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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF REMDESIVIR IN BULK AND INJECTION DOSAGE FORM

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Abstract: For the quantitative determination of Remdesivir in bulk and injection dosage form, a new validated HPLC method was validated in the present work. The mobile phase composed of Acetonitrile:Water in 0.1% Triethylamine (60:40 v/v) with a flow rate of 1 ml/min. The column was a Phenomenex Luna C_{18} column (150 mm × 4.6 mm id; 5 µm particle size). UV detectors at 238 nm were used to monitored. The calibration curve (R^2 value: 0.9998) was linear in the concentration range of 20 to 100 µg/ml. The suggested method was validated in accordance with ICH guidelines and successfully applied for the assay of Remdesivir in injection dose form.

Index Terms **- Remdesivir, RP-HPLC method, Method development, Validation**

I. INTRODUCTION

Remdesivir is chemically (2S)-2-{(2R,3S,4R,5R)-[5-(4-Aminopyrrolo[2,1-f] [1,2,4] triazin-7-yl)-5-cyano-3,4-dihydroxy-tetrahydro-furan-2-ylmethoxy]phenoxy-(S)-phosphorylamino} propionic acid 2-ethyl-butyl ester. Remdesivir triphosphate can also inhibit viral RNA synthesis due to incorporation into the viral RNA template [1,2,3,4,5]. A comprehensive literature survey revealed that, no method have been developed for the estimation of Remdesivir in bulk drugs and injection dosage form by using this mobile phase. The method described is rapid, economical, precise, and accurate and can be effectively used for routine quality control analysis of injection dosage form. The developed method was validated as per ICH norms [6-7].

II. METHODS AND MATERIALS:

Materials and Reagents

Remdesivir injection dosage form that was marked to contain 100 ml/20 mg of Remdesivir used in this method. The mobile phase utilized was Acetonitrile: Water in 0.1% Triethylamine (60:40 v/v).

The Instrument and chromatographic conditions

A manual injecting facility with a 20 μL capacity per injection was used with the Shimadzu HPLC system (Shimadzu corporation Kyoto, Japan), which included a pump (LC - 20AD solvent deliver module, SPD-20A UV- Visible detector), both of which were operated by Lab solutions software. Phenomenex Luna C_{18} (150 mm × 4.6 mm, 5.0 µm particle size) was the column that was used. Different mobile phases were tried in order to find the best condition for separation of Remdesivir. The mobile phase composed of Acetonitrile:Water in 0.1% Triethylamine (60:40, v/v) which had a flow rate of 1.0 ml/min. At 238 nm, UV detection was determined. A 0.45 µm membrane filter was used to filter the mobile phase and samples. Before usage, mobile phase was degassed with a Sonica ultrasonic cleaner (model 2200 MH). The other instrument used are hot air oven. The Shimadzu AUX-220 electronic balance was used for all weighing.

Preparation of standard and sample solutions

Mobile phase

Acetonitrile: Water in 0.1% Triethylamine (60:40 v/v) is programmed as RP-HPLC method.

Preparation of standard stock solution

Accurately weighed quantity of 100 mg of Remdesivir API was transferred into 100 ml volumetric flask and dissolved in methanol and diluted up to mark to get concentration of 1000 μg/ml.

Preparation of working standard solution

From above standard stock solution of Remdesivir 0.4ml of solution was taken into 10 ml volumetric flask and was made to the mark with the methanol to get 40 μg/ml of Remdesivir.

Quantification of Intravenous formulation

Intravenous formulation containing 100 mg of Remdesivir per 20 ml. A quantity of Injection formulation equivalent to about 100 mg of Remdesivir was transferred to a clean 100 ml standard flask; added 70 ml methanol, further methanol was added and made up to the mark. Pipetted out 0.4ml and transferred to 10ml volumetric flask, made up to with methanol to get the final concentration of 40 μg/ml.

Validation

The proposed method was validated as per ICH guidelines.

Linearity

Different aliquots of 0.2 - 1.0 ml of standard stock solution was transferred into series of 10 ml volumetric flasks, separately and the volume was made up to the mark with mobile phase to get concentrations 20, 40, 60, 80 and 100 µg/ml, respectively.

Accuracy

To the preanalysed sample solution, a known amount of standard stock solution was added at different levels i.e. 50, 100 and 150%. The solutions were reanalyzed by proposed method.

Precision

The reproducibility of this method was determined by analyzing tablets at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision).

II. Results and Discussion

Method development and optimization The HPLC procedure was optimized with a view to develop a suitable LC method for the determination of Remdesivir in injection dosage form. Initially, methanol and water in different ratios were tried. But Remdesivir gave broad peak with tailing, so acetonitrile was replaced with methanol and mixtures of acetonitrile and water in different ratios were tried. It was found that Acetonitrile:Water in 0.1% Triethylamine (60:40, v/v) gave acceptable retention times (3.959 min) with flow rate of 1.0 ml/min as shown in figure 1.

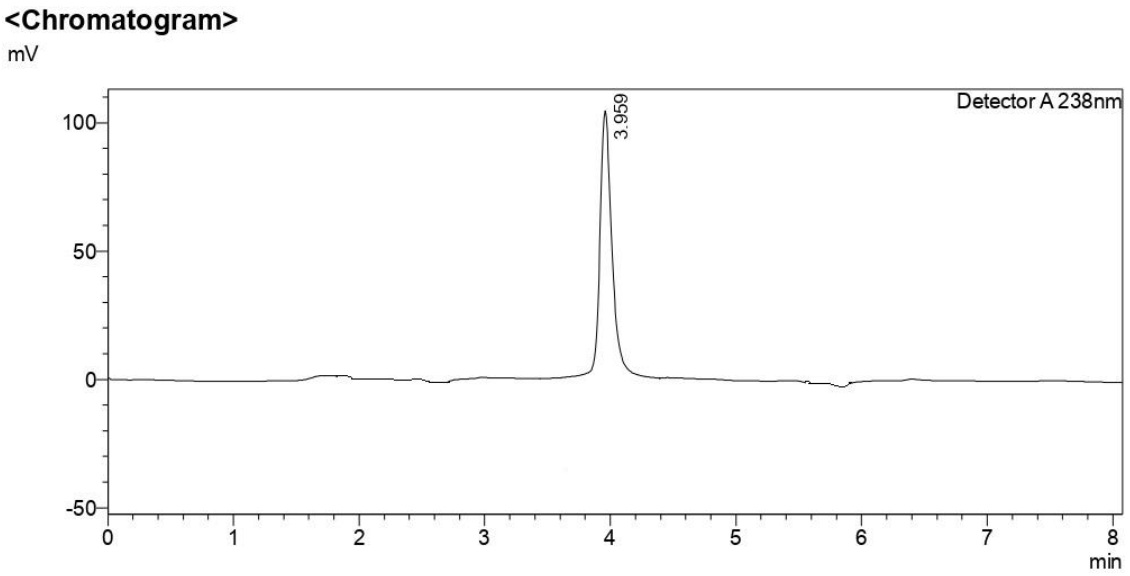

Figure 1: Chromatogram of Remdesivir obtained at optimum chromatographic conditions.

Method Validation

The described method has been validated which include parameters like linearity, system suitability, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification)

System suitability

System suitability and chromatographic parameters were validated such as tailing factor and theoretical plates was calculated. The results are given in table 1

Table I: Quantification of DESREM LQ Formulation

Parameters	Remdesivir	Recommended limits
Retention time	3.959	RSD ≤2
Peak area	876131	RSD ≤2
Tailing factor (T)	1.024	T <2
Theoretical plate (N)	4365	> 2000

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solution of Remdesivir at different concentration in the range of 20 -100 µg/ml with correlation coefficient (r^2) of 0.9998. Results are given in figure 2.

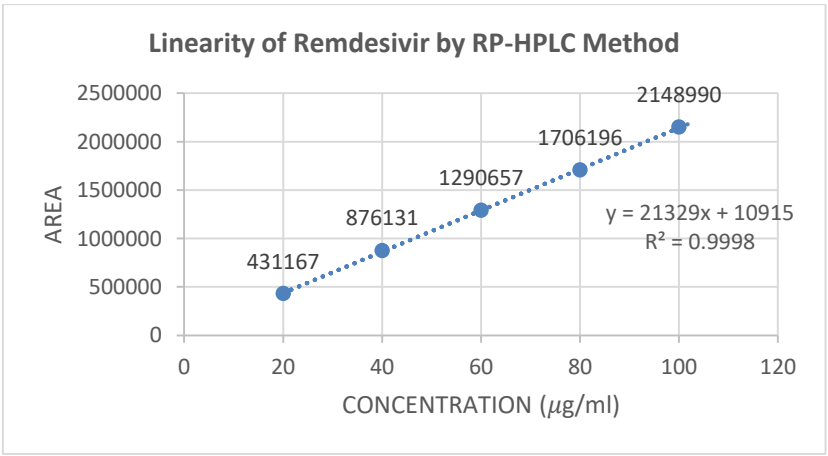

Figure- 2: Linearity of Remdesivir.

Accuracy

Accuracy of the proposed method was determined by performing the recovery experiment. The recovery experiment was studied by adding known amount of standard Remdesivir to the pharmaceutical product and calculating the recovered standard amount. At 50%, 100% and 150% standard addition level mean recovery of Remdesivir found to be 100.18%, 100.28% and 100.62% respectively.

Precision

Precision was evaluated at the repeatability and intermediate precision levels. For repeatability analysis, six independent portions of injection dosage form were processed through the full analytical method and results was evaluated obtaining a % RSD value of 0.6038 and average % purity of 100.22.

Robustness

Robustness study was conducted by deliberate changes in mobile phase composition and flow rate, revealed that there was no significant variation in % assay.

Limit of detection (LOD) and limit of quantification (LOQ)

The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration.

IV. CONCLUSION

The Remdesivir in injection formulation was determined using the HPLC method, which was developed and validated in accordance with ICH guidelines. According to the validation studies, the developed method was found to be quick, easy, accurate, precise, selective and economical. Therefore, this method is simple to use for regular analysis of Remdesivir in injection dosage form.

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