A comparative study of HPLC and UV spectrophotometric methods for remdesivir quantification in pharmaceutical formulations

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ABSTRACT
UV spectrophotometric and HPLC methods have been developed and validated for the quantitative determination of remdesivir in pharmaceutical formulations. The HPLC analyzes have been performed on a C-18 column with a mobile phase consisting of 20 mM KH₂PO₄ solution and acetonitrile (50:50, v/v), at a flow rate of 1.2 mL min⁻¹. UV spectrum has been recorded between 200 and 800 nm using deionized water as solvent and the wavelength of 247 nm has been selected. These methods have been validated according to the procedures described in ICH guidelines Q2(R1). Both methods demonstrated good linearity, precision and recovery. No spectral and chromatographic interferences from non-medicinal ingredients were found in both methods. Correlation coefficients were greater than 0.999 within a concentration range of 10–60 mg mL⁻¹. Therefore both analytical methods gave the most reliable results for the quantification of remdesivir in pharmaceutical formulation.

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1. Introduction
Remdesivir (RDV) has been determined as the most useful drug in in vitro and in vivo studies due to its antiviral activity. It is a prodrug of adenosine triphosphate analogue and has antiviral potential against a number of RNA viruses (Figure 1). It was developed by the company Gilead Sciences Biopharmaceutical and acts as a therapeutic against Ebola virus in rhesus monkeys [1–3].

Some analytical methods for the quantitative determination of RDV in biological matrix were described in the literature, such as UHPLC-ESI(+)-MS/MS and Liquid Chromatography-Mass Spectroscopy (LC-MS). A LC-MS/MS analysis method for the pharmacokinetic study of RDV in uninfected male rhesus monkeys was developed in a 2016 study [4]. In a 2020 study, an analysis method was developed in which RDV and its metabolite were determined in human plasma using an LC-MS/MS method and a simple protein precipitation step using a mixture of methanol and zinc sulphate [5]. In addition, an LC-ESI (+)-MS/MS method was developed to determine the concentration of RDV in plasma in a 2020 study [6]. In a recent study, an analysis method for determining RDV in human plasma was developed using the UHPLC-ESI (+)-MS/MS device, which has the advantage of high sensitivity and sample processing when compared to conventional LC-MS systems [7]. The same UHPLC system was then used for pharmacokinetic evaluation of RDV in two critically ill patients who recovered from COVID-19 [8].

Although recoveries obtained by LC-MS/MS and UPLC-MS/MS were similar, UHPLC gave significantly better precision. The literature review shows that only LCMS methods are introduced for the determination of RDV in biological samples, which significantly increases the cost of analysis. So far, all studies reported in the literature have been performed on biological fluids, particularly plasma samples.

According to the literature, both LC-MS/MS and UPLC-MS/MS methods provide adequate analytical performance for RDV measurement, but UPLC has a higher efficiency and sensitivity, as well as a shorter analysis time and better resolution. MS device was operated in a positive polarity in all cases. In addition, all existing methods use the protonated molecular ion [M + H]⁺ as a precursor ion for quantification. ESI is the most commonly used ion source in the study of RDV and its metabolites in biological matrices by LC-MS/MS methods, according to the cited literature.

These approaches provide a powerful analytical instrument for RDV clinical therapeutic monitoring. MS apparatuses, on the other hand, are often very costly, and this expense can be prohibitive for clinical laboratories. As a result, considering the many benefits of MS detection, using MS-based separation methods in practice can be difficult.

RDV is produced as a single stereoisomer and has six chiral centres. Chromatography is considered as the best one among various analytical techniques. It’s
because it’s reliable, cost-effective, repeatable, human-friendly, efficient and selective. Liquid chromatography, Gas chromatography, Capillary electrochromatography, Micellar electrokinetic chromatography, Nanoliquid chromatography and Supercritical fluid chromatography are the several forms of chromatography. These have been used to separate distinct compounds based on their chirality [9,10].

Unlike complex analytical techniques, HPLC and UV-spectrophotometric techniques for RDV quantification are low-cost, effective, easy-to-use and on-site. In order to ensure the safety and effectiveness of drugs in various matrices, qualitative and quantitative analysis is crucial. As a result, developing HPLC and UV-Spectrophotometric methods that display selective and validated stability in accordance with International Conference on Harmonization guidelines is critical.

To our knowledge, no reliable method for estimating RDV in pharmaceutical formulations has been published. The LC is a more commonly used tool in quality control laboratories due to its high sensitivity and true-ness. The UV method is very simple because it does not require any reagents, pH adjustments or extraction techniques.

As a result, UV-spectrophotometric and LC-chromatographic methods for quantifying RDV in pharmaceutical preparations were developed and validated. The results of these methods have been compared statistically using variance analysis. They also evaluated the methods’ effectiveness and viability, concentrating on quality control research.

2. Experimental

2.1. Equipments

Chromatographic analysis was carried out with the use of an Agilent 1260 series liquid chromatograph equipped with an UV-Visible detector, a quaternary pump, a vacuum degasser, a column oven and Chemstation software. The present study also utilized a Mettler-Toledo electronic balance (Mettler-Toledo, Switzerland), a Milli-Q water purification system (Millipore, USA), and UV-Visible spectrophotometer with a double beam using 1.0 cm quartz cells and UV-Probe software (Shimadzu UV-1800 spectrophotometer, Japan).

2.2. Chemicals

Analytical grade chemical compounds were used without further purification in these analyses. Potassium dihydrogen phosphate, methanol and acetonitrile were bought from Sigma-Aldrich. Deionized water was purified using a Milli-Q system (Millipore). Pure RDV and Covifor injectable (100 mg per vial) were supplied from Atabay Pharmaceuticals and Fine Chemicals Inc. (Istanbul, Turkey).

2.3. Standard solutions

For the creation of the calibration curve, stock standard solution of RDV (500 µg mL\(^{-1}\)) was prepared in methanol. The subsequent stock solution has been sonicated and filtered through a 0.22-µm filter. Further, the stock solution was diluted with methanol to obtain standard solutions at concentrations in the range (10–60 µg mL\(^{-1}\)) prior to analyses.

2.4. Sample solution

50 mg of Covifor injectable powder was transferred to a 100 mL calibrated flask and dissolved in 30 mL of methanol. The content was shaken for 10 min. The volume was completed with methanol to get a concentration of 500 µg mL\(^{-1}\). The final solution was filtered using a Whatman filter paper (No. 42).

2.5. Determination of \(\lambda_{\text{max}}\)

First, the spectrophotometer was calibrated to zero. Then the maximum absorption wavelength of RDV solution (30 µg mL\(^{-1}\)) was determined by scanning in the range of 200 and 800 nm.

2.6. Conditions

Chromatographic analysis was performed on a liquid chromatograph (Agilent 1260) with a UV-visible detector. RDV was analysed at a flow rate of 1.2 mL min\(^{-1}\) using a mobile phase composed of 20 mM potassium dihydrogen phosphate solution and acetonitrile (50:50, v/v). Before use, the mobile phase was filtered and degassed through a 0.22-µm membrane filter. An Agilent Extend C18 (4.6 mm × 250 mm, 5.0 µm particle size) column was used and operated at 25°C. RDV was detected with the UV detector at 247 nm. The run time under these conditions was 10 min. UV spectrophotometric method was carried out on a double beam spectrophotometer at 247 nm using 1.0 cm quartz cells for all absorbance measurements.
2.7. Method validation

Remdesivir is very slightly soluble (0.35 mg mL\(^{-1}\)) at pH 2, practically insoluble (0.04 mg mL\(^{-1}\)) at pH 4, and practically insoluble (0.03 mg mL\(^{-1}\)) at pH 7. The partition coefficient (log P) is 3.2 and the pKa is 3.3. The pH (7.50) of mobile phase A (20 mM potassium dihydrogen phosphate solution) was not changed because the solubility of RDV at low pH is very low. Pharmaceutical forms of RDV (Veklury and Covifor powder for solution for infusion) contain betadex sulfobutyl ether sodium as non-medicinal ingredients to increase the water solubility and bioavailability of RDV [11].

Analytical methods have been validated in compliance with the recommendations of the International Harmonization Conference on the validity of analytical procedures [12,13]. Validation parameters (linearity, specificity, trueness, precision, limit of detection and quantification, and robustness) have been investigated.

Standard calibration curves in both methods were obtained by analysing a series of standard solutions. These standard solutions have been prepared in triplicate and linearity was assessed using linear regression analysis.

Specificity of both methods was assessed by comparison of the spectrums and chromatograms obtained from standard and sample preparations which take part in the pharmaceutical preparations.

Limit of detection and quantification have been determined using the slope of the calibration curve (m) and standard error (s) as displayed in following equations:

\[
\text{LOD} = \frac{3.3 \times s}{m} \quad (1) \\
\text{LOQ} = \frac{10 \times s}{m} \quad (2)
\]

A standard solution at LOQ concentration was prepared. For precision and trueness tests, this solution was analysed six replicates on the same day. The precision test result was determined as R.S.D. %. Trueness test results were calculated as recovery %.

Precision of both methods were analysed in terms of both repeatability (intra-day precision) and intermediate precision (inter-day precision). The repeatability was determined from five replicate injections of a freshly prepared RDV solution (40 µg mL\(^{-1}\)) in the same equipment on the same day. In order to determine intermediate precision, the experiment was also replicated by analysing the newly prepared solution at the same concentration on three consecutive days. Precision was expressed as R.S.D. % of a series of measurements.

The percentage recovery was determined by using three preparations of three different levels of the reference drug of RDV for accuracy. The findings were expressed as the percentage of RDV recovered in the sample and R.S.D. %.

The robustness of analytical methods was evaluated by making small changes in method conditions. For LC method, samples have been analysed under different circumstances like changes in the flow rate of mobile phase (±0.1 mL min\(^{-1}\)) and in acetonitrile content (±2%) in the mobile phase and the effect of system suitability parameters have been observed. For the UV method, samples have been analysed under different circumstances like changes in solvents used and detection wavelengths.

2.8. Analysis of pharmaceutical formulations.

Freshly prepared stock sample solution was diluted with methanol to obtain sample solution (30 µg mL\(^{-1}\)). This freshly prepared sample solution was filtered using a filter of 0.22 µm and then analysed.

2.9. Statistical comparison of LC and UV methods

Both analysis methods were applied for pharmaceutical formulations of RDV and the %RSD values for the percent recovery of each level (80%, 100% and 120%) were statistically compared.

3. Result and discussion

3.1. LC method

A reversed-phase LC method for estimating RDV in pharmaceutical forms has been proposed. In order to get a successful result, chromatographic conditions were adapted. The LC procedure has been optimized to develop a reliable and repeatable method. Different conditions such as mobile phase compositions, different columns and configurations were tested to achieve a sharp peak. The mobile phase was chosen considering the peak parameters (tailing, symmetry), analysis time, easy preparation and cost. Figure 2 displays chromatogram produced of RDV standard and sample solutions using the developed method. RDV was eluted to form symmetrical peak, as seen in this figure. The observed retention time (3.865 min) enables the rapid detection of RDV, which is essential for routine research. The resulting RDV peak showed that the flow rate of 1.2 mL min\(^{-1}\) of the mobile phase consisting of 20 mM potassium dihydrogen phosphate and acetonitrile in the ratio of 50:50 (v/v), on the column used was appropriate. In this developed method, the peak was eluted with a capacity factor of 3.87, a tailing factor of 0.895 and a number of theoretical plates of 8937. The chromatogram of the sample solution was given in Figure 2(D). RDV peak and non-medicinal ingredients in their pharmaceutical forms did not show any interaction.

The equation of the calibration curve was obtained from linear regression analysis of the peak area versus the concentration of RDV. Regression equations of the calibration curves for RDV were calculated as
$y = 22.335x + 3.9467$ at the range of 10–60 µg mL$^{-1}$. Correlation coefficient ($r^2$: 0.9998) indicates a good linearity and high sensitivity (Table 1). Limit of detection and quantification have been found to be 2.40 and 7.30 µg mL$^{-1}$, respectively. A standard solution was prepared at LOQ concentration (7.30 µg mL$^{-1}$). For precision and accuracy tests, six replicates were analysed at LOQ concentration (7.30 µg mL$^{-1}$) on the same day. As a result of the precision test, R.S.D was determined as 3.50%. The accuracy test results at LOQ concentration were between 98.25% and 101.65% Figure 3.

Precision of this method has been determined by repeatability (intraday) and intermediate precision (interday). Precision was expressed as R.S.D. % of a sequence of measurement. Precision study data were presented in Table 2. The result obtained shows a good intra-day precision. Inter-day precision was also calculated from assays on 3 days.

### Table 1. Linearity study data.

| Parameter                                           | UV method | LC method  |
|-----------------------------------------------------|-----------|------------|
| Concentration range (µg mL$^{-1}$)                  | 10–60     | 10–60      |
| Limit of detection and quantification (µg mL$^{-1}$)| 3.00/9.00 | 2.40/7.30  |
| Slope                                               | 0.0514    | 22.335     |
| Standard error (Slope)                              | 0.0005    | 0.2700     |
| Intercept                                           | 0.0191    | 3.9647     |
| Standard error (Intercept)                          | 0.0188    | 6.6852     |
| Correlation coefficient                             | 0.9996    | 0.9997     |
| Standard deviation (Residuals)                      | 1.00      | 0.29       |
intense absorbance peaks have been observed at 247 and 276 nm. The most intense absorbance peak (λ_max) was observed at 247 nm. Several assays were carried out, and the best results have been achieved when using the amplitude from the valley at a wavelength of 247 nm to the zero base line. The overlay spectrum of RDV standard solutions and spectrum of sample solution was given in Figure 4(C,D).

A good linearity was achieved in the concentration range of 10–60 µg mL$^{-1}$ of standard solutions of RDV (Figure 5). The exact data obtained for the evaluated methods are presented in Table 2. Less than 0.5 of R.S.D. % values have been determined. This shows that both methods provide good sensitivity, but the LC method is more sensitive compared to the UV method. Accuracy was studied by means of recovery experiments using the methods developed. Both spectrophotometric and chromatographic methods displayed mean recoveries of close to 100%, showing adequate trueness (Table 3).

The method’s robustness was evaluated by testing the effect of minor variations on experimental variables like changes in different solvents and detection wavelengths on the analytical performance. The minor differences in each of the factors did not affect the findings dramatically. This indicates that the method developed for routine analysis is reliable (Table 4).

### 3.3. Application of these methods to pharmaceutical preparations.

Both methods developed and validated have been successfully applied for the determination of RDV in pharmaceutical formulations. Test results for a tablet containing RDV sold in pharmacies were presented in Table 5. The results are very close to the amounts indicated on the label of the tablets. The UV and LC methods recommended in this report can be applied appropriately for the analysis of RDV in pharmaceutical preparations.

### 3.4. Statistical comparison of LC and UV methods

From the validation results, it was determined that these methods described above are suitable for routine quality control analysis of RDV in pharmaceutical formulations. Both analysis methods were applied
Figure 4. (A) Spectrum of standard RDV solution (40 μg mL\(^{-1}\)), (B) spectrum of blank solution, (C) overlap spectrum of standard solutions (10–60 μg mL\(^{-1}\)) and (D) spectrum of sample solution (20 μg mL\(^{-1}\)).

Figure 5. Calibration curve for UV method.

For pharmaceutical formulations of RDV and the %RSD values for the percent recovery of each level (80%, 100% and 120%) were statistically compared (Table 6). The Student’s t-test and F-test were performed and showed no significant difference between the experimental values obtained during the analysis by the two methods. The calculated t-value and F-value were found to be less than the tabulated values of both methods in the 95% confidence interval. It was clear from this report that the recommended LC and UV methods were applicable to RDV in pharmaceutical preparations.

### 4. Conclusions

The LC method and UV spectroscopy method were developed and validated for the analysis of RDV in

### Table 5. Method application results.

| Formulation     | Label claim mg | UV method | LC method |
|-----------------|----------------|-----------|-----------|
| Covifor injectable | 100 mg        | 99.96 mg  | 100.05 mg |

| Level of drug taken % | % RSD for % content | LC method | UV method | F-test\(^a\) | t-test\(^b\) |
|-----------------------|---------------------|-----------|-----------|-------------|-------------|
| 80                    | 0.117               | 0.162     | 0.911     | 1.054       |
| 100                   | 0.171               | 0.230     | 1.013     | 1.108       |
| 120                   | 0.225               | 0.265     | 1.403     | 1.204       |

\(^{a,b}\)Limits of 95% confidence interval.
pharmaceutical preparations were found to be reliable, simple, fast, true and precise. The findings suggest that UV and LC methods are appropriate methods for quantifying RDV in pharmaceutical dosage forms. The results of UV method showed no significant difference from the HPLC method. The purpose to develop the new spectroscopic method is not to replace the available methods for the content analysis of RDV in pharmaceutical formulations, but to use as an alternative method where advanced instruments like HPLC and GC are not available for routine analysis. Hence, it was concluded that there is no need for an extraction procedure for sample preparation and less time consuming. The spectroscopy method requires only the wavelength scan, so it can be utilized for frequent analysis RDV in pharmaceutical dosage forms than the other sophisticated methods. Statistically compared, the LC method is more precise and accurate than the UV method. Because both recommended methods are specific, simple, fast, precise and accurate, they can be successfully applied for routine quality control analysis in pharmaceutical dosage forms of RDV.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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