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CHROMATOGRAPHIC TECHNIQUES FOR THE ESTIMATION OF REMDESIVIR: A COMPREHENSIVE REVIEW

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ABSTRACT

Remdesivir (RDV), a broad-spectrum antiviral initially developed for Ebola, became a pivotal therapeutic agent during the global COVID-19 pandemic. As a direct-acting nucleotide prodrug administered intravenously, its quality, purity, and concentration in biological systems are of paramount importance. This review provides an exhaustive examination of the chromatographic methodologies employed for the estimation of Remdesivir. We explore a wide spectrum of techniques, ranging from routine **Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)** used in quality control, to advanced **Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)** for pharmacokinetic bioanalysis, and eco-friendly **High-Performance Thin Layer Chromatography (HPTLC)**. Furthermore, this review delves into the specific separation of chiral isomers, stability-indicating assays for degradation products, and the shift towards Ultra-Performance Liquid Chromatography (UPLC) for high-throughput analysis.

KEYWORDS: Remdesivir, HPLC, LC-MS/MS, HPTLC, Stability Indicating Method, Bioanalysis, Chromatography.

1. INTRODUCTION

The emergence of the SARS-CoV-2 virus in late 2019 necessitated the rapid repurposing of existing antiviral drugs. **Remdesivir (GS-5734)**, developed by Gilead Sciences, quickly rose to prominence due to its ability to inhibit the viral RNA-dependent RNA polymerase (RdRp). Biologically, Remdesivir is a "prodrug." This means it is not the active warrior itself; rather, it is a delivery vehicle. Once it enters human cells, it is metabolized into its active triphosphate form. This active form mimics adenosine (a natural genetic building block).

When the virus attempts to use this mimic to replicate its genetic material, the process is arrested, effectively stopping the infection in its tracks [1, 2].

However, the chemical complexity of Remdesivir—specifically its phosphoramidate core and multiple chiral centers—presents a significant challenge for analytical chemists. Ensuring the drug is free from impurities and identifying its metabolites in the human body requires highly specific and sensitive tools. Chromatography, the science of separating mixtures, provides the solution. This review details how scientists utilize various chromatographic platforms to ensure the safety and efficacy of this life-saving medication. The precision of these techniques ensures that the drug supplied to hospitals is free from toxic degradation products [3, 31].

2. PHYSICOCHEMICAL PROFILE

Before developing a chromatographic method, one must understand the molecule. Remdesivir is practically insoluble in water, which poses a challenge for mobile phase selection. It dissolves freely in organic solvents like methanol and ethanol. The molecule has pKa values around 3.8 and 9.8. This informs the choice of buffer pH in chromatography; keeping the pH between 3.0 and 4.0 typically ensures the molecule remains in a non-ionized state, improving retention on non-polar columns [4, 29].

Furthermore, the molecule possesses a chromophore that absorbs ultraviolet light, with a maximum absorption (\lambda_{max}) typically observed at **246 nm**. This property is the basis for UV detection in HPLC. Understanding these physical traits is crucial for selecting the right solvents and columns during method development [5, 33].

3. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

3.1 The Standard for Quality Control

RP-HPLC is the workhorse of pharmaceutical analysis. It is favored in industrial "Quality Control" (QC) labs because it is robust, precise, and widely available. The primary goal in this setting is to assay the drug content in injection vials to ensure it meets the label claim (usually 100 mg/vial). The reproducibility of HPLC allows manufacturers to guarantee that every batch released to the market is identical in potency [6, 30].

3.2 Mobile Phase Strategies

Since Remdesivir is hydrophobic (water-hating), it requires an organic solvent to move through the column. A blend of **Acetonitrile** and **Water** (or buffer) is the most common choice. Acetonitrile is often preferred over Methanol because it produces lower back-pressure

and sharper peaks. To prevent peak tailing (where the peak shape becomes asymmetrical), researchers often add **Phosphate Buffer** or **0.1% Formic Acid** to the water. A typical composition found in successful validations is **Acetonitrile : Phosphate Buffer (50:50 v/v)**, adjusted to pH 3.5 [7, 26].

3.3 Stationary Phase (Column) Selection

The industry standard is the **C18 Column** (Octadecylsilane). The long 18-carbon chains act like "molecular grease," interacting with the non-polar parts of Remdesivir. Popular commercial columns include the *Agilent Zorbax Eclipse Plus C18* or the *Kromasil 100-5-C18*. A standard setup uses a column length of 250 mm with an internal diameter of 4.6 mm and a particle size of 5 microns (5 \mum). This specific geometry provides enough surface area to separate Remdesivir from any manufacturing impurities [4, 21].

3.4 Method Performance

Under these conditions, Remdesivir typically elutes (comes out of the column) at a retention time between **4 to 12 minutes**. Validated methods generally show a linearity range of **10–100 \mug/mL** with a correlation coefficient (r^2) greater than 0.999, proving high reliability. The "Tail Factor"—a measure of how symmetrical the peak is—must be less than 2.0. If the peak trails off (tails), it usually means the pH is wrong or the column is aging [5, 24].

4. ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

4.1 The Need for Speed

During the pandemic, the demand for Remdesivir skyrocketed. Manufacturers needed to test thousands of batches daily. Standard HPLC, with run times of 15 minutes, was a bottleneck. To address this, many labs transitioned to UPLC, which allows for high-throughput analysis without sacrificing accuracy [8].

4.2 The UPLC Solution

Ultra-Performance Liquid Chromatography (UPLC) utilizes columns packed with sub-2-micron particles (e.g., 1.7 \mum). This allows for much faster flow rates without losing resolution. Using a **BEH C18 column** (50 mm x 2.1 mm), researchers have achieved complete separation of Remdesivir in **less than 1.5 minutes**. UPLC often provides sharper peaks, leading to lower Limits of Detection (LOD) compared to HPLC, making it efficient for detecting trace impurities [9, 10].

5. BIOANALYTICAL TECHNIQUES: LC-MS/MS

5.1 Analyzing Blood Samples

When Remdesivir is injected into a patient, it rapidly metabolizes. Doctors and researchers need to measure the concentration of both the parent drug and its primary metabolite (**GS-441524**) in blood plasma. Standard UV detection is insufficient here because blood plasma is a "dirty" matrix full of interfering proteins that create background noise [11, 34].

5.2 Mass Spectrometry (MS)

LC-MS/MS (Liquid Chromatography with Tandem Mass Spectrometry) is the gold standard for bioanalysis. Instead of using light, the detector weighs the molecules. It isolates the specific mass of Remdesivir (Precursor Ion) and smashes it into fragments (Product Ions). For Remdesivir, the mass transition typically monitored is **m/z 603.3 \rightarrow 200.0**. For the metabolite GS-441524, it is **m/z 292.1 \rightarrow 147.1**. This specificity acts like a molecular fingerprint, ensuring no other substance is mistaken for the drug [12, 14, 15].

5.3 Sample Preparation

Before injection, the plasma must be cleaned. The most common method involves adding **Methanol** or **Acetonitrile** to the plasma sample. This causes the blood proteins to solidify and fall out of the solution (Protein Precipitation), leaving the drug in the clear supernatant. These methods are incredibly sensitive, capable of detecting drug concentrations as low as **1 to 5 ng/mL** [13, 25].

6. CHIRAL CHROMATOGRAPHY

Remdesivir contains a phosphorus center that is "chiral," meaning it can exist in two forms that are mirror images of each other (Sp and Rp isomers). Only the **Sp-isomer** is the active drug; the Rp-isomer is an impurity. Standard C18 columns cannot separate these mirror images because they have identical physical properties in non-chiral environments [2, 35].

The solution is the use of **Chiral Columns** (e.g., *Chiralpak IA* or *Chiralpak IE*). These columns are packed with amylose or cellulose derivatives that interact differently with the two isomers, allowing them to be separated and quantified individually. This ensures that the final product contains only the therapeutically active form of the molecule [35].

7. HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

7.1 The Green Alternative

HPTLC is gaining traction as an eco-friendly alternative to HPLC. It consumes significantly less solvent and energy. Samples are spotted onto **Silica Gel 60 F254** plates, and the mobile

phase moves up the plate via capillary action. A mixture of **Ethyl Acetate : Ethanol** is often used, avoiding toxic solvents like chloroform [16, 17].

7.2 Advantages

It allows for the simultaneous analysis of dozens of samples on a single plate, making it highly cost-effective for stability testing in resource-limited settings. A "Densitometer" scans the plate with UV light to measure the darkness of the spots. Recent studies have proven HPTLC is accurate enough for routine factory checks, providing a sustainable option for developing nations [18, 19].

8. STABILITY-INDICATING ASSAYS

To determine the shelf-life of Remdesivir, researchers perform "Forced Degradation Studies." The drug is intentionally exposed to stress to see how it breaks down. Conditions include hydrolysis (Boiling in Acid or Base), Oxidation (Exposure to Hydrogen Peroxide), and Photolysis (Exposure to UV light) [20, 22].

Findings show that Remdesivir is notably unstable in alkaline (basic) conditions, rapidly degrading into the nucleoside GS-441524. A valid chromatographic method must be able to resolve the peak of the intact drug from these degradation peaks to ensure accurate purity testing. If the method cannot distinguish between the drug and its spoiled byproducts, it is considered unsafe for stability testing [23, 24].

9. CONCLUSION

The analytical estimation of Remdesivir is a multi-faceted discipline that underpins its safe global distribution. **RP-HPLC** remains the industry standard for routine formulation analysis due to its precision. **LC-MS/MS** is indispensable for clinical pharmacokinetics due to its sensitivity in biological fluids. **UPLC** offers a pathway for high-throughput manufacturing, while **HPTLC** provides a sustainable, low-cost option for stability testing. As the pharmaceutical landscape evolves, we anticipate a further shift toward automated, greener, and faster chromatographic methods to support the lifecycle of antiviral therapeutics [1, 28].

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