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Method Development And Validation For The Simultaneous Estimation Of Cabotegravir And Rilpivirine In Bulk And Pharmaceutical Dosage Form And Stability Studies By Uplc

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Abstract:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cabotegravir and Rilpivirine in pharmaceutical dosage form. Chromatogram was run through Hibar C18 100 x 2.1 mm, 2µm. Mobile phase containing Water: Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 0.3 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 257 nm. Retention time of RPN and CGV were found to be 0.618 min and 1.152. %RSD of the CGV and RPN were found to be 0.3 and 1.5 respectively. %Recovery was obtained as 99.79% and 99.58% for CGV and RPN respectively. LOD, LOQ values obtained from regression equations of CGV and RPN were 0.43, 1.30 and 0.78, 2.37 respectively. Regression equation of CGV is y = 4028.3x + 977.59, and RPN is y = 4057.2x + 1673.2. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: RP-UPLC, Cabotegravir(CGV) and Rilpivirine(RPN), Method development, ICH Guidelines.

INTRODUCTION

CGV Chemical name is *N*-((2,4-Difluorophenyl)methyl)-6-hydroxy-3-methyl-5,7-dioxo-2,3,5,7,11, 11a-hexahydro(1,3) oxazolo(3,2-a) pyrido(1,2-d) pyrazine-8-carboxamide. RPN Chemical name is 4-{[4-({4-[(*E*)-2-cyanovinyl]-2,6-dimethylphenyl}amino)pyrimidin-2-

yl]amino}benzonitrile. CGV and RPN is indicated as a complete regimen for the treatment of HIV type 1 infection in adults to replace a current antiretroviral regimen in those who are virologically suppressed on a stable antiretroviral regimen with no history of treatment failure. In 2021, the U.S. Food and Drug Administration (FDA) approved the use of CGV in combination with RPN to treat patients with complete regimen for HIV-infected adults that is administered once a month.[1-7] In reported LCMS and FTIR[8] method the separation was done by using a Symmetry C18 (4.6×150 mm, 3.5) column, a high-performance liquid chromatographic method for quantifcation of Rilpivirine and Cabotegravir in active pharmaceutical ingredients was developed and validated. The mobile phase is made up of buffer, acetonitrile, and 0.1 percent formic acid in a 20:80v/v ratio. The fow rate was kept constant at 1.0 ml/min, and detection was accomplished through absorption at 231 nm with a photodiode array detector. In another reported UHPLC-MS/MS[9] method the four analytes were eluted in less than 3 minutes using a reversed-phase chromatography method coupled with triple quadrupole mass spectrometry detection. This bioassay was fully validated following international guidelines and achieved good performances in terms of trueness (94.7%-107.5%), repeatability (2.6%-11%), and intermediate precision (3.0%-11.2%) over the clinically relevant concentration ranges (from 30 to 9000 ng/mL for bictegravir, cabotegravir, and doravirine and from 10 to 1800 ng/mL for rilpivirine). Literature review discloses that very few different methods were reported for the analysis of CGV and RPN in bulk and formulations by LCMS and FTIR[8], UHPLC-MS/MS[9] and other methods [10-12] After detailed studies no method was reported to estimate CGV and RPN by Ultra Performance Liquid Chromatography (UPLC); hence our present plan is to develop a new, sensitive, economical method for its analysis in bulk and formulation and validated as per ICH norms. [13-15]

MATERIALS AND METHODS

Materials

CGV and RPN pure drugs (API), Combination CGV and RPN Tablet formulation (Cabenuva), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All chemicals, HPLC grade, Merck, are purchased from local distributor.

Instruments

UPLC instrument used was of WATERS ACQUITY SYSTEM UPLC 2965 with Auto Injector and Acquity TUV detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance of CGV and RPN. Sonicator (Ultrasonicator-BVK enterprises), P^H meter (Thermo scientific), Micro balance (Sartorius), Vacuum filter pump (Welch) are the other instruments used for this study.

Analytical methodology

Preparation of buffer(0.01N KH_2PO_4 Buffer)

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Standard/ Working solution preparation

Preparation of Standard stock solutions: Accurately Weighed and transferred 37.5mg of RPN, and 25mg of CGV working Standards into 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (750µg/ml RPN, and 500µg/ml of CGV)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (75µg/ml RPN of and 50µg/ml of CGV)

Preparation of Sample stock solutions: Pippete out 1ml of RPN and CGV injection sample into a 100 volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by filters. (3000µg/ml RPN, and 2000 µg/ml of CGV).

Preparation of Sample working solutions (100% solution): 0.25ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(75µg/ml of RPN and 50µg/ml of CGV)

Diluent: Based up on the solubility of the drugs, diluent was selected, Water and Acetonitrile taken in the ratio of 50:50.

Linearity

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of CGV and RPN are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 12.5ppm, 25ppm, 37.5ppm, 50ppm, 62.5ppm, 75ppm of CGV and 18.75ppm, 37.5ppm, 56.25ppm, 75ppm, 93.75ppm, 112.5ppm of RPN respectively.

Precision

Accurately Weighed and transferred 37.5mg of RPN, and 25mg of CGV working Standards into 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (750µg/ml RPN, and 500µg/ml of CGV). 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (75µg/ml RPN of and 50µg/ml of CGV)

Accuracy

Accurately Weighed and transferred 37.5mg of RPN, and 25mg of CGV working Standards into 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (750µg/ml RPN and 500µg/ml of CGV). From this solution 0.5, 1.0 and 1.5ml was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent to produce 50, 100, 150% of spiked solution respectively.

Validation Procedure [24]

The analytical method was validated as per ICH Q2(R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ) and forced degradation.

System Suitability
System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits.

Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and the amount of the drug present, percentage of recovery and related standard deviation were calculated.

Precision
The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the present method was assessed in terms of repeatability, intra-day and inter-day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity and range
The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample within a given range. The six series of standard solutions were injected for assessing linearity range. The calibration curve was plotted using peak area with concentration of the standard solution and the regression equations were calculated.

LOD and LOQ
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were separately determined based on the calibration curve. The LOD and LOQ of CGV and RPN determined by injecting progressively low concentrations of standard solutions by using the developed method. The LOD and LOQ were calculated as 3.3s/n and 10s/n respectively as per ICH guidelines, where s/n indicates signal-to-noise ratio.

Stress degradation
Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q1A (R2).The degradation peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions (acid, alkali, oxidation, thermal, UV, water) to obtain the degradation of about 20%.

Robustness
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the UPLC system and altered chromatographic conditions such as Flow minus, Flow plus, mobile phase minus, mobile phase plus, temperature minus and temperature plus.

The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

METHOD DEVELOPMENT

Optimized method

Trials were performed for the method development by using different column like CHS C18, BEH C18, SB C8, HSS C18, Hibar C18 etc., and the best peak were eluted at1.152min and 0.618 min for CGV and RPN respectively with good resolution and Plate count. Optimized chromatographic conditions were shown in Table 1 and optimized chromatogram was shown in figure 2.

System suitability

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. System suitability parameters were shown in table 2 and chromatogram was shown in figure 3.

METHODS FOR VALIDATION

Linearity

To demonstrate the linearity of assay method, Six linear concentrations of CGV (12.5-75µg/ml) and RPN (18.75-112.5µg/ml) were injected in a duplicatemanner. Average areas were mentioned above and linearity equations obtained for CGV was y = 4028.3x + 977.59 and of RPN was y = 4057.2x + 1673.2. Correlation coefficient obtained was 0.999 for the two drugs.

1.1. Precision

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.3% and 1.5% respectively for CGV and RPN.As the limit of Precision was less than “2” the system precision was passed in this method. System precision values were shown in Table 4.

Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.72% and 99.77% for CGV and RPN respectively. Recovery study values were shown in Table 5.

Robustness

Robustness conditions like Flow minus (0.27ml/min), Flow plus (0.33ml/min), mobile phase minus (60W:40A), mobile phase plus (50W:50A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. Robustness data were shown in table 6.

LOD and LOQ

LOD and LOQ were estimated from the signal-to-noise ratio. The LOD of CGV and RPN were found to be 0.43 & 0.78µg/ml and the LOQ were 1.3 & 2.37µg/ml respectively. LOD

and LOQ values were shown in table 7. LOD and LOQ Chromatograms were shown in figure 5 & 6 respectively.

Degradation Data

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. Degradation values were shown in table 8.

Assay of marked formulation

Cabenuva, bearing the label claim CGV 600mg, RPN 400mg. Assay was performed with the above formulation. Average % Assay for CGV and RPN obtained was 99.26% and 99.45% respectively Assay Data of Marked Formulation were shown in table 9.

RESULTS AND DISCUSSION

Literature review depicts that no UPLC analytical method reported for CGV and RPN. Up-to-date only very few different methods were reported for the analysis of CGV and RPN in bulk and formulations by LCMS and FTIR[8], UHPLC-MS/MS[9] and other methods.[10-12] The aim and objectives of the present study was to develop a new UPLC method for rapid, simple and simultaneous quantification, validation and stability studies of CGV and RPN. The present method was developed with trials and error method by using different mobile phases and different columns like CHS C18, BEH C18, SB C8, HSS C18, Hibar C18 etc., The mobile phase containing Water: Acetonitrile taken in the ratio 55:45 %v/v produced the optimized separation chromatogram (Fig. 2) using Hibar C18 (100 x 2.1 mm, 2µm) column. The developed method was validated as per ICH guidelines. The validation parameters such as specificity, linearity (R^2 as 0.999 & 0.999 for the two drugs), precision (0.3% and 1.5% for CGV and RPN), accuracy(99.72% and 99.77% for CGV and RPN), robustness and system suitability results were achieved and were within the ICH guidelines [13-15]. The retention time was showed in this proposed method were eluted at1.152min and 0.618 min for CGV and RPN respectively. The calibration curve was linear over the concentration range of CGV (12.5-75µg/ml) and RPN (18.75-112.5µg/ml). The LOD of CGV and RPN were found to be 0.43 & 0.78µg/ml and the LOQ were 1.3 & 2.37µg/ml respectively. For the assay of marked Formulation, the average % Assay for CGV and RPN obtained was 99.26±1.25% and 99.45±0.5% respectively is under the limits. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method. Hence it was concluded that the RP-UPLC method developed was very much suit for routine analysis.

CONCLUSION

In the present investigation, from the above experimental results it was concluded that, the newly developed RP-UPLC method was simple, specific, accurate and precise. The method was effectively validated in terms of system suitability, linearity, precision, accuracy, range, LOD, LOQ and robustness and stability indicating studies according to ICH guidelines. Hence the developed method can use for estimation of CGV and RPN in quality control departments of pharmaceutical industries and testing laboratories.

CONFLICTS OF INTEREST

The authors have no conflict of interest.

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REFERENCES

- https://go.drugbank.com/drugs/DB11751
- https://go.drugbank.com/drugs/DB08864
- "Cabenuva Product information". Health Canada. 25 April 2012. Retrieved22 January 2021.
- "Cabenuva- cabotegravir and rilpivirine kit". DailyMed. Retrieved13 February 2021.
- "FDA Approves First Extended-Release, Injectable Drug Regimen for Adults Living with HIV". U.S. Food and Drug Administration (FDA)(Press release). 21 January 2021. Retrieved 21 January 2021.
- "Drug Trials Snapshot: Cabenuva". U.S. Food and Drug Administration (FDA). 20 January 2021. Retrieved 17 February 2021.
- "Drug Approval Package: Cabotegravir". U.S. Food and Drug Administration(FDA). 3 March 2021. Retrieved 14 September 2021.
- Anuradha Vejendla, Subrahmanyam Talari, Raju Moturu , S. N. Murthy Boddapati and Emmanuel Kola A. Method development and validation for Cabotegravir and Rilpivirine by using HPLC and its degradants are characterized by LCMS and FTIR. Futur J Pharm Sci (2021) 7:226; 1-18.
- Courlet P, Alves Saldanha S, Cavassini M, Marzolini C, Choong E, Csajka C, Günthard HF, André P, Buclin T, Desfontaine V, Decosterd LA. Development and validation of a multiplex UHPLC-MS/MS assay with stable isotopic internal standards for the monitoring of the plasma concentrations of the antiretroviral drugs bictegravir, cabotegravir, doravirine, and rilpivirine in people living with HIV, J Mass Spectrom ,2020 Mar 11; 55:4506.
- Kumar BMS, Rajkamal B, Chandramowli B (2019) Development and validation of Rilpivirine in pharmaceutical formulation by RP-HPLC. Am J PharmTech Res 9(03):345–353
- Veeraswami B, Naveen VMK (2019) Development and validation n of RP-HPLC method for the estimation of Dolutegravir and Rilpivirine in bulk and pharmaceutical dosage form and its application to rat plasma. Asian J Pharm Clin Res 12(2):267–271.
- Rubesh Kumar et al…, RP-HPLC method development & validation of rilpivirine pharmaceutical dosage form, IJPAR,Vol.8, Issue 3, Jul - Sep – 2019.
- ICH Harmonised Tripartite Guideline. Validation of analytical procedures, Text and methodology, Q1 R2. IntCGVational Conference on Harmonization, 2005, 1-13.
- ICH Harmonised Tripartite Guideline, Stability Testing of New Drug Substances and Products, Q1A (R2). IntCGVational Conference on Harmonization, 2003, 1-18.
- ICH Harmonised Tripartite Guideline. Validation of analytical procedures, Text and methodology, Q2 R1. IntCGVational Conference on Harmonization, 2005, 1-17.