Significance of HPLC in the Development and Production of the Antiviral Drug Remdesivir

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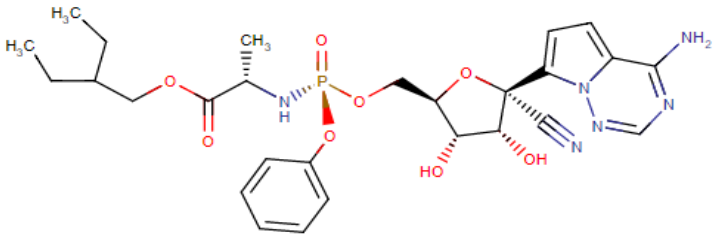
Overview

The disease COVID-19 caused by the new coronavirus SARS-Cov-2 is now classified as a pandemic outbreak by the World Health Organization (WHO). After Chinese researchers identified the pathogen and shared viral genomic sequences in a short period of time, many drug-research institutes began screening different classes of antiviral drugs. However, no specific single therapeutic has appeared to fully treat the virus infection so far. On January 31, 2020, the New England Journal of Medicine (NEJM) published an article about the first case of a COVID-19 infected person being cured in the United States[1], which raises hope for the development of new anti-coronavirus specific drugs.

Remdesivir, the drug mentioned in the article, is also known as GS-5734. It is a nucleoside analog antiviral drug developed by Gilead Sciences. According to an article published in the Journal of Medicinal Chemistry[2], within more than 20 years of research spanning multiple antiviral drug projects, GS-5734 stands out from the drug library of nearly 1,000 different nucleosides and nucleoside phosphates as it demonstrates high potency in multiple cell lines and has the potential to scale up due to its rapid synthesis process.

Figure 1. Structure of Remdesivir (*Drug Bank*)



Nucleoside analogs are activated by intracellular nucleoside kinases to produce their respective nucleoside triphosphate (NTP). The pharmacologically active NTP then competes with the endogenous natural nucleotide library to be incorporated into replicated viral RNA and acts as a RNA chain terminator. As the first phosphorylation step to generate nucleoside monophosphate is usually a rate-limiting step, monophosphate prodrugs, especially phosphoramidates, have been widely studied in the screening of nucleoside analogs. Structure-wise, Remdesivir is obtained by the monophosphorylation of nucleoside analogs, and the 1'-CN group and C-linked nucleobases are important for selective resistance to host polymerase.

This article[2] proposes a more efficient route for the synthesis of the single Sp diastereomer- GS-5734 by the crystallization of a key reagent. The synthesized GS-5734 molecule was then subjected to in-vivo model study. HPLC was used for purity assessment during the whole synthesis process. The column used in this article was Kinetex® C18, 2.6 µm, 100 × 4.6 mm (Part No. 00D-4462-E0). A gradient from 2% mobile phase B to 98% mobile phase B was performed under the conditions of 0.1% TFA in water as mobile phase A, 0.1% TFA in acetonitrile as mobile phase B, and a flow rate of 1.5 mL/min. The core-shell structure of Kinetex column results in narrower peak shapes and shorter run time for purity assessment.

Meanwhile, LC-MS equipped with a Gemini® 5 µm C18 30 × 4.6mm (Part No. 00A-4435-E0) column was also used to monitor the progress of the reaction during synthesis. During this synthesis of different prodrugs and intermediates, HPLC preparative columns (ex. AXIA™) and silica gel flash columns (ex. Claricep™) were widely used to separate and purify compounds.

In the first-generation synthesis route, compound 4a was a 1: 1 mixture of two diastereomers of Sp 4b (GS-5734) and Rp 4c. It was necessary to use the fully porous polysaccharide chiral column Lux® Cellulose-2 to separate them under polar organic separation mode. The running condition was isocratic elution with 95% acetonitrile / 5% methanol. 4c was the first eluate and 4b (GS-5734) was the second one. Without the assistance of HPLC the synthesis and separation of prodrugs and intermediates will be much of a challenge.

In addition to the prodrug synthesis and separation applications, HPLC has played an important role in in-vivo study and pharmacokinetic analysis. The article "Therapeutic Efficacy of the Small Molecule GS-5734 against Ebola Virus in Rhesus Monkeys", which was published in Nature (2016) demonstrated the studies on PK and intracellular metabolism of GS-5734 (Remdesivir). To determine the content of alanine metabolite, nucleoside, nucleoside monophosphate, nucleoside diphosphate and nucleoside triphosphate, a Luna® C18(2) HST 2.5 µm 50 × 2.0 mm (Part No.: 00B-4446-B0) column, coupled with API 4000 MS / MS had been used. The ion pair reagent 10 mM dimethylhexylamine and 3 mM ammonium formate (pH 5.0) in water was used as mobile phase A. A gradient from 10% acetonitrile to 50% acetonitrile at a flow rate of 150 µL/min in 8 minutes was chosen to separate the analytes.

In the pharmacokinetic analysis, the plasma sample from three uninfected male rhesus monkeys after administration were treated with a mixture of methanol and acetonitrile for protein precipitation. After adding an internal standard, the sample was reconstituted in a mixture containing 1% acetonitrile and 99% water with 0.01% formic acid. A Synergi™ Hydro-RP 4 µm 75 × 2.0 mm (Part No.: 00C-4375-B0) column was used at a flow rate of 0.26 mL/min. The gradient was from 99% water with 0.2% formic acid and 1% acetonitrile, to 95% acetonitrile, 5% water with 0.2% formic acid over 4.5 minutes. This study was mentioned in "Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruse"[3] (2017). Although GS-5734 (Remdesivir) was only used in clinical studies of Ebola before the outbreak of COVID-19, research has found that it can inhibit the replication of SARS-CoV and MERS-CoV in a variety of in-vitro systems. In a mouse model for the pathogenesis of SARS-CoV[3], through prophylactic and early therapeutic administration of GS-5734, it significantly reduced lung viral load, reduced clinical signs, and improved respiratory function. These data indicate that Remdesivir is likely to have a good inhibitory effect on COVID-19.

Conclusion

To summarize, the HPLC technique plays an important role in drug synthesis, screening, separation, and purification, as demonstrated in the case of Remdesivir. By using columns with different selectivity, the target analytes can be fully separated in a short amount of time. In addition, low or medium pressure flash chromatography and high pressure preparative HPLC is also important in the scale up of drug precursor and intermediate production. In the preclinical cell experimentation and animal experimentation, HPLC has also become the main method in pharmacokinetic research. From February 6th, 2020, Remdesivir is being tested in Phase III clinical trials[4]. As the study on Remdesivir continues, HPLC will continue to be an effective analytical and purification method in the development and production of antiviral drugs.

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