



# DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF REMDESIVIR IN BULK AND INJECTION DOSAGE FORM

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**Abstract:** A simple, sensitive, rapid, economic and accurate first order derivative spectrophotometric method has been developed for estimation of Remdesivir in bulk and injection dosage form. The wavelengths selected for quantitation was 249 nm Beer's law was obeyed in the concentration range of 15-75 µg/ml. The method was validated as per ICH guidelines. Statistical analysis proved that the method was accurate, precise, and reproducible for analysis of Remdesivir in injection dosage form. The wide linearity range, sensitivity, accuracy and simple procedure imply that the proposed technique demonstrated to be appropriate for routine analysis and quality control assay of injection dosage form.

**Index Terms - Remdesivir, First order derivative 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. [1,7,8,10,11]. A comprehensive literature survey revealed that, method have also been developed for the estimation of Remdesivir in bulk drugs and pharmaceutical formulation and in reverse phase high performance liquid chromatography (RP-HPLC) [2,3,6,9]. 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 [4-5].

## II. MATERIALS AND METHODS

### Instrumentation

The instrument used in the present study was Lab india UV/Visible spectrophotometer (Model UV- 3000) with spectral band width of 1 nm. All weighing was done on electronic balance (Model Shimadzu AUX -220).

### Selection of solvent

Selection of solvent in UV analysis is a key component of method development. The solvent used was methanol for the estimation of Remdesivir for the first order derivative method.

### Preparation of standard stock solutions

Standard stock solution of Remdesivir (150 µg/ml) was prepared by dissolving 100 mg of Remdesivir in 100 ml of methanol in 100 ml clean volumetric flask with vigorous shaking. Further pipetted out 15ml and transferred into a 100ml volumetric flask, make up to with methanol.

### Selection of wavelengths for estimation

In First order derivative method, solution of Remdesivir (10 µg/ml), was prepared separately by appropriate dilution of standard stock solution with methanol and scanned in the spectrum mode from 200 nm to 400 nm. The absorption spectra thus obtained was derivatized for first order. From the spectra of these drug, the wavelength selected for quantitation was 249.0 nm for Remdesivir.

### Preparation of calibration graph

From the above standard stock solution, aliquots were drawn and suitably methanol was added to get the final concentration range of 15, 30, 45, 60 and 75 µg/ml for Remdesivir. Absorbances was measured at 249nm.

### 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. 15 ml was withdrawn and diluted to 100 ml using methanol to get 150 µg/ml. Further pipetted out 2ml and transferred to 10ml volumetric flask, made up to with methanol to get the final concentration of 30 µg/ml. The concentration of Remdesivir was determined by measuring absorbance of sample solution in first order derivative at 249 nm. Amount of Remdesivir was calculated. This procedure was repeated six times.

### Validation

The proposed method was validated as per ICH guidelines.

#### Linearity

The aliquots of five different concentration ranging 15-75 µg/ml for Remdesivir was prepared and calibration graph was plotted between absorbance of drug and concentration. The linearity was calculated by the least square regression method and calculated optical parameters.

#### Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (50%, 100% and 150%). The results of recovery studies, expressed as percent recovery, were satisfactory and are presented in Table 2.

#### LOD and LOQ

LOD is smallest quantity of analyte that can be detected in a sample. The quantitation limit is lowest amount of analyte which can be quantified in a sample with the suitable accuracy and precision. These two parameters were calculated using the formula  $LOD = (3.3 \times SD/S)$  and  $LOQ = (10 \times SD/S)$ .

#### Precision

The reproducibility of this method was determined by analyzing tablet at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision). Coefficient of variance for intra-day assay precision was found to be 0.7455. Inter-day assay precision coefficient of variance was found to be 0.7456.

#### Ruggedness

Ruggedness is the measure of reproducibility of the test results obtained for identical samples under normal (but variable) test conditions. In the present study, determination of Remdesivir was carried out by using different analysts and different instruments and it was carried out in DESREM LQ injection formulation.

## III. RESULTS AND DISCUSSION

An accurate, simple, fast, and precise first order derivative UV method was developed and validated. Methanol was chosen as a solvent for the estimation of REM.

The standard solutions of 10 µg/ml of REM in the corresponding solvent was prepared and the solution was scanned in the UV region of 200 to 400 nm by using methanol. The zero order UV spectrum were derivatized into first order derivative UV spectrum. The first order derivative UV spectrum of REM was recorded, and the spectrum was shown in Figures 1. From the UV spectrum, 249 nm was chosen for the determination of REM without any intervention.

The preparation of calibration graph was repeated six times for drug at the selective wavelength. The calibration graph was plotted using absorbance against concentration. Correlation coefficient, Sandell's sensitivity, LOD, LOQ, Molar absorptivity and Standard error are the examples for optical parameters, and it was calculated for drug. The correlation coefficient of REM was found to be 0.9998. Hence the calibration graph was found to be linear.

Desrem LQ injection formulation containing 100 mg was selected for estimation. From the linearity, the nominal concentration of 30 µg/ml of REM was prepared. The absorbances of the solution was measured at 249 nm and the amount of six test solutions were determined. The % purity present in injection dosage form was found to be  $100.14 \pm 0.7469$ . The % RSD values were found to be very less. Hence the method has good precision. The results of analysis are shown in Table - 1.

Precision of the developed UV method was studied by making repeated analysis (Intraday and Interday). Intraday and interday analysis of % RSD values was found to be 0.7455 and 0.7455. The results show the developed UV method was very high.

In ruggedness studies, the % RSD values for different Instrument 1 and Instrument 2 were found to be 0.7385 and 0.7356. The % RSD values for different Analyst 1 and Analyst 2 were found to be 0.7537 and 0.7299. Lower % RSD values indicated; method was more rugged.

Accuracy studies of the developed UV method was confirmed. The % recovery was found from 99.99 to 100.26. Lower % RSD values indicated that the developed UV method was more accurate. The results were shown in Table-2.

Table 01: Quantification of DESREM LQ Formulation

Sample	Sample number	Label claim (mg/20ml)	Amount present (mg/20ml) *	% Purity* (% w/w)	Mean purity (% w/w)	SD	% RSD
REM	1	100	99.12	99.12	100.14	0.7469	0.7467
	2	100	100.87	100.87			
	3	100	100.87	100.87			
	4	100	100.00	100.00			
	5	100	100.87	100.87			
	6	100	99.12	99.12			

\*Mean of six observations

Table 02: Recovery Study

Sample	% Concentration	Sample amount* (µg/ml)	Amount spiked* (µg/ml)	Estimated amount* (µg/ml)	Recovered amount* (µg/ml)	Average* % recovery	SD	% RSD
REM	50	30.26	15	45.25	14.99	99.99	0.7381	0.7385
	100	30.26	30	60.34	30.08	100.26	0.7453	0.7454
	150	30.26	45	75.25	44.99	99.99	0.7381	0.7386

\*Mean of three observations

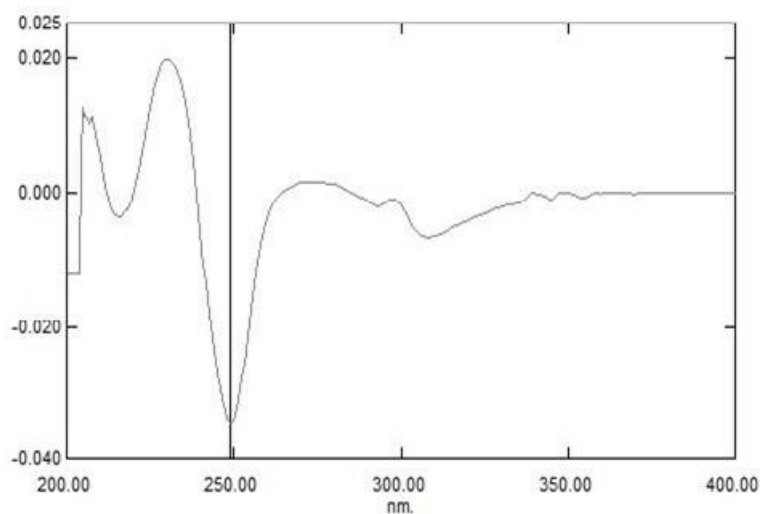


Figure 1: First order derivative spectrum of REM (10µg/ml)

#### IV. CONCLUSION

The validated First order derivative method employed here proved to be simple, rapid, precise, accurate and economic and the developed method are suitable for determination of Remdesivir as a bulk drug and in marketed dosage form without any interference from the excipients. Statistical analysis proves that this method was repeatable and selective for the analysis of Remdesivir

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