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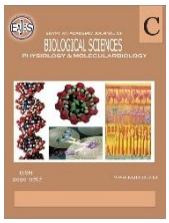

Recent Analytical Methodologies for Determination of Anti-Viral COVID-19 Drugs: A Review

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ABSTRACT

Since the first discovery of the first case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019 in Wuhan, China, the virus quickly spread throughout the world and became a pandemic. The virus has continued to pose challenges, with new variants emerging and evolving, necessitating ongoing adaptation and response from governments, healthcare systems, and communities worldwide. Various drug regimens were introduced with immediate implementation and there is a need for the study of Review of Quality Control aspects of drugs employed in COVID-19 treatment is critical for ensuring their safety, efficacy, and reliability. With the proliferation of pharmaceutical interventions aimed at combating the pandemic, such as antiviral, immunomodulators, and repurposed medications, a thorough review is essential. Harnessing immune modulators, monoclonal antibodies, and antiviral agents like the ritonavir (RTN)/nirmatrelvir (NTV) combination, molnupiravir (MLP), and favipiravir (FVP) presents a multifaceted approach to combating COVID-19. These pharmaceutical interventions offer diverse mechanisms of action aimed at suppressing viral replication, mitigating immune responses, and enhancing the body's ability to combat the virus Improving analytical methods to achieve precise and sensitive quantification of these drugs is paramount for advancing quality control measures. The study delved into a variety of analytical techniques for assessing the concentrations of antiviral drugs utilized in COVID-19 treatment. The techniques covered by these methods include Ultra High-Performance Liquid Chromatography (UHPLC-MS/MS) or Ultraviolet detectors (HPLC-UV), High-Performance Thin Layer Chromatography (HPTLC), Reversed Phase-High Performance Liquid Chromatography (RP-HPLC), High-Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS) or Ultraviolet detectors (TLC-UV), and Micellar Liquid Chromatography (MLC). With regard to sensitivity, selectivity, and efficiency in measuring drug concentrations, each of these methods has special benefits. The TLC and HPTLC techniques offer reasonably easy sample preparation at a reasonable price, whereas the RP-HPLC, HPLC-MS/MS, UHPLC-MS/MS, and UPLC-UV techniques offer more sensitivity and precision, especially in complex sample types. Furthermore, precise identification and quantification of pharmacological molecules at low concentrations are made possible by the application of UV and mass spectrometric detection.

The purpose of the study is to assess the effectiveness of the RP-HPLC method and validate the chosen medications. The results of the study will be further examined, and the development of a novel RP-HPLC method will be optimized. These analytical techniques can be refined to satisfy legal specifications and guarantee accurate anti-COVID-19 medication quantification. Here, we focus on clarifying modern, straightforward, fast, accurate, and eco-friendly analytical techniques that demonstrate great sensitivity, selectivity, and accuracy for the analysis of anti-COVID-19 medications.

INTRODUCTION

The World Health Organization (WHO) declared the COVID-19(https://www.drugs.com/ condition/covid-19.html) epidemic, which began in Wuhan, China, at the end of 2019 to be a global pandemic by March 2020 due to its rapid global spread. People in the US and around the world faced a new dilemma as a result of the extraordinary scope of COVID-19. Initially, the urgency of the issue was exacerbated by the lack of precise treatments or vaccines, which caused considerable uncertainty and fear among communities worldwide.

Many nations have enacted safety precautions, such as requiring people to wear masks while they are in public, instituting lockdowns to prevent the spread of viruses, switching to virtual work and schooling, and making outdoor meetings the new norm. Even in modern times, some social distancing practices endure, even if the virus's intensity has decreased. In order to properly manage the condition, research into new antiviral drugs and the repurposing of existing medications has increased due to the COVID-19 outbreak. The pressing need to create pharmaceutical treatments that can both fight the virus and lessen its effects on public health is reflected in the increased emphasis on research.

A wide array of drug classes, spanning antibiotics, antiviral, non-steroidal anti-inflammatory drugs (NSAIDs), anticancer agents, antimalarials, and immune suppressants, are undergoing rigorous evaluation to determine their efficacy and safety in managing symptoms associated with SARS-CoV-2 infection. Among these, antiviral drugs play a crucial role in hindering the infection and replication of SARS-CoV-2 through diverse mechanisms. (Fig. 1).

Medications that impede virus entry into the cell do so by blocking the virus from interacting with the host cell. This disruption typically involves interfering with the binding of the virus's spike protein to the host receptor. Among these drugs, two classes are notable: Protease Inhibitors and Angiotensin-Converting Enzyme 2 (ACE) Inhibitors: (Fig. 1A)(Mahdavia R, Talebpoura Z;2023) Drugs that hinder the replication and dissemination of viral genomes following membrane fusion are pivotal in combating viral infections. (Fig. 1B).

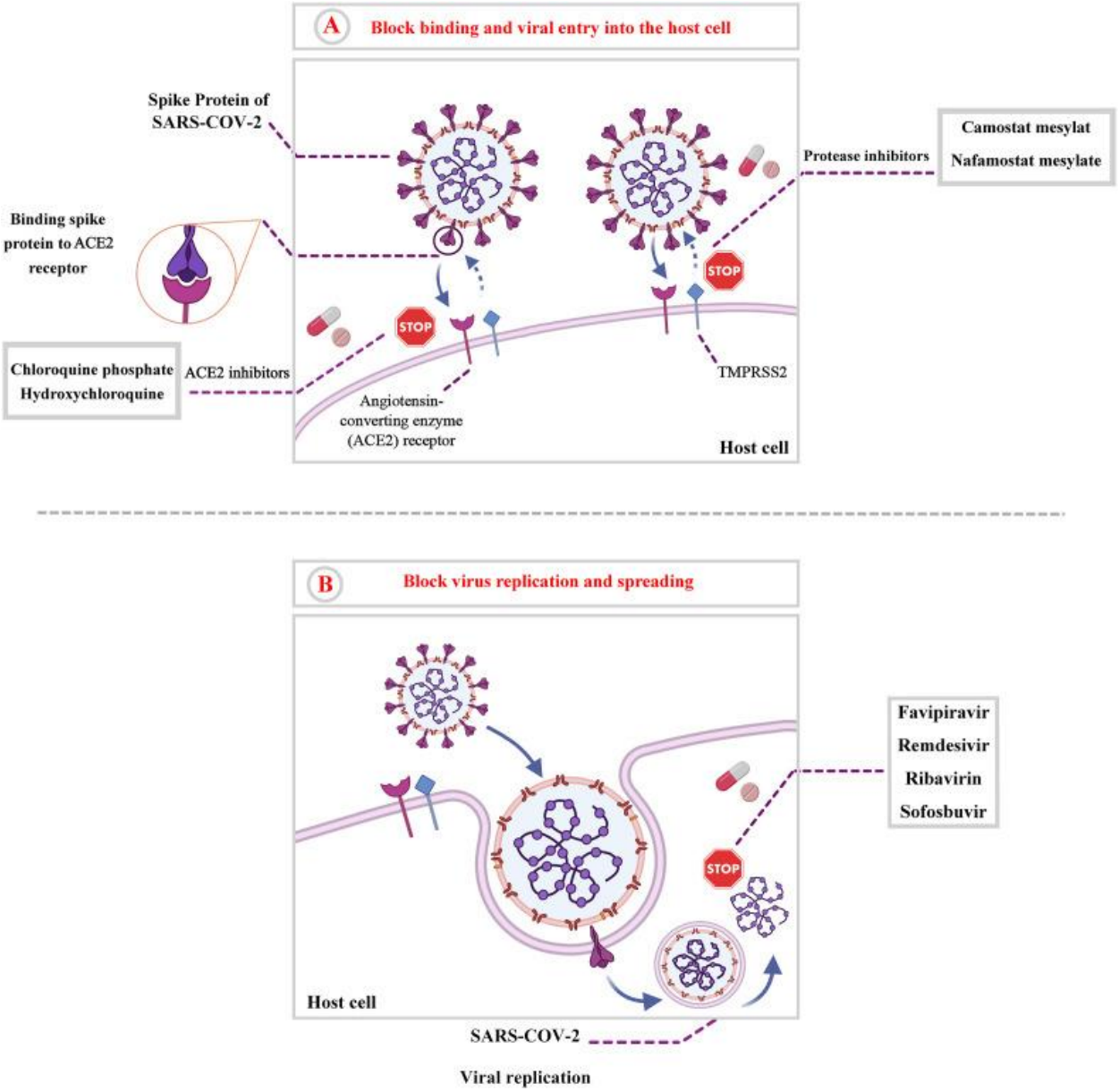


Fig. -1A-1B-Mechanism of Action

As the COVID-19 pandemic progresses into its fourth year, surveillance efforts have significantly waned. Despite weekly reported cases and deaths reaching their lowest levels since the pandemic's onset, millions of individuals are still contracting or re-contracting SARS-CoV-2, with thousands succumbing to the virus each week.

Given the significance of this juncture, the World Health Organization (WHO) has revised its Strategic Preparedness and Response plan to address the evolving landscape from 2023 to 2025. The revised two-year strategy builds upon the goals outlined in the 2022 SPRP and provides assistance to countries as they endeavor to shift their crucial emergency response efforts toward sustainable, long-term prevention, control, and management of COVID-19. It is essential to read this strategy alongside pertinent WHO guidance on COVID-19,(Wang Q, Li Z, Ho J;2022)

In response to the novel coronavirus (COVID-19) outbreak in March 2020, In response to the urgent need for effective treatments, the FDA swiftly initiated the Coronavirus Treatment Acceleration Program (CTAP).

Ctap Program:

Corona Virus Treatment Acceleration Program(CTAP)(US Food and Drug Administration) was initiated with the primary objective of the Coronavirus Treatment Acceleration Program (CTAP) is to expedite the development and evaluation of drugs and biologics (excluding vaccines) intended for the treatment of COVID-19. To achieve this goal, CTAP has facilitated collaboration between the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) within the FDA. This collaboration involves the consolidation of scientific resources and expertise from both CDER and CBER, streamlining the development and evaluation process for COVID-19 therapeutics. By pooling their knowledge and capabilities, CDER and CBER can leverage their collective experience in drug and biologics evaluation to accelerate the assessment and approval of potential treatments for COVID-19. Through this coordinated effort, CTAP aims to expedite the advancement of promising COVID-19 therapeutics, ensuring that safe and effective treatments are made available to patients as quickly as possible. By facilitating cooperation between CDER and CBER, CTAP maximizes the efficiency of the regulatory review process, ultimately benefiting public health efforts to combat the COVID-19 pandemic. CTAP has been instrumental in achieving significant milestones in the ongoing response to the COVID-19 pandemic. The COVID-19 Therapeutics Accelerator Program (CTAP) has played a pivotal role in expediting the development and availability of essential treatments for COVID-19. These initiatives have significantly bolstered the global response to the pandemic, guaranteeing prompt access to effective therapies for those in need. Overall, CTAP has played a pivotal role in expediting the advancement of COVID-19 therapeutics, underscoring its importance in the broader efforts to combat the pandemic and mitigate its impact on public health.

CTAP Status:

As of April 16, 2020, the CTAP has been actively managing the influx of proposals and inquiries related to COVID-19 treatments, particularly during the second wave of the pandemic. Within a short timeframe, CTAP received 950 inquiries and proposals for the development of COVID-19 treatments. By May 11, 2020, the program had facilitated 144 ongoing trials of COVID-19 treatments, with an additional 457 COVID-19 development programs in the planning stages. The CTAP's commitment to expeditious review is evident in its swift turnaround times, with most requests processed within one day or less.

Companies seeking FDA input for their proposed COVID-19 treatment protocols are encouraged to utilize the CTAP mailbox if their treatments meet specific criteria. These criteria include being in late Phase 1, Phase 2, or Phase 3 of development, demonstrating clear proof of concept and/or significant efficacy, and possessing a favorable benefit-risk profile. Additionally, treatments falling under FDA jurisdiction but lacking clarity on the appropriate review center are eligible for CTAP consideration.

While early-stage proposals are not excluded from CTAP review, they may be directed to standard Pre-IND meeting pathways. Conversely, treatments in later stages of development without clear proof of concept or with weaker efficacy signals may receive lower priority within the CTAP pipeline. The FDA emphasizes the importance of existing evidence regarding proof of concept, efficacy, safety, and benefit-risk profile for strategic assessment and regulatory pathway determination.

Overall, the FDA remains committed to expediting the review of COVID-19 treatment programs based on factors such as completeness, scientific merit, stage of

development, and benefit-risk profile, ensuring timely evaluation and potential approval of promising investigational treatments.

Line of Treatment :

There are various treatments for covid -19 . Among those we have discussed about anti-viral , immunomodulators , mono clonal antibodies.

Table 1: line of Treatment

COVID-19 PANDEMIC		
DRUGS	**IMMUNOMODULATORS**	**MONOCLONAL ANTI BODIES**
Anti-viralDrugs • Remdesivir (Veklury) • Paxlovid(nirmatrelvir, ritonavir) • Molnupiravir • Favipiravir **Anti-Anti-Inflammatory Drugs** • Corticosteroids • Tocilizumab • Baricitinib • Colchicine • Anakinra • Vilobelimab	• Dexamethasone • Tocilizumab (or sarilumab) • Abatacept • Infliximab	• Convalescent Plasma • Casirivimab and imdevimab • Bamlanivimab and etesevimab • Sotrovimab • Bebtelovimab

Antiviral Drugs :

Numerous antiviral drugs have been utilized or studied for the treatment of COVID-19. These include:

Remdesivir: (JOHN J FARLEY,2020)

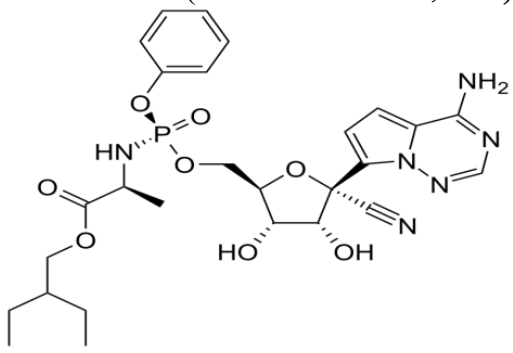
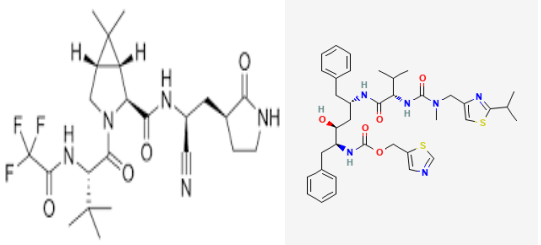


Fig. 2 : Structure of Remdesivir.

It's noteworthy that Remdesivir underwent a series of regulatory milestones since its initial authorization for inpatient use in May 2020. In October 2020, it received full FDA approval for individuals aged 12 and older. Subsequently, in January 2022, its usage was expanded to include outpatients of all ages. Additionally, in April 2022, it received full approval for specific children aged 28 days and older. Remdesivir, initially formulated for Ebola, is an antiviral drug that the FDA has granted emergency use authorization (EUA) for, in the treatment of COVID-19 among certain hospitalized patients. These regulatory decisions underscore the evolving landscape of COVID-19 therapeutics and the ongoing efforts to expand treatment options for patients of various ages and conditions.

Paxlovid (Nirmatrelvir/Ritonavir): (U.S. FDA. 2022)



Nirmatrelvir Ritonavir

Fig. 3: Structure of Nirmatrelvir and Ritonavir

Paxlovid, a combination therapy consisting of nirmatrelvir, a SARS-CoV-2 protease inhibitor, and ritonavir, a booster, was initially authorized for use in December 2021. In May 2023, the authorization for this combination therapy was further broadened to encompass full FDA approval for adults. Emergency use authorization for this treatment in individuals at high risk with mild-to-moderate COVID-19 has been granted. This approval highlights its potential as a therapeutic solution to alleviate the effects of COVID-19, particularly among vulnerable populations.

Molnupiravir (Lagevrio):(https://www. fda. gov/media/155054/download**;2023)**

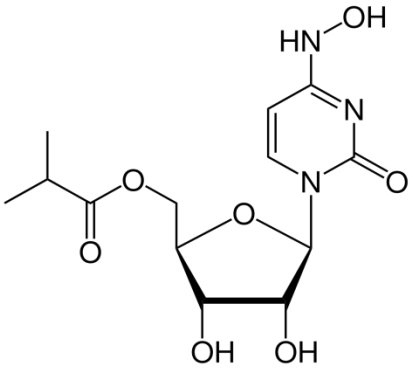


Fig. 4: Structure of Molnupiravir.

Molnupiravir was first authorized in December 2021. An oral antiviral medication, molnupiravir, has shown effectiveness against COVID-19 in clinical trials. It works by introducing errors into the genetic material of the virus, inhibiting its replication. It seems that Paxlovid and Remdesivir have shown greater effectiveness compared to other treatments for COVID-19.

Favipiravir:(Furuta Y, Gowen BB, 2013)

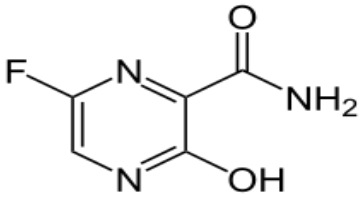


Fig. 5 : Structure of Favipiravir.

Developed and manufactured by Toyama Chemical (a subsidiary of Fujifilm), this antiviral drug obtained medical approval in Japan in 2014. In 2016, Fujifilm licensed it to Zhejiang Hisun Pharmaceutical Co. Later, it transitioned into a generic drug in 2019. Approved for influenza treatment in multiple countries, this antiviral has also undergone investigation for potential use in treating COVID-19.

Anti-Inflammatory Drugs :(RECOVERY; 16 June 2020)

Several anti-inflammatory drugs have been investigated or utilized in the management of COVID-19 to address the inflammatory response associated with the disease. These medications aim to modulate the immune system and reduce inflammation, potentially mitigating the severity of symptoms and improving patient outcomes. Some of these include:

Vilobelimab (Gohibic): Vilobelimab received initial emergency use authorization in April 2023. (Gohibic) is an injectable medication administered intravenously. Similar to tocilizumab, it belongs to the category of biologic medications. However, it targets a distinct aspect of the immune system to mitigate inflammation.

Corticosteroids :(Jonathan A. C. Sterne , Srinivas Murthy ,2020)

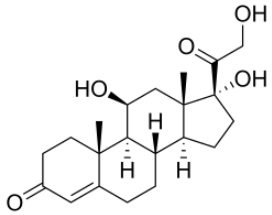


Fig. 6: Structure of Corticosteroids.

Medications like dexamethasone, prednisone, and methylprednisolone have been extensively utilized in the treatment of severe cases of COVID-19, especially in hospitalized patients requiring supplemental oxygen or mechanical ventilation. Corticosteroids help reduce inflammation and can improve outcomes in severe cases.

Tocilizumab :(Reference ID: 5097900) Tocilizumab was initially authorized for use in June 2021 and later received full approval in December 2022. This monoclonal antibody targets the interleukin-6 (IL-6) receptor, a key player in the inflammatory response. By inhibiting IL-6 signaling, tocilizumab aims to

dampen the excessive inflammation observed in severe cases of COVID-19, potentially improving patient outcomes.

Baricitinib:(Obbina Abani, Ali Abbas, Fatima Abbas;Lancet,2022)

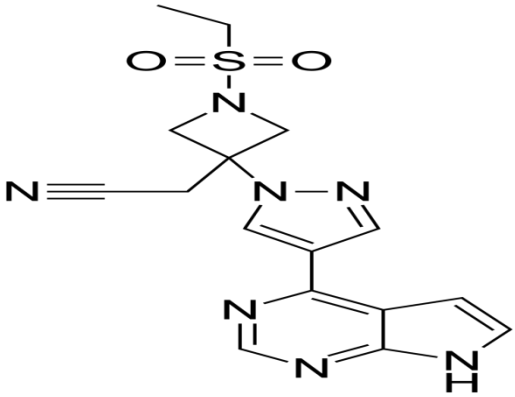


Fig. 7: Structure of Baricitinib**.**

Baricitinib was first authorized for use in November 2020 and later received full FDA approval for the treatment of COVID-19 in May 2022. This Janus kinase (JAK) inhibitor has shown promise in reducing inflammation and improving outcomes in severe cases of COVID-19 by targeting key pathways involved in the inflammatory response. Baricitinib has been utilized in combination with remdesivir in hospitalized COVID-19 patients, particularly those in need of supplemental oxygen. This combination therapy aims to address both the viral replication and the inflammatory response associated with severe cases of COVID-19, potentially improving patient outcomes.

Colchicine : (Thompson PL, Nidorf SM;2018)

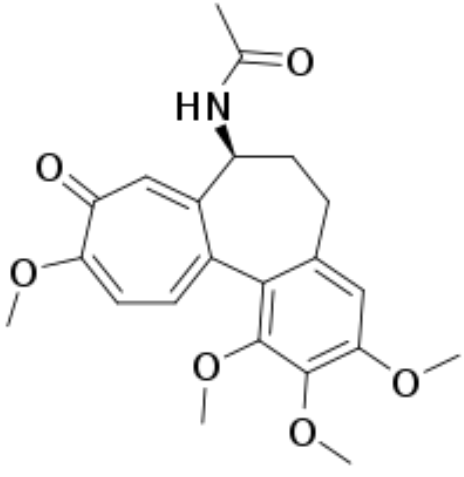


Fig. 8: Structure of Colchinine.

It was first approved on July 29, 2009, for Acute Gout, Mediterranean Fever. On October 22, 2009, the FDA approved Colcrys for the prevention of gout flares. This anti-inflammatory drug is commonly used to treat gout and other inflammatory conditions. It has been studied in COVID-19 patients and has shown potential benefits in reducing clinical deterioration and hospitalizations, particularly when used early in the disease course.

Anakinra:(Lennie P.G. Derde,2021) Anakinra received emergency use authorization in early November 2022. Anakinra is an interleukin-1 (IL-1) receptor antagonist used in the treatment of inflammatory conditions like rheumatoid arthritis. It has been investigated in COVID-19 patients to mitigate the cytokine storm associated with severe disease. It's crucial to note that although these drugs have demonstrated potential in managing inflammation associated with COVID-19, their efficacy and safety can vary, and they may not be suitable for all patients. Treatment decisions should be made on a case-by-case basis by healthcare professionals based on the patient's condition and available evidence.

Mono clonal Antibodies:

Monoclonal antibodies have been developed and utilized in COVID-19 treatment to neutralize the virus and alleviate the severity of symptoms. Some monoclonal antibodies that have been authorized or approved for emergency use in COVID-19 treatment include:

Convalescent Plasma:

Convalescent plasma, derived from individuals who have recovered from COVID-19, contains antibodies that can aid in combating the virus in those currently infected. However, there is conflicting evidence regarding its effectiveness. While some studies suggest that convalescent plasma may reduce the risk of death in specific hospitalized individuals with severe COVID-19, other research findings have not consistently supported this conclusion. However, conflicting reports indicate it may

not provide any additional benefit. According to the NIH, certain individuals with a compromised immune system may potentially benefit from it, although the supporting evidence is weak.

Casirivimab and imdevimab : (Hurt AC, Wheatley AK,2021)

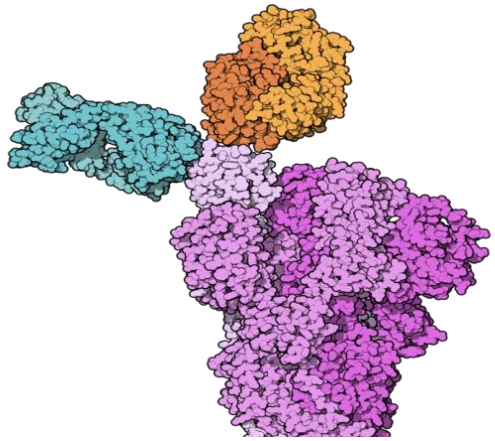


Fig. 9: REGN10933 (blue) and REGN10987 (orange) bound to SARS-CoV-2 spike protein (pink).

REGEN-COV, a combination therapy consisting of two monoclonal antibodies, casirivimab, and imdevimab, which target the spike protein of the SARS-CoV-2 virus, was initially authorized for use in November 2020. However, as of January 2022, it is no longer authorized for use in the U.S. This therapy, also referred to as REGEN-COV, had been granted emergency use authorization by the FDA for the treatment of mild to moderate COVID-19 in non-hospitalized patients at high risk of progressing to severe disease.

Bamlanivimab and Etesevimab:(Berlin DA, Gulick RM;2020) Bamlanivimab was initially granted emergency use authorization in November 2020. Subsequently, the FDA amended its EUA in February 2021 to permit its combination with etesevimab. However, as of January 2022, this combination is no longer authorized for use in the U.S. Bamlanivimab and etesevimab target distinct epitopes on the spike protein of the SARS-CoV-2 virus, which enhances their efficacy against the virus. This combination therapy had received emergency use authorization from the FDA for the treatment of mild to moderate COVID-19 in non-hospitalized patients at high risk of progressing to severe disease.

Sotrovimab :(Canada 30 july 2021). Sotrovimab was first authorized for use in May 2021; however, it is presently not authorized in any region of the U.S. This monoclonal antibody targets a conserved epitope on the spike protein of the SARS-CoV-2 virus. It initially received emergency use authorization from the FDA for the treatment of mild to moderate COVID-19 in non-hospitalized patients at high risk of progressing to severe disease, as well as in hospitalized patients with COVID-19.

Bebtelovimab :(Dani Barnhizer, Kevin Hern;2022).

Bebtelovimab was initially granted emergency use authorization in February 2022; however, it is no longer authorized for use. This monoclonal antibody targets the receptor-binding domain of the spike protein of the SARS-CoV-2 virus. While it has been authorized for emergency use by some regulatory authorities for the treatment of COVID-19, its current authorization status may vary across regions**.** These monoclonal antibodies function by binding to the spike protein of the SARS-CoV-2 virus, thereby hindering its entry into human cells and replication. They have demonstrated efficacy in reducing viral load, alleviating symptoms, and mitigating the risk of hospitalization in specific high-risk patients. However, their use may be subject to specific regulatory approvals and guidelines in different regions, and treatment decisions should be made by healthcare professionals based on individual patient factors.

Immuno Modulators:

According to the COVID-19 Treatment Guidelines Panel, for hospitalized patients with COVID-19, the choice of immunomodulators depends on the severity of the disease. Here are some of the recommended immunomodulators:

Dexamethasone: (Jonathan A C Sterne , Srinivas Murthy ,2020).

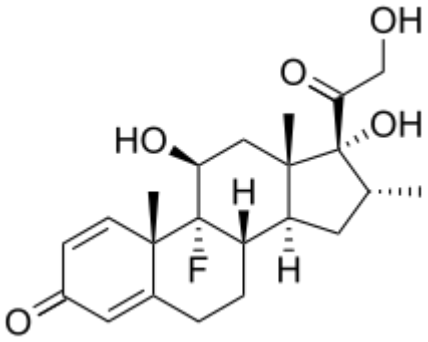


Fig. 10: Structure of Dexamethasone.

The COVID-19 Treatment Guidelines Panel recommends the use of dexamethasone, a corticosteroid, in hospitalized patients with COVID-19 who require supplemental oxygen. This recommendation is supported by a rating of B-IIa, indicating moderate certainty in the evidence supporting its efficacy and safety for this indication. Dexamethasone is considered a cornerstone of COVID-19 treatment protocols due to its ability to suppress the hyperactive immune response associated with severe disease and reduce inflammation in the lungs, potentially improving clinical outcomes.

Tocilizumab (or sarilumab):(Obbina Abani, Ali Abbas, Fatima Abbas;Lancet,2022) Tocilizumab was initially authorized for use in June 2021 and later received full approval in December 2022. This monoclonal antibody targets the interleukin-6 (IL-6) receptor, a key player in the inflammatory response. By inhibiting IL-6 signaling, tocilizumab aims to dampen the excessive inflammation observed in severe cases of COVID-19, potentially improving patient outcomes.

Baricitinib (or tofacitinib) :(Obbina Abani, Ali Abbas, Fatima Abbas; Lancet, 2022)

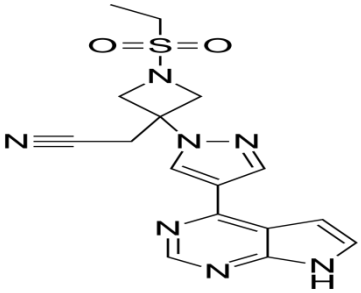


Fig. 11: Structure of Baricitinib.

Baricitinib was first authorized for use in November 2020 and later received full FDA approval for the treatment of COVID-19 in May 2022. This Janus kinase (JAK) inhibitor has shown promise in reducing inflammation and improving outcomes in severe cases of COVID-19 by targeting key pathways involved in the inflammatory response. Baricitinib has been utilized in combination with remdesivir in hospitalized COVID-19 patients, particularly those in need of supplemental oxygen. This combination therapy aims to address both the viral replication and the inflammatory response associated with severe cases of COVID-19, potentially improving patient outcomes.

Abatacept :(Reference ID: 4904773). Abatacept, a T-cell co-stimulation modulator, was first approved on December 23, 2005. It may be considered in hospitalized COVID-19 patients.

Infliximab:**(**Del Valle DM, Kim-Schulze S ;2020). On August 24, 1998, the monoclonal antibody Remicade® (infliximab; Janssen Biotech, Horsham, PA, USA) was approved by the US Food and Drug Administration (FDA) for the treatment of Crohn's disease (CD), marking the first availability of a tumor necrosis factor-alpha (TNF) inhibitor for clinical use. This approval represented a significant milestone in the management of CD, offering patients a novel therapeutic option to target the underlying inflammatory pathways associated with the disease.

Analytical Method Development:

Certainly, understanding the physicochemical properties of candidate drugs is crucial for selecting appropriate analytical methods and experimental conditions.

Molecular Weight: This indicates the mass of a molecule. It's important for determining

Log P (Partition Coefficient): This represents the lipophilicity of a compound, which influences its solubility and distribution within biological systems.

pKa: The negative logarithm of the acid dissociation constant (Ka), indicating the ionization state of the drug molecule at different pH levels. This influences drug

solubility, absorption, and distribution.

Protein Binding Percentage: The percentage of drug molecules bound to proteins in the blood plasma. This affects the concentration of free drugs available for pharmacological activity. These properties are vital for selecting appropriate analytical methods such as chromatography (e.g., HPLC, GC) and sample preparation techniques (e.g., liquid-liquid extraction, solid-phase extraction) for quantifying drug levels in biological samples. Additionally, these properties can inform pharmacokinetic studies and aid in understanding the drug's behavior within the body.

Considering the physicochemical characteristics of the candidate drugs for COVID-19 treatment, most compounds have molecular weights exceeding 300 Da and log P values below 5. Due to these factors, gas chromatography (GC) is limited, as it requires analytes to be volatile, thermo-stable, and either non-polar or semi-polar. Therefore, there are limited reports on the analysis of these drugs by GC or GC-MS. In contrast, liquid chromatography techniques offer more flexibility and are widely used for analyzing drugs in complex biological environments. Liquid chromatography allows for a wide range of stationary and mobile phases, no limitations in terms of polarity and volatility, and the use of various modes from normal to hydrophilic interaction chromatography (HILIC). Additionally, liquid chromatography methods can employ suitable temperature programs. Among the liquid chromatography techniques, high-performance liquid chromatography (HPLC) stands out as the most practical choice. HPLC methods offer expedited, effective, and eco-friendly separations. Furthermore, ultra-high performance liquid chromatography (UPLC), miniaturized liquid chromatography (capillary and nano-LC), and multidimensional liquid chromatography (MD-LC) have gained prominence for their successful applications in various fields. Therefore, based on the study, an HPLC method is selected as the most suitable analytical technique for analyzing drugs used in COVID-19 treatment due to its versatility, efficiency, and wide range of applications.

HPLC Methods:

High-performance liquid chromatography (HPLC) is indeed a widely used technique in pharmaceutical analysis, including the determination of antiviral drugs used in the treatment of COVID-19. HPLC is valued for its ability to separate, identify, and quantify components within a mixture, making it particularly useful for analyzing complex pharmaceutical formulations and biological samples. While specific methods may vary depending on the drug being analyzed, here are some general considerations and techniques used in developing HPLC methods for antiviral drugs:

Selection of Chromatographic Conditions: This includes choosing the appropriate stationary phase (reverse-phase C18 is commonly used), mobile phase (usually a mixture of water and organic solvent such as acetonitrile or methanol), and the detection wavelength based on the drug's properties.

Sample Preparation: Samples may need to be prepared prior to analysis to remove interfering substances and to ensure accurate quantification. This could involve techniques such as filtration, dilution, centrifugation, or extraction.

Column Selection: The column used in HPLC plays a crucial role in separating the components of the sample. The choice of column dimensions, particle size, and chemistry can affect resolution, sensitivity, and analysis time.

Optimization of Parameters: Parameters such as flow rate, column temperature, and injection volume need to be optimized to achieve the best separation and sensitivity for the target analytes.

Detection Method: UV-Vis detection is commonly used in HPLC analysis. However, for some drugs, especially those with low UV absorbance, other detection methods such as mass spectrometry (MS) may be necessary for higher sensitivity and selectivity.

Validation: HPLC methods need to be validated to ensure accuracy, precision,

specificity, and robustness. This involves testing parameters such as linearity, range, accuracy, precision, and specificity according to regulatory guidelines.

Quantification: Calibration curves are often used for quantification by plotting peak area or height against analyte concentration. Standard solutions of known concentrations are used to generate these curves.

Forced Degradation Studies: In the case of stability-indicating methods, forced degradation studies may be conducted to identify and characterize degradation products and demonstrate the specificity of the method.

Documentation and Reporting: Proper documentation of method development, validation, and results is essential for regulatory compliance and scientific integrity.

Compliance with Regulatory Guidelines: HPLC methods used for analyzing antiviral drugs for COVID-19 treatment need to comply with regulatory guidelines set by organizations such as the FDA (Food and Drug Administration) or EMA (European Medicines Agency).

For anti-viral Drugs :

It's important to highlight that tailored HPLC methods for each antiviral drug employed in COVID-19 treatment would need to be developed and validated based on the chemical properties of the drug and the requirements of the regulatory authorities. These methods are often published in scientific journals or provided by regulatory agencies.

There are several antiviral drugs have been used or investigated for the treatment of COVID-19.

Here are some examples along with brief descriptions of their HPLC methods for analysis:

Remdesivir:

Description: Remdesivir is a broad-spectrum antiviral medication initially developed for Ebola virus disease.

HPLC Method: **(**Padhye Hemangi, Sonawane B;2022, Valeria Avataneo , Amedeo de Nicolò;2020)HPLC methods for the analysis of remdesivir typically involve reverse-phase chromatography using C18 columns and a mobile phase Ammonium acetate buffer pH (4.6) and acetonitrile (60:40) %v/v and it is also performed by using a mixture of buffer and organic solvents such as acetonitrile or methanol. Detection is often performed using UV-Vis detection at wavelengths around 260 nm.

Favipiravir:

Description: Favipiravir is a broad-spectrum antiviral medication that has shown some efficacy against RNA viruses.

HPLC Method: (13)(Furuta Y, Gowen BB;2013) Reverse-phase chromatography using a C18 column and a mobile phase comprising a buffer and organic solvent mixture is commonly used in HPLC analysis of favipiravir. Quantification frequently uses UV detection.

Paxlovid :(Nirmatrelvir/Ritonavir):

Description: Nirmatrelvir/ritonavir is a combination drug utilized in the management of mild to moderate COVID-19

HPLCMethod:(https://www.ema.europa.eu/en/medicines/human/EPAR/paxlovid;2022)

HPLC methods for nirmatrelvir and ritonavir analysis involve reverse-phase chromatography with C18 columns. The mobile phase can be made up of ethanol, buffer, and organic solvent mixed with water; UV-Vis is used for detection.

Molnupiravir:

Description: In preclinical trials, molnupiravir has shown broad-spectrum antiviral effectiveness against a number of RNA viruses, including coronaviruses like SARS-CoV-2. It functions by inducing mistakes in the replication of viral RNA, leading to the introduction of mutations in the viral genome and ultimately inhibiting viral replication.

HPLC Method: (Reçber T, Timur SS;2022) Molnupiravir analysis using HPLC methods involves reverse-phase chromatography using a C18 column. The mobile phase, which is detectable by UV-Vis detection, may be composed of methanol, water, and buffer containing 0.1% OPA and acetonitrile.

RESULTS AND DISCUSSION

Table 2: RP-HPLC Methods for antiviral drugs used for covid-19**.**

Drug	Method	Retention Time	Linearity Range
Remdesivir	1. The Inert Sustain C18 (4.6 mm x 100 mm, 3 µm) column is utilized to achieve the separation, and the mobile phase consists of acetonitrile (60:40) %v/v and ammonium acetate buffer pH (4.6). 2. The separation is achieved by using C18 RP (250mm ×4.6mm,5µm) as a column. Phosphate (0.02M) buffer and Acetonitrile 48:52 with pH 2.80 are used as a mobile phase. 3. The C18 (250mm x 4.6mm, 5µm) column and buffer with a pH of 5.0 and acetonitrile (30:70) as the mobile phase are employed to perform the separation.	7.3 min 3.665 min 4.402 min	1. Range is between 5-100µg/ml. The regression coefficient is R^2=0.9996 2. Range is between 30-70µg/ml. Regression coefficient is R^2=0.999 3. Range is between 10-30µg/ml. Regression coefficient is R^2=0.9998
Paxlovid (Nirmatrelvir) and (Ritonavir)	1. Several columns, including the C18, C8, and Cyano columns, are used to create the separation. According to the study, HPLC analysis performed better using the C18 column. A chosen column was subjected to isocratic elution using a mobile phase. ethanol Water v/v (80:20) 2. The Zorbax XBD -C18 2.1×50mm analytical column, which had a particle size of 3.5µm, was safeguarded by a security guard system that had a 4mm×2mm C -18 filter insert. Gradient elution was used to separate the analytes, with 90% aqueous buffer and 10% acetonitrile.	Nirmatrelvir-4.9 min. Ritonavir-6.8min Nirmatrelvir - 8.2 min Ritonavir -9.2 min	1. Nirmatrelvir range is between 1.0-20.0µg/ml and regression coefficient is R^2=0.9999 Ritonavir range is between 1.0-20.0µg/ml and regression coefficient is R^2=0.9998 2.Nirmatrelvir range is between 10-10000ng/ml and regression coefficient is R^2=0.9995 Ritonavir range is between 2-2000ng/ml and the regression coefficient is R^2=0.9996
Favipiravir	The C 18 column and ammonium acetate buffer (pH 6.5) with methanol as the mobile phase were utilized to achieve the separation.	2.65 min	Favipiravir range of 20.0–60.0 µg/mL with regression coefficient (r2) = 0.9999.
Molnupiravir	1. The separation was accomplished with a column made of stain-free steel Lichrosphere 100 RP-C18 (250 mm x 4 mm, 5 µm) and a mobile phase of methanol and water (65:35%) v/v. 2. Using a C18 column and acetonitrile-triple-distilled water (28:72%) v/v as the mobile phase, the separation was accomplished. 3. Utilizing a Kromasil 100-5-C18 (150 mm x 4.6 mm, 5 µm) column and a buffer solution containing 0.1% ortho phosphoric acid and aceto nitrile in a ratio of 85:15 % v/v, the separation was accomplished.	5 min 4.2 min 10 min	1.Molnupiravir range is between 0.1-60µg/ml and regression coefficient R^2=0.9999 2.Molnupiravir range is between 10-70µg/ml and the regression coefficient is R^2=0.9993 3.Molnupiravir range is between 50-150µg/ml and regression coefficient is R^2=0.999

CONCLUSION:

While the pandemic continues to spread worldwide, there is an immediate necessity to comprehend the advantages and drawbacks of each treatment. The current review is based on the overview of COVID-

19 drugs that are effectively used for the treatment. The review also focused on various RP-HPLC Methods for the determination of anti-viral drugs such as Remdesivir, Paxlovid, Molnupiravir, Favipiravir and the chromatographic conditions which were adopted based on their ionic interactions and physicochemical properties the detection wavelength, buffer pH ratio of organic solvent, flow rate and elution time were studied. Linearity, Specificity, Accuracy, Precision, LOD, and LOQ were researched as validation metrics. The selected few analytical methods that were reported in the article were based on the usage of different columns such as C 8, C18 and cyano columns studied the review method was focused on the different modes of elutions such as isocratic as well as gradient modes. The drugs such as Nirmatrelvir and ritonavir were recently approved and recommended and these drugs belong to anti-viral agents hence there is a need to study the drugs coming under the category such as Remdesivir, Molnupiravir, Favipiravir. Based on this review the objective is to develop RP-HPLC Methods for these selected drugs using solvents that are eco-friendly and optimize the methods based on DOE and factorial designs which can be an economical and reliable approach.

Future Prospective:

The author's aim is to evaluate the chromatographic conditions and optimize the method based on system suitability further the author is interested in focusing on the ionic interactions and physicochemical properties of recently approved drugs for their estimation using the mobile phase which is eco friendly and adoption of DOE to minimize time and expenditure.

List of Abbreviations:

LOD: stands for Limit of Detection.

RP-HPLC: stands for Reverse Phase High Performance Liquid Chromatography.

LOQ: stands for Limit of Quantitation

DOE: stands for Design of Experiment

US FDA: stands for United States Food Drug Administration

CTAP: stands for Coronavirus Treatment Acceleration Program

SPRP: stands for Strategic Preparedness and Response Plan

Declarations:

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: The authors declare that they have no competing interests.

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