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Development and validation of a RP-HPLC Method for the Estimation of Remdesivir in Bulk and Pharmaceutical Formulation

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ABSTRACT

A simple, reproducible and efficient reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed for estimation of the broad-spectrum antiviral prodrug, Remdesivir in raw material and its injection dosage form. Separation was done by using mobile phase consisting of Acetonitrile : 0.1% Triethylamine, TEA (70:30). The separations were carried out on a Column X-Bridge phenyl (150x4.6mm, 3.5µ) at a flow rate of 1 mL/min. The injection volume was 10 µl and the peaks were detected at 235 nm. The linear dynamic response was found to be in the concentration range of 50µg/mL-300µg/mL and coefficient of correlation was found to be 0.9989. The %RSD value was below 2.0 µg/mL for intraday and interday precision indicated that the method was highly precise. The LOD and LOQ were found to be 6.0 µg/mL and 20 µg/mL respectively which revealed that the method was highly sensitive. The percentage recovery value was higher than 100 %, indicating the accuracy of the method and absence of interference of the excipients present in the formulation. The proposed method was simple, fast, accurate, precise and reproducible and hence can be applied for routine quality control analysis of Remdesivir in bulk and pharmaceutical formulation.

Keywords: Remdesivir, Estimation, Injection, RP-HPLC.

INTRODUCTION

Remdesivir is a broad-spectrum antiviral medication. It is administered via injection into a vein. During the COVID-19 pandemic, Remdesivir was approved or authorized for emergency use to treat COVID-19 in numerous countries. Remdesivir was originally developed to treat hepatitis C[17], and was subsequently investigated for Ebola virus disease and Marburg virus infections[18] before being studied as a post-infection treatment for COVID-19[19]. Remdesivir is a prodrug that is intended to allow intracellular delivery of GS-441524 monophosphate and subsequent biotransformation into GS-441524 triphosphate, a ribonucleotide analogue inhibitor of viral RNA polymerase[14].

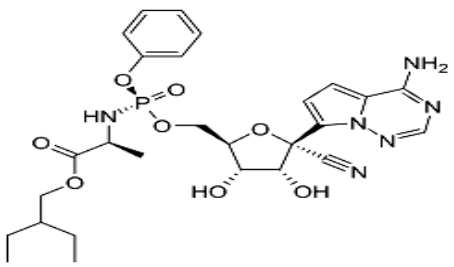

Fig 1: Molecular structure of Remdesivir

Various HPLC and LC-MS methods have been reported for the determination of Remdesivir in biological fluids [3-9]. A literature survey revealed that very few HPLC methods have been reported for the determination of Remdesivir from pharmaceutical dosage form [10,11]. So In this present investigation an attempt has been made to develop an accurate, precise and economically viable reversed phase HPLC method for the s estimation of Remdesivir in bulk drug and in pharmaceutical dosage form.

EXPERIMENTAL SECTION

Apparatus and chromatographic condition

The chromatographic separation was performed on a high performance liquid chromatographic instrument, ALLIANCE, Waters e 2695 Empower software 2.0 versions equipped with a X-Bridge phenyl (150x4.6mm, 3.5µ) integrated with UV detection at 235 nm .The mobile phase consisting of Acetonitrile: 0.1% TEA (70:30) and was prepared freshly, filtered and sonicated before use and delivered at a flow rate of 1 mL/ min. The volume of each injection was 1 0 µl. The column and the HPLC system were kept in ambient temperature.

Chemicals and reagents

Remidsiver was obtained as a gift sample from Hetro drug Ltd. acetonitrile and triethanolamine used were of HPLC grade. The commercially available remedisvir injection vial claimed to contain 100 mg of active ingredients were procured from local market.

Preparation of stock solution

Accurately weigh and transfer 200 mg of Remdesivir working standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (200ppm of Remdesivir).and the solution was sonicated for 20 min. Subsequent dilutions of this solution were made with mobile phase to get concentrations of 5 0-300 µg/mL. The standard solutions prepared as above were injected into the 20 µ L loop and the chromatogram was recorded (Figure 3).

Analysis of injection formulation

A quantity equivalent to 200 mg of remedisvir was weighed accurately and transferred to a 100 mL volumetric flask. Then 50mL of the mobile phase was added to it and the mixture was sonicated for 20 min and then diluted up to the mark with the same solvent. The resulting solution was filtered through a membrane filter. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity as previously discussed forthe pure drug. Sample solution was injected under the chromatographic conditions as mentioned above and the chromatogram was recorded.

RESULT AND DISCUSSION

All of the analytical validation parameters for the proposed method were determined according to Conference on Harmonization (ICH) guidelines [12].

Linearity

The linearity of this method was determined at ten concentration levels ranging from 50 µg/mL - 300 µg/mL. The plot of peak area of each sample against respective concentration of remedisvir was found to be linear (Figure 1) in the range of 50–300 µg/mL. Beer's law was found to be obeyed over this concentration range. The regression equation was found to be Y = 10065.94x + 1055.46Y and the correlation coefficient (r) of the standard curve was found to be 0.9992 (Table 1).

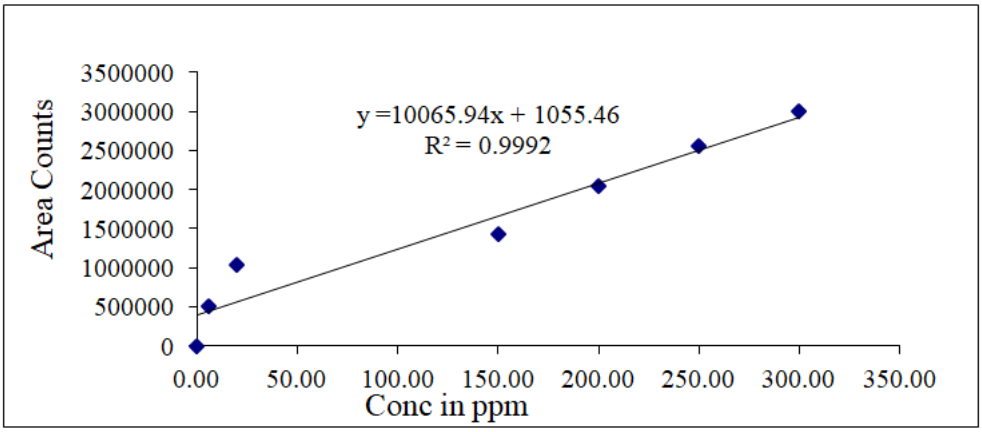

Fig 2: Calibration curve of remedisvir

Precision

The precision is a measure of the ability of the method to generate reproducible results. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as %RSD. For this, 120 µg/mL of the solution was measured three timesin a day and the same was repeated in next three days. The precision (measurements of intraday and interday) results showed (Table 2) good reproducibility with percent relative standard deviation (% RSD) was below 2.0%. This indicated that method was highly precise.

Table 1: Validation Parameters

Parameters	Results	
Linearity range (µg/ml)	50 to 300	
Standard Regression equation	Y = 10065.94x + 1055.46	
Correlation coefficient	0.9992	
LOD (µg /ml)	6.00	
LOQ (µg /ml)	20.00	
Precision (at 150µg/ml)	Intraday (% RSD)	Interday (%RSD)
	0.354	0.265

Recovery studies (Accuracy)

Recovery studies were performed to judge the accuracy of the method. The studies were carried out by adding a known quantity of pure drug to the pre-analyzed formulation and the proposed method was followed. From the amount of drug found, the percent recovery was calculated. Recovery study was carried out at three levels 80%, 100% and 120% for the formulation concentration of 150µg/mL. The percentage recovery value (Table 2), which was higher than100 %, indicated that the accuracy of the method and absence of interference of the excipients present in the formulation.

Table 2: Recovery study

Level of Addition (%)	Formulation (µg/mL)	Addition of pure drug (µg/mL)	% Recovery of pure drug	Recovery (%) ± S.D.
80	150	120	101.33	
100	150	150	101.35	101.55±0.37

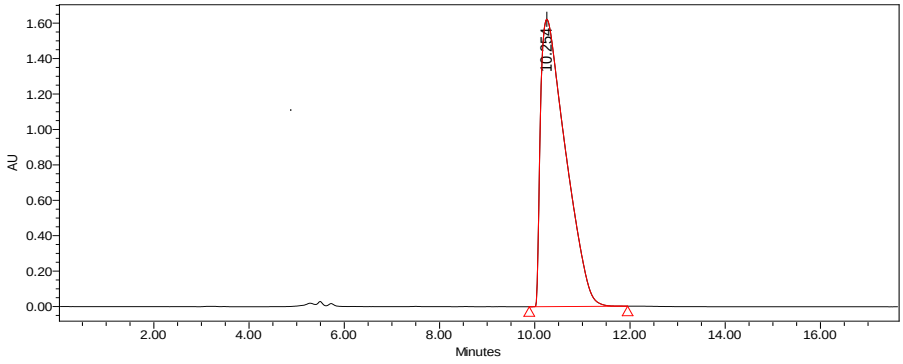

Fig 3: Typical chromatogram of remidsivir

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) were calculated by the using the equation given in ICH guidelines. This may be expressed as LOD = 3.3 σ /S and LOQ = 10 σ / S, where σ is the standard deviation of the response, S is the slope of the calibration curve whichmay be estimated from the calibration curve of the analyte. The LOD and LOQ for remidsivir were found to be 6.00 **µg** /mL and 20.00 **µg** /mL respectively (Table 1), this demonstrated that the method was highly sensitive.

System suitability test

The system suitability tests were carried out on freshly prepared standard stock solution of remdesivir to evaluate the suitability of the system and the parameters that were studied presented in Table 3. From the typical chromatogram of remdesivir as shown in Fig 3, it was found that the average retention time ± standard deviation for remdesivir was found to be 2.863 ±0.001 min for five replicate injections. The asymmetry factor was found to be 1.78, which indicated asymmetric nature of the peak. The number of theoretical plates was found to be 5327 , which suggested an efficient performance of the column. The absence of additional peaks in the chromatogram indicated non-interference by the common excipients used in the injection formulation. To optimize the chromatographic conditions, various combinations of Acetonitrile: 0.1% TEA 70:30 v/v afforded peak withgood shape and resolution.

Table 3: System Suitability Parameters

Retention time (min) ± S.D.	2.863 ± 0.001
No. of theoretical plates	5327
Asymmetric factor	1.78

Robustness

Robustness was performed by small but deliberate variation in the chromatographic conditionsand was found to be unaffected by small variations like ±2% in volume of mobile phasecomposition, ±0.1 mL/min in flow rate of mobile phase and ±1% change in column temperature. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed

method was robust.

Table 4: Determination of active ingredients in tablets

Sample	Label claimed	Amount found	% Amount found*
remedisivir	100 mg/vial	101.72±0.67	101.09

(Average of three determinations)*

CONCLUSION

From the above discussion it is clear that the proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method was also applied for the assay of remedesvir in injection formulation (in triplicate) and the results are shown in Table 4. The results obtained were in good agreement with the label claims. Hence, this method can be used for the routine determination of remedsvir pure sample and in injection formulations.

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