To Study Validated Uplc Method and the Pharmacokinetic Properties of Molnupiravir and Favipiravir

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I. INTRODUCTION

One instance of an antiviral medication is favipiravir, which is sold under the brand name Avigan among others. Ongoing research is being conducted on its efficacy as a treatment for SARS-CoV-2 [1,2] and various other viruses [3]. Like the investigational antiviral drugs [4,5] T-1105 and T-1106, it is a derivative of pyrazinecarboxamide. Favipiravir is a modified pyrazine analog that may be utilized to treat influenza patients when the virus has developed resistance [6,7]. This antiviral drug specifically targets RdRp, an enzyme crucial for the transcription and replication of viral genomes [8,9]. Favipiravir has the potential to treat avian influenza, inhibit the replication of influenza A and B, and may serve as an alternative for influenza strains resistant to neuraminidase inhibitors. The application of favipiravir in treating potentially life-threatening infections such as COVID-19, Ebola, and Lassa viruses is currently under investigation [10,11]. According to existing data, its use during pregnancy may pose risks to the unborn child, as results from studies on four different animal species indicated that it was teratogenic and embryotoxic [12]. Another antiviral drug that inhibits the replication of certain RNA viruses is molnupiravir, marketed under the brand name Lagevrio. This medication is administered orally to individuals infected with the SARS-CoV-2 virus to treat coronavirus disease 2019. Molnupiravir exerts its antiviral effects by inducing errors in viral RNA replication. It acts as a prodrug of the synthetic nucleoside derivative N4-hydroxycytidine. Initially, molnupiravir was intended for the treatment of influenza. It is indicated for mild-to-moderate COVID-19 patients who have tested positive for SARS-CoV-2 and possess risk factors for severe disease conditions. It is advised not to use this medication if you are pregnant, as there is insufficient human data available to evaluate the risks to the mother or fetus. Sub-therapeutic exposure to drugs and instability related to impurities are widely acknowledged as significant contributors to the emergence of antiviral resistance. As a result, rigorous pharmaceutical analysis is crucial to guarantee therapeutic efficacy and the success of long-term treatment. Strong analytical control not only protects product quality but also ensures consistent clinical performance throughout the product's lifecycle (Andersson & Hughes, 2014).Precise analytical assessment is vital for confirming correct dosage strength, ensuring batch-to-batch consistency, providing reliable stability evaluations, and effectively detecting and quantifying degradation products, in addition to identifying impurities related to the manufacturing process (ICH, 2003; ICH, 2005). Even slight variations in the concentration of active pharmaceutical ingredients (APIs) can jeopardize therapeutic effectiveness and may foster resistance. Similarly, the presence of degradation products or leftover impurities can modify drug performance and affect pharmacokinetic behavior.Advanced analytical methods such as High-Performance Liquid Chromatography (HPLC), LC–MS, and validated stability-indicating techniques are essential for upholding drug quality, safety, and

compliance with regulatory standards. Especially in the face of emerging viral infections, maintaining stringent analytical control is critical for ensuring reliable antiviral efficacy. Antiviral resistance is not merely a virological issue; it is also intricately connected to pharmaceutical quality. Inadequate characterization of impurities and a lack of sufficient analytical oversight can undermine drug performance. Therefore, systematic profiling of impurities and the adoption of validated analytical methodologies are fundamental to contemporary antiviral quality assurance.

II. METHODOLOGY

Various types of analytical methods are required to be validated in accordance with currentregulatory requirements. These methods are generally classified as identification tests, limittests, and quantitative tests. Identification tests are used to confirm the identity of asubstance, while limit tests are performed to control the presence of impurities or otherspecified components within defined limits. Quantitative tests are designed to determine theexact amount of an analyte present in a sample, such as assay, impurity content, ordissolution. For quantitative analytical methods, specific validation characteristics must beevaluated to demonstrate that the method is suitable for its intended purpose. As per currentregulatory guidance, the typical validation parameters to be considered include accuracy,

100 mg of active ingredient was carefully weighed and dissolved in roughly 20 mL of ultrapure water before being transferred to a 100 mL measuring flask. The volume of the stock solution was increased to 100 mL using ultrapure water to obtain 1 mg mL-1. The resulting stock solution was filtered through a 0.45 µm filter and sonicated. The stock solution was further diluted with deionized water before being fed into the system for analysis to obtain the needed standard solution concentration. (1-10 µg mL-1).2.3Preparation of Sample SolutionFive FVP tablets were sensitively weighed before being transferred to a dry, clean mixture and pounded into a fine dust. The average weight of five tablets was 0.280 grams. Following that, 200 mg faripiravir tablet powder was placed to a 100 mL volumetric flask. 100 mL deionized water was added to the flask, which was then shaken for 10 minutes to completely disperse the components. After 30 minutes of sonication, the mixture was diluted to volume with ultrapure water to obtain a 500 µg mL-1solution, which was then filtered through a 0.45 µm filter.2.4Determination of λmaxA UV spectrophotometer was used to scan a standard solution (10 g mL-1) between 200 and 800 nm. (Perkin Elmer Lambda-35 UV-VIS spectrophotometer). The UV spectra of standard solution was used to calculate λ max.

Chromatographic ConditionsOn a Poroshell column, chromatographic analysis was done. 120 EC-C18 (4.6 mm × 50 mm, 2.7 µm). The mobile phase contained of methanol and H2O with formic acid % 0.1 (20:80, v/v). Before analysis, a 0.45 µm membrane filter was used to filter and degas the mobile phase, which was then pumped at 0.8 mL min-1. The column's temperature has been set to 40 °C. The run time was 2 minutes under these conditions.2.6Mass Spectroscopy ConditionsThe MS analysis was performed on UPLC-MS/MS instrument (Agilent 6460, USA) equipped with electrospray jet stream ionization source (AJS ESI). The data acquisition was under the control of Masshunter (Agilent, USA). First, the mass spectrometer was optimized for FVR and the daughter ion, fragmentor voltage and collusion energy were determined. Analyses were performed in positive and negative ion modes. A standard solution of Favipiravir at a concentration of 500 µg/mL was prepared andscanned in the 200–400 nm range using a photodiode array (PDA) detector. The maximumabsorbance (λmax) was observed at 275 nm, which was selected for UPLC analysis (Snyder et al., 2012; International Council for Harmonisation, 2023).

III. TRIALS FOR MOBILE PHASE SELECTION

The mobile phase was selected based on critical analytical consideration s to ensure optimal chromatographic performance. The primary criteria included compatibility with liquidchromatography–mass spectrometry (LC-MS), in case hyphenated analysis was required;high UV transparency within the selected wavelength range to avoid baseline interference; and the ability to achieve a stable baseline with complete separation of the Favipiravir peak from its potential impurities and degradation

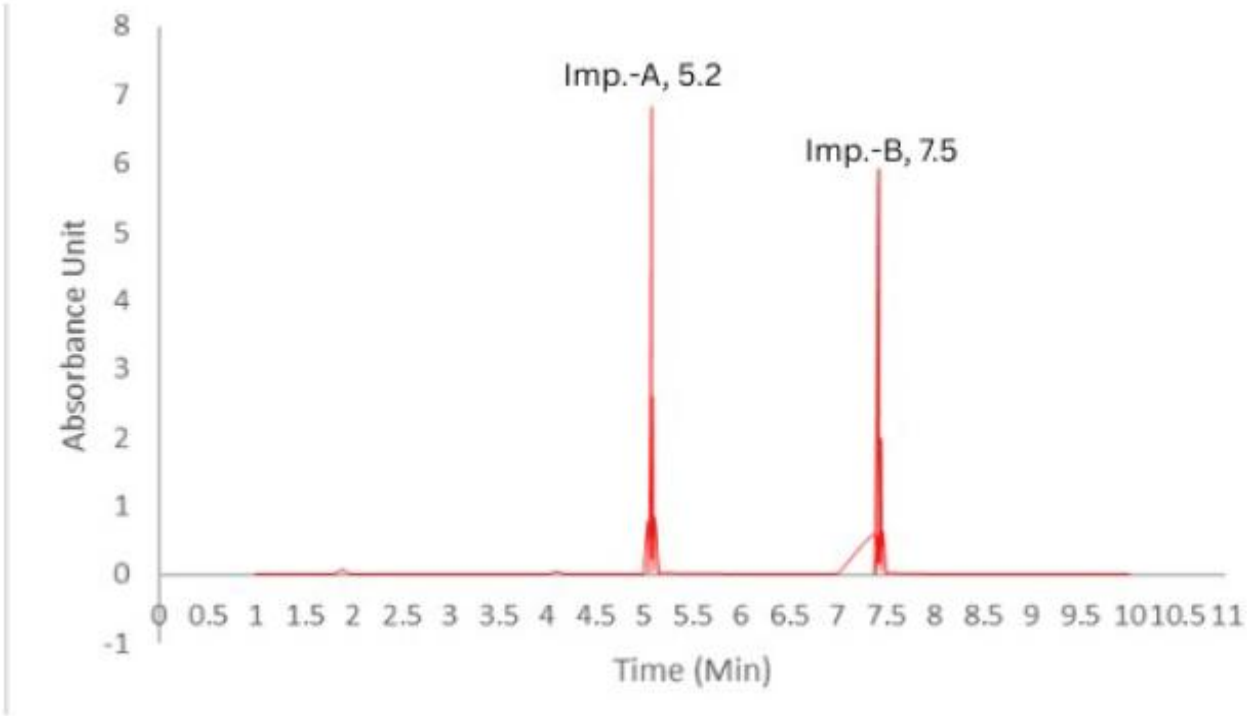
products (Snyder et al., 2012; International Council for Harmonisation, 2023). To optimize chromatographic conditions, several experimental trials were conducted using different combinations of aqueous buffers and organic solvents, such as water–acetonitrile and phosphate buffer–methanol systems. These trials were systematically evaluated to obtain improved resolution, symmetrical peak shape, and an appropriate retention time, ensuring the development of a robust and reliable analytical method (Snyder et al., 2012).

IV. SELECTION OF STATIONARY PHASE

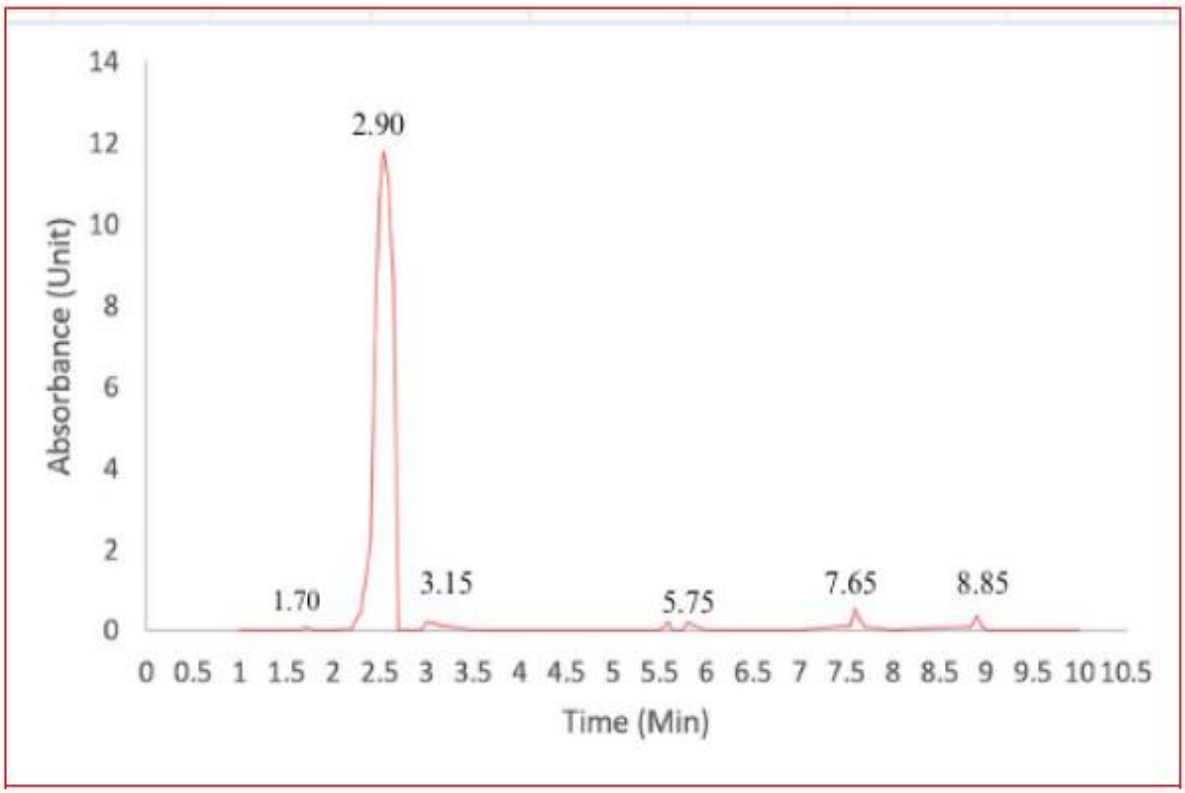
Since Favipiravir is a mixed-polar compound, retention of the analyte and its potential impurities require a polar-embedded stationary phase. The CSH Phenyl-Hexyl column provides alternative selectivity compared to traditional reversed-phase columns and is valuable for method development. The trifunctionally bonded C6 phenyl-ligand is a robust, low-bleed sorbent that selectively retains polyaromatic and polar compounds through π-π interactions, improving resolution of Molnupiravir from impurities (Snyder et al., 2012; International Council for Harmonisation, 2023).

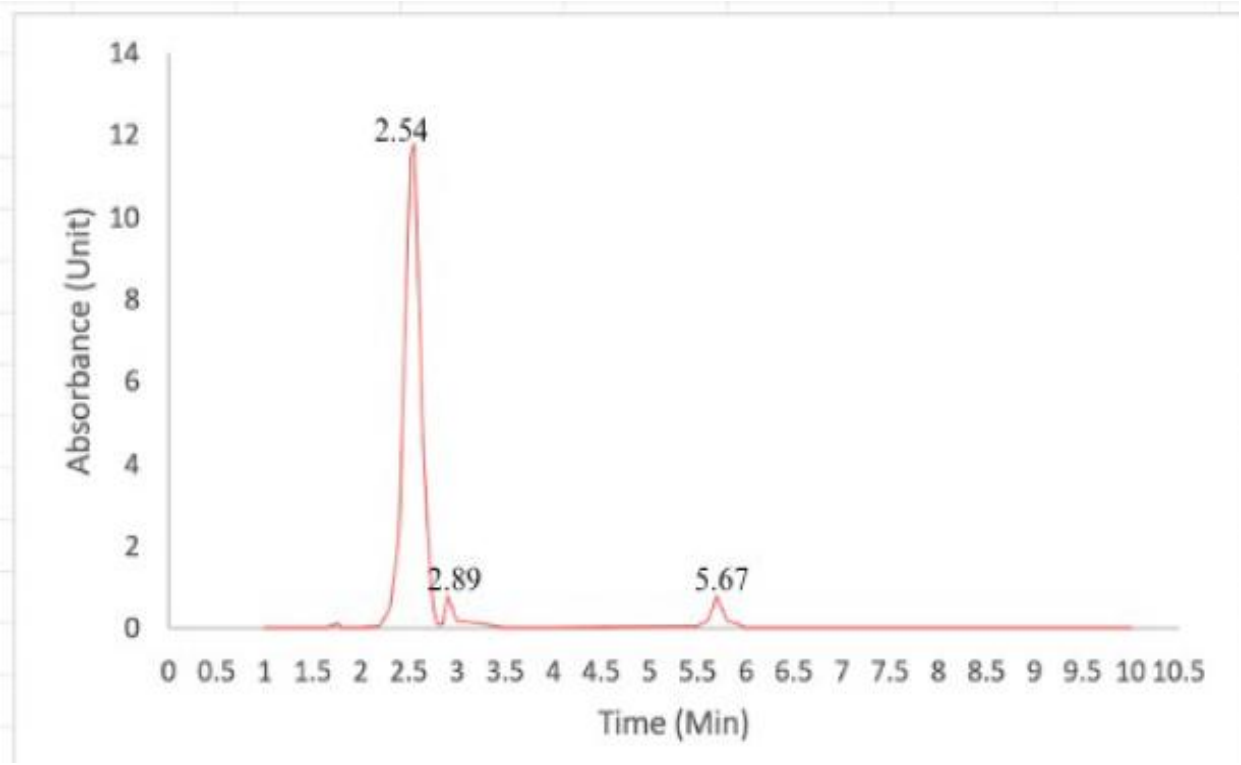
The developed UPLC method is a fast, reliable and robust method for medical and chemical use. It differs from other studies in the literature with its speed and simplicity. Degradation studies are important for chemical studies, drug production techniques and processes, and further studies, as they show the conditions under which the active substance remains stable and degrades. In this study, the active substance was exposed to different stress conditions and under which conditions it remained stable and under which conditions it degraded was examined. As a result of the decomposition studies, it was observed that the active substance was completely decomposed in the basic conditions and to a large extent in the acidic conditions. The study conducted in terms of drug production, packaging and storage conditions has been presented to the literature as a fast, robust and reliable analysis method

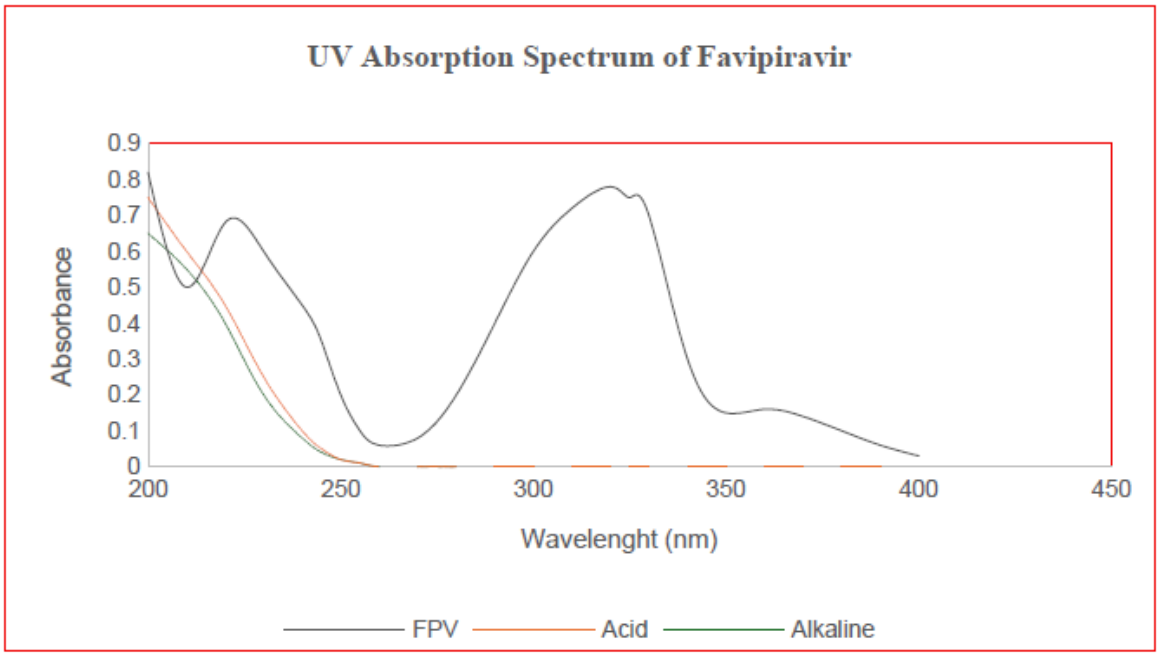
V. RESULTS

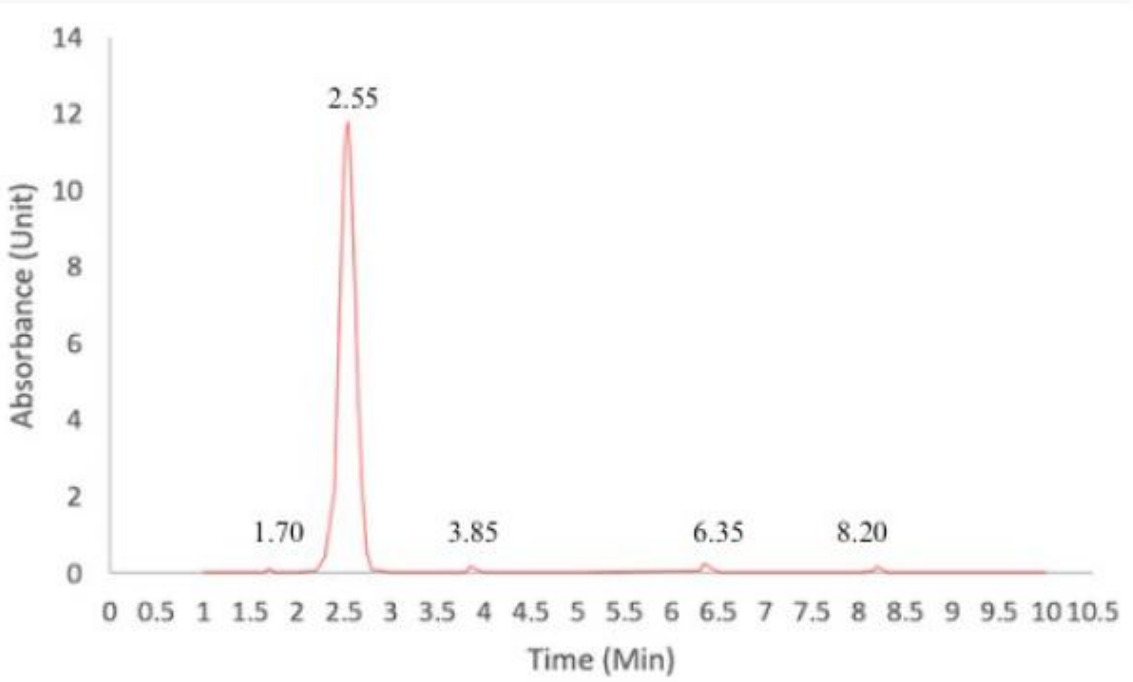


near 2.89 and 5.67 min.









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