0.207 µg/ml and quantization limits as 0.634 µg/ml and 0.690 µg/ml. This method is explicit having RSD values as 0.097% Pibrentasir & 0.232% Glecaprevir showing an accuracy of between 98.82 and 100.07% for Pibrentasir 99.31, Glecaprevir 100.45% recovery values. During the investigation of degradation, peaks elution times of degradants greatly varied with the elution times of Glecaprevir and Pibrentasvir thus, proving method’s power of stability indication and specificity. The validation and degradation stability studies were carried out according to ICH and ICH Q1B Guidelines.

Keywords: Hepatitis; HCV; Pibrentasvir; Glecaprevir; HPLC; Cosmicsil; PDA analysis; ICH.

INTRODUCTION

When liver cells get infected and vandalized, the condition is termed as Hepatitis. Although there are varied reasons for its occurrence and types, similar symptoms may be exhibited [1]. The major service of liver is to detoxify blood, store vitamins and manufacture hormones. Disruption of previously stated liver functions may lead to severe health issues in total body [2]. The acute and major kinds are Hepatitis A, B, C caused because of various viruses [3, 4]. Multi-class combination drugs refer to a single pill or pill pack combination of drugs. The combination of used drugs approved is represented in (Table 1). Pibrentasvir acts on NS3A proteases are indispensable to replication of hepatitis C virus RNA and virus assembly. These processes are clogged and hence virus growth is held in by Pibrentasvir [5-7]. Glecaprevir Proteases NS4A and 5A are preconditions for RNA replication and virus assembly of hepatitis C virus. Hence, blocks these two processes and thus virus development is suppressed [8-10].
To the finest of our information, handful studies use HPLC and UPLC to assess pibrentasvir together with glecaprevir [11-17].

The main aim of this investigation is an effort to establish the RP-HPLC method which is stability indicating for testing pibrentasvir and glecaprevir which is economically friendly, fast and have a wide accurate range. The established method is validated for parameters such as sensitivity, linearity, specificity, selectivity, accuracy, robustness and precision according to ICH Guidelines [18]. And degradation studies were carried out to represent the method sensitivity according to ICH Q1B Guidelines [19].

MATERIALS AND METHODS

Materials used: The materials employed were Pibrentasvir, Glecaprevir, Methanol, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide, and Potassium dihydrogen phosphate.

The apparatus used were Waters alliance HPLC system, Photodiode array detector and Cosmosil analytical column, measuring C18, 250 x 4.6 mm, 5 µm

HPLC technique conditions: The Mobile phase flow rate was 1.0ml/Min, Temperature was maintained at 25°C, Volume subjected was 10µl, Run time was 5min, detected at the wave length of 230nm and maintained pH was 4.5.

Preparation of mobile phase
KH₂PO₄ of 0.1 M is blended in 65:35 parts with methanol: Orthophosphoric acid is used to alter pH to 4.5. This mixture is also applied as a solvent in the development of standard solutions.

Preparation of stock solution
Implicated in the preparation of stock solution of pibrentasvir and glecaprevir, a properly weighed 40 mg pibrentasvir and 100 mg glecaprevir in a 100 ml volumetric flask and exactly diluted with mobile phase. Concentration of stock solutions: pibrentasvir 400 µg/ml and glecaprevir 1000 µg/ml.

Preparation of sample solutions for validation
The standard solution to validate pibrentasvir and glecaprevir was performed in which mobile phase was used to dilute one ml of stock of pibrentasvir and glecaprevir to ten ml in the flask of capacity 10 ml. Standard solution concentration for validation is pibrentasvir 40 µg/ml and glecaprevir 100 µg/ml.

Optimized method
After several trials a method was optimized with following conditions represented in (Table 2) and results are represented in (Figure 3).

Assay of Pibrentasvir and Glecaprevir in Maviret tablet
Involved in the preparation of pibrentasvir and glecaprevir stock tablet solution, a properly weighed finely powdered Marivet tablet equivalent to 40 mg pibrentasvir and 100 mg glecaprevir in a 100 ml volumetric flask with 30 ml mobile phase were mixed. Concentrations of stock tablet solutions are 400µg/ml and 1000 µg/ml of pibrentasvir & glecaprevir respectively. Test tablet solution concentrations are 40µg/ml (pibrentasvir) and 100 µg/ml (glecaprevir).

A sample solution amounting 20 µl was 3 times infused into HPLC. The peak areas are measured at 230 nm and concentrations of pibrentasvir and glecaprevir in tablet specimens were determined with the help of linear regression equation or calibration graphs.

RESULTS AND DISCUSSIONS

A stability indicating method development & validation of Pibrentasvir and Glecaprevir was done by RP-HPLC method. The estimation was done by the analysis in RP-HPLC employing Cosmosil C18 (250×4.6mm, 5µm) chromatographic column. The mobile phase was mixture of Phosphate Buffer and Acetonitrile (65:35). The flow rate was 1.0 ml/ min and detection was performed at 230 nm.

Validation
The new technique has been assessed to meet the International Conference on Harmonization (ICH) Q1B criteria, including sensitivity, linearity, selectivity, accuracy, specificity, robustness and precision.
Table 1: Multi-class drug combination to treat Hepatitis C virus

| Brand Name | Generic Name                  | Status  | Pharmaceutical Company |
|------------|-------------------------------|---------|------------------------|
| Epclusa*   | Sofosbuvir + Velpatasvir      | Approved| Gilead Sciences        |
| Harvoni*   | Ledipasvir + Sofosbuvir       | Approved| Gilead Sciences        |
| Mavyret    | Glecaprevir + Pibrentasvir    | Approved| Abbvie                 |
| Vosevi     | Sofosbuvir/Velpatasvir/ Voxilaprevir | Approved| Gilead Sciences        |
| Zepatier   | Elbasvir + Grazoprevir        | Approved| Merck                  |
| n/a        | Daclatasvir + Asunaprevir + Beclabuvir | Phase III| Bristol-Myers Squibb   |

Table 2: Optimized method conditions

| Combo and ratio in blend of mobile phase | KH2PO4 (65%) and Acetonitrile (35%) |
|----------------------------------------|-------------------------------------|
| Flow rate trial                        | 1.0 ml/min                          |
| Size of vol. sample injected            | 10 µl                               |
| Column temperature trial                | 25°C                                |
| Time run                               | 5 min                               |
| Eluents checked at                     | 230 nm                              |

Figure 4: Chromatogram of optimized method

Figure 5: Chromatograms of selectivity
Selectivity

The selectivity evaluation was conducted by incorporating into the chromatographic system a volume of 20 μg solution standard (40 μg/ml pibrentasvir and 100 μg/ml glecaprevir), tablet sample (40 μg/ml pibrentasvir and 100 μg/ml glecaprevir) blank diluent and placebo. No peaks that interfere with peaks when pibrentasvir and glecaprevir are retained. These results are represented in (Figure 4).

Linearity

Linearity solutions were prepared with concentrations pibrentasvir and glecaprevir each solution for concentration was incorporated into the HPLC instrument and assessed according to the similar conditions. The results are represented in (Table 3) and linearity graphs are shown in (Figure 5).

| Table 3: Peak area and concentration data |
|------------------------------------------|
| Conc of Glecaprevir μg/ml | Area response | Conc of Pibrentasvir μg/ml | Area response |
|---------------------------|---------------|---------------------------|---------------|
| 50                        | 1353890       | 20                        | 469419        |
| 75                        | 2038068       | 30                        | 704724        |
| 100                       | 2712858       | 40                        | 939681        |
| 125                       | 3392084       | 50                        | 1171910       |
| 150                       | 4073013       | 60                        | 1408097       |

The linearity range of Pibrentasvir was found to be HPLC 20-60 μg/ml, with R² value of 0.9995 and linearity range of Glecaprevir was found to be HPLC 60-150 μg/ml, with R² value of 0.9997. The %RSD for intra precision was <2%. The % recovery varies in the range of 95-105. The method also passes the specifications for robustness parameters

Sensitivity

The sensitivity measurement (LOD and LOQ) is performed by the signal to noise (S/N) ratio for both the drugs. Values proved good sensitivity. Results are represented in (Figure 6). LOD and LOQ was calculated by using following formula

\[ \text{LOD: } \frac{3.3}{\sigma} \times 100, \quad \text{LOQ: } \frac{10}{\sigma} \times 100 \]

LOD: 0.190 μg/ml for Pibrentasvir and 0.20 μg/ml for Glecaprevir. LOQ: 0.634 μg/ml for Pibrentasvir and 0.690 μg/ml for Glecaprevir.

Precision

For precise measurement six area response measurements of standard solution (40 μg/ml pibrentasvir and 100 μg/ml glecaprevir) were used. The RSD was calculated and it was precise.

| Table 4: Area response of Glecaprevir and Pibrentasvir for precision |
|---------------------------------------------------------------|
| Area response | Area response |
|               |               |
| Glecaprevir  | Pibrentasvir  |
| Sample I     | 2708652       | 939412       |
| Sample II    | 2716419       | 938701       |
| Sample III   | 2705033       | 939606       |

Accuracy

The precision was evaluated using tablets by pibrentasvir and glecaprevir recovery research. Each level concentration was prepared and analyzed for three times. For replicate specimens, the recovery percentage of added analytes was calculated. The result revealed the accuracy.

Robustness

Robustness was evaluated by inspecting the impacts in assay circumstances generated by minor alternations. The Theoretical plate count, asymmetry factor, resolution of analytes and peak area of glecaprevir and pibrentasvir in every condition shows that there was no major changes observed. Hence, the method is robust.

Degradation Studies for Pibrentasvir and Glecaprevir

Acid Hydrolysis - Degrading With 0.1n Hcl

10 ml solution of tablet with 400 µg/ml concentration of Pibrentasvir and 1000 µg/ml concentration of Glecaprevir was blended to 10 ml of Hcl with normality 0.1N at 27°C up to 30 min by sonication.

Base hydrolysis degrading with 0.1N NaOH

10 ml tablet solution (strength of 400 µg / ml pibrentasvir and 1000 µg / ml glecaprevir) was combined at 27°C to 10 ml 0.1N NaOH for 30 min by sonication.

Oxidative hydrolysis degrading with 30% hydrogen peroxide

10 ml of tablet solution (concentration of 400 µg/ml pibrentasvir and 1000 µg/ml glecaprevir) was combined for 1/2 an hour with 10 ml 30 percent H₂O₂ at 27°C through sonication.

Thermal analysis degrading with dry heat at 105°C

Tablet solution at temperature of 105°C (concentration 400μg/ml pibrentasvir and 1000 μg/ml glecaprevir) is applied for 30 min to 10 ml in hot air oven.

Photolysis Degrading with sunlight

10 ml tablet solution (400 µg/ml pibrentasvir concentration and 1000 μg/ml glecaprevir concentration) is held in sunlight for 6 hours.

Pibrentasvir and glecaprevir was more degraded in dry heat condition and less degraded in peroxide condition. The peak elution times of the degradants are...
**Figure 6**: Sensitivity test chromatograms

**Table 5**: Recovery data for Pibrentasvir for accuracy

| Level         | Area of response | Conc. of Pibrentasvir added (µg/ml) | Conc. of Pibrentasvir found (µg/ml) | Percentage of Pibrentasvir Recovered |
|---------------|------------------|------------------------------------|------------------------------------|------------------------------------|
| 50% spiked    |                  | 468954                             | 19.8                               | 19.78                              | 99.89                              |
|               |                  | 468666                             | 19.8                               | 19.77                              | 99.83                              |
|               |                  | 469413                             | 19.8                               | 19.80                              | 99.99                              |
| 100% spiked   |                  | 938544                             | 39.6                               | 39.58                              | 99.96                              |
|               |                  | 938907                             | 39.6                               | 39.60                              | 99.99                              |
|               |                  | 939645                             | 39.6                               | 39.63                              | 100.07                             |
| 150% spiked   |                  | 1397221                            | 59.4                               | 58.93                              | 99.20                              |
|               |                  | 1391781                            | 59.4                               | 58.70                              | 98.82                              |
|               |                  | 1400703                            | 59.4                               | 59.07                              | 99.45                              |

**Table 6**: Recovery data for Glecaprevir for accuracy

| Level         | Area of response | Conc. of Glecaprevir added (µg/ml) | Conc. of Glecaprevir found (µg/ml) | Percentage of Glecaprevir Recovered |
|---------------|------------------|------------------------------------|------------------------------------|------------------------------------|
| 50% spiked    |                  | 1343575                            | 49.5                               | 49.16                              | 99.31                              |
|               |                  | 1350505                            | 49.5                               | 49.41                              | 99.82                              |
|               |                  | 1341895                            | 49.5                               | 49.10                              | 99.16                              |
| 100% spiked   |                  | 2703946                            | 99.0                               | 98.93                              | 99.93                              |
|               |                  | 2710263                            | 99.0                               | 99.16                              | 100.16                             |
| 150% spiked   |                  | 4066263                            | 148.5                              | 148.77                             | 100.18                             |
|               |                  | 4068818                            | 148.5                              | 148.87                             | 100.25                             |
|               |                  | 4076857                            | 148.5                              | 149.16                             | 100.45                             |

**Table 7**: Robustness data of Pibrentasvir

| Sample Name | Peak Name | RT   | Area   | USP Tailing | USP Plate Count |
|-------------|-----------|------|--------|-------------|-----------------|
| FLOW-1      | Pibrentasvir | 1.376| 787370 | 1.38        | 4713            |
| FLOW-2      | Pibrentasvir | 1.499| 859477 | 1.39        | 4946            |
| TEMP-1      | Pibrentasvir | 1.823| 1059372| 1.40        | 5582            |
| TEMP-2      | Pibrentasvir | 2.054| 1191705| 1.40        | 5973            |
| COMP-1      | Pibrentasvir | 1.376| 787370 | 1.38        | 4713            |
| COMP-2      | Pibrentasvir | 1.823| 1059372| 1.40        | 5582            |
| pH-1        | Pibrentasvir | 1.657| 942412 | 1.38        | 4997            |
| pH-2        | Pibrentasvir | 1.654| 939701 | 1.37        | 4973            |
distinct from the time of glecaprevir and pibrentasvir being eluted. So interference will not occur. Results proved stability indicating ability.

CONCLUSION

Stability study on Pibrentasvir and Glecaprevir was carried out was an efficient HPLC method for the quantification of Pibrentasvir and Glecaprevir and identification of its degradation products and validated. The results of stress testing of API, undertaken to the ICH Q1B guidelines, revealed that degradation products were formed under acidic, alkaline, oxidizing and thermal conditions.

The results show the method is accurate, precise, sensitive, and economic friendly and rapid. Hence, the method can be successfully applied to the pharmaceutical dosage form and can be used for routine analysis.

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