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A REVIEW ON ANALYTICAL METHOD OF DRUGS USED FOR PANDEMIC COVID-19 TREATMENT

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ABSTRACT

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. Drugs such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs) including tocilizumab, have been used to treat autoimmune conditions used in the treatment of covid-19. **Remdesivir** is a nucleoside analog used to treat RNA virus infections including COVID-19 and is validated using the LC-MS method Favipiravir is an antiviral used to manage influenza, and that has the potential to target other viral infections and favipiravir was validated using the HPLC-UV method. Azithromycin is a macrolide antibiotic used to treat a variety of bacterial infections. Method development and validation for azithromycin were performed using the HPLC method. Dexamethasone is a glucocorticoid available in various modes of administration that is used for the treatment of various inflammatory conditions, including bronchial asthma, as well as endocrine and rheumatic disorders. Dexamethasone was validated using the RP-HPLC method. Hydroxychloroquine is an antimalarial drug used to treat uncomplicated malaria patients and it was validated using the HPLC method. Omicron is a new variant in covid-19, the rise of the alpha, beta, and delta SARS-CoV-2 VOCs were related to new waves of contaminations, once in a while across worldwide.

Keywords: Covid-19 drugs, Remdesivir, Favipiravir, LC-MS, HPLC, variants of Covid 19

INTRODUCTION:

HISTORY:

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. SARS-CoV was transmitted to humans from exotic animals in wet markets. Most people infected with the virus will experience mild to moderate respiratory illness and recover without requiring special treatment. However, some will become seriously ill and require medical attention. The coronavirus disease-2019 (COVID-19) outbreak all over the world has led researchers to strive to develop drugs or vaccines to prevent or halt the progression of this ailment. To hasten the treatment process, repurposed drugs are being evaluated **[1-2]**.

Since the first few patients with COVID-19 were reported in December 2019, it had rapidly spread all over the world. Till June 26, 2020, COVID-19 broke out in 216 countries, with more than 9 million confirmed cases **[3]**. The most common symptoms of COVID-19 are coughing, and breathing problems.

COVID-19 TREATMENT:

- If you're on angiotensin-converting enzymes (ACE) inhibitors, angiotensin receptor blockers (ARBs), or statins for another reason, your doctor will advise you to keep taking them.
- The FDA has given monoclonal antibodies an emergency use authorization (EUA) to treat COVID-19. High-risk patients who have recently been diagnosed with mild to moderate disease can be administered sotrovimab or a combination of casirivimab and imdevimab (REGEN-COV) to lower viral levels in their bodies and reduce the chance of hospitalizations. EUA has also been awarded to REGEN-COV for preventative treatment in high-risk individuals who have been exposed to COVID-19 **[3]**.
- Several clinical trials are currently underway to investigate and develop therapies for COVID-19 that have been used to treat other diseases. The FDA has also approved an EAU for blood plasma from COVID-19 survivors to aid patients with severe or life-threatening cases.
- Other drugs, such as tocilizumab, which has been used to treat autoimmune diseases and an inflammatory condition is known as cytokine release syndrome, are currently being tested in clinical studies **[4]**.

• The FDA has revoked an emergency authorization for the use of hydroxychloroquine and chloroquine to treat COVID-19 patients who are hospitalized, citing major concerns about their safety and effectiveness against the virus. The drugs have been approved to treat malaria as well as autoimmune diseases such as rheumatoid arthritis and lupus.

• Dexamethasone, a popular steroid medicine, was reported to aid persons who were hospitalized with severe asthma in one trial.

• If you test positive for COVID-19 and want to participate in a clinical trial for treatment, you should do so as soon as possible, while the virus is still active **[4]**.

ANALYTICAL METHOD FOR COVID-19 DRUGS:

REMDESIVIR:

- **Remdesivir** is a nucleoside analog used to treat RNA virus infections including COVID-19. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19), which is a respiratory disease that is capable of progressing to viral pneumonia and acute respiratory distress syndrome (ARDS); COVID-19 can be fatal.
- Remdesivir (GS-5734) is an adenosine triphosphate analog that was first identified as a potential Ebola treatment in the literature in 2016. The National Institute of Allergy and Infectious Diseases in the United States revealed on April 29, 2020, that remdesivir was superior to placebo in shortening recovery time in hospitalized patients with advanced COVID-19 and lung involvement, based on the Adaptive COVID-19 Treatment Trial **[5]**.
- Remdesivir is now being studied as a COVID-19 specific medication, and it has been approved for emergency use in persons with severe symptoms in the United States **[6]**.

Remdesivir has been tested in various COVID-19 clinical trials and has been confirmed as a non-obligate chain terminator of RdRp from SARS-CoV-2 and related SARS-CoV and MERS-CoV.

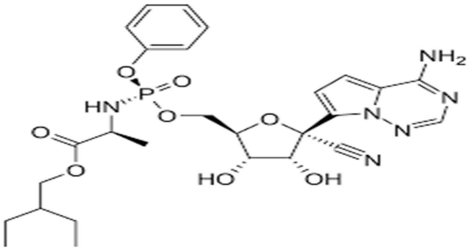


Figure 1: Structure of Remdesivir

Mechanism of action:

COVID-19 is caused by coronavirus 2, a positive-sense RNA virus that causes severe acute respiratory syndrome (SARS-CoV-2). Replication of the viral genome is

a crucial phase in the infectious cycle of RNA viruses including the Filoviridae, Paramyxoviridae, Pneumoviridae, and Coronaviridae, and is carried out by viral RNA-dependent RNA polymerase (RdRp) enzymes or enzyme complexes. Remdesivir is a phosphoramidite prodrug of a 1'-cyano-substituted adenosine nucleotide analo that competes with ATP for inclusion by the matching RdRp complex into newly generated viral RNA. Remdesivir penetrates cells before being cleaved to its monophosphate form by carboxylesterase 1 or cathepsin A and then phosphorylated by undescribed kinases to produce its active triphosphate form, remdesivirg triphosphate (RDV-TP or GS-443902) **[7-8]**. The SARS-CoV-2 RdRp complex efficiently incorporates RDV-TP, with a 3.65-fold selectivity for RDV-TP over endogenous ATP. SARS-CoV, SARS-CoV-2, and MERS-CoV all have the same mechanism, and genetic analysis confirmed it. Genomic comparisons reveal that Ser-861 is conserved across alpha-, beta-, and deltacoronaviruses, suggesting remdesivir may possess broad antiviral activity **[9]**.

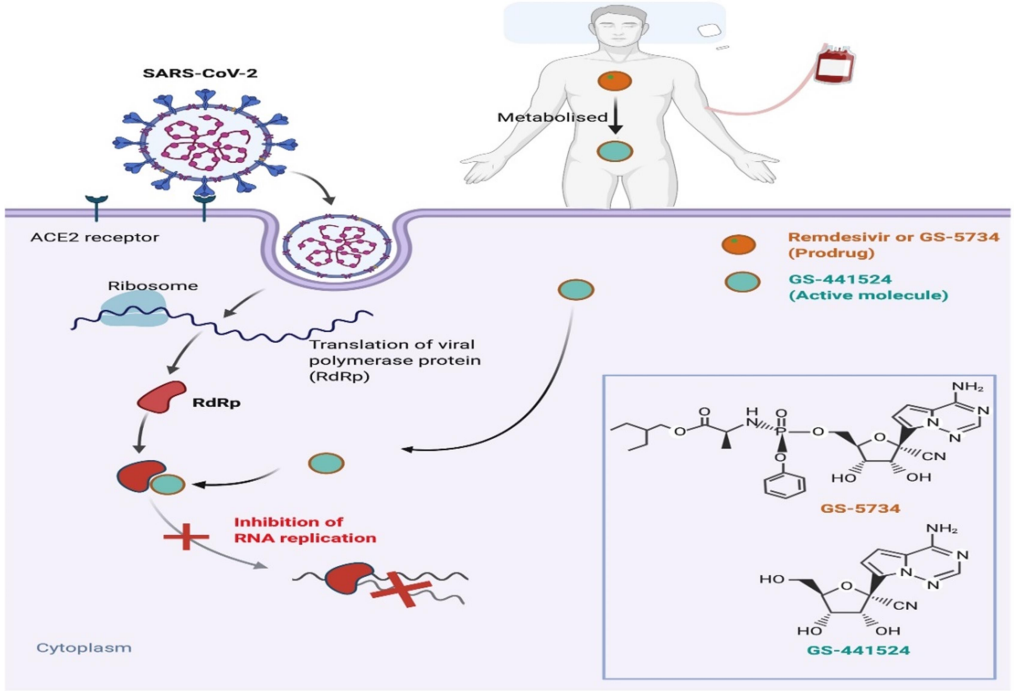


Figure 2: Mechanism of action of Remdesivir [10]

Validation of Remdesivir Using LC-MS Method:

- LC-MS/MS techniques were approved for assurance of the 3 analytes in human plasma that elaborate two vital angles to ensure their accuracy, exactness, and strength. To begin with, the precariousness issues of the analytes were overwhelmed by weakened

formic corrosive (FA) treatment of the plasma tests. Furthermore, a different infusion for each analyte was performed with various ESI modes and natural angles to accomplish affectability and limit vestige **[11]**.

- Chromatographic partition was accomplished on an Acquity UPLC HSS T3 section (2.1 50 mm, 1.8 m) with a run season of 3.4 min. The adjustment ranges were 44000, 22000, and 22000 ng/mL, individually for RDV, GS-441524, and GS-704277. The intraday and interday accuracy (%CV) across approval run at 3 QC levels for each of the 3 analytes were under 6.6%, and the exactness was inside 11.5%. The drawn-out capacity soundness in FA-treated plasma was set up to be 392, 392, and 257 days at 70 C, separately for RDV, GS-441524, and GS-704277. The approved strategy was effectively applied in COVID-19 related clinical investigations **[11]**.

Favipiravir:

Favipiravir is an antiviral used to manage influenza, and that has the potential to target other viral infections. Discovered by Toyama Chemical Co., Ltd. in Japan, favipiravir is a modified pyrazine analog that was initially approved for therapeutic use in resistant cases of influenza. The antiviral targets RNA-dependent RNA polymerase (RdRp) enzymes, which are necessary for the transcription and replication of viral genomes.

Not only does favipiravir inhibit replication of influenza A and B, but the drug has shown promise in the treatment of avian influenza, and maybe an alternative option for influenza strains that are resistant to neuraminidase inhibitors. Favipiravir has been investigated for the treatment of life-threatening pathogens such as the Ebola virus, Lassa virus, and now COVID-19 **[11]**.

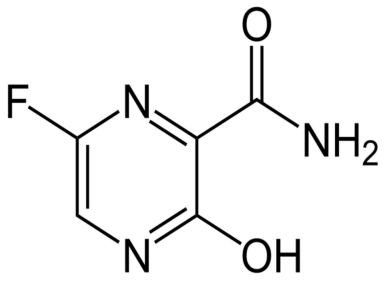


Figure 3: Structure of Favipiravir

Mechanism of action:

- The mechanism of action of favipiravir is novel compared to existing influenza antivirals that primarily prevent the entry and exit of the virus from cells. The active favipiravir-RTP selectively inhibits RNA polymerase and prevents replication of the viral genome.
- There are several hypotheses as to how favipiravir-RTP interacts with RNA-dependent RNA polymerase

(RdRp). Some studies have shown that when favipiravir-RTP is incorporated into a nascent RNA strand, it prevents RNA strand elongation and viral proliferation.

- Studies have also found that the presence of purine analogs can reduce favipiravir's antiviral activity, suggesting competition between favipiravir-RTP and purine nucleosides for RdRp binding.
- Although favipiravir was originally developed to treat influenza, the RdRp catalytic domain (favipiravir's primary target), is expected to be similar for other RNA viruses. This conserved RdRp catalytic domain contributes to favipiravir's broad-spectrum coverage.

Validation of Favipiravir Using HPLC-UV Method:

Favipiravir (FVP), a pyrazine simple, has shown antiviral movement against a wide assortment of infections. It is viewed as worth further examination as a potential up-and-comer drug for COVID-19. It isn't authoritatively accessible in any pharmacopeia. A quick, straightforward, exact, precise, and isocratic elite execution liquid chromatography (HPLC) technique has been created for routine quality control of favipiravir in drug details **[12]**.

Partition was done by the C18 segment. The versatile stage was a combination of 50 mM potassium dihydrogen phosphate (pH 2.3) and acetonitrile (90:10, v/v) at a stream pace of 1 mL min1. The bright (UV) discovery and segment temperature were 323 nm, and 30 8C, separately. The run time was 15 min under these chromatographic conditions.

An incredible direct connection between the top region and favipiravir fixation in the scope of 10100 mg mL1 has been noticed (r2, 0.9999). The created technique has been viewed as delicate (restrictions of location and quantification were 1.20 mg mL 1 and 3.60 mg mL 1, separately), exact (the interday and intraday relative standard deviation (RSD) values for the top region and maintenance time were under 0.4 and 0.2%, individually), precise (recuperation, 99.19100.17%), specific and vigorous (% RSD were under 1.00, for framework reasonableness boundaries). The proposed technique has been effectively applied for the quantification of favipiravir in drug plans **[13]**.

Antibiotics:

Azithromycin:

Azithromycin is a macrolide antibiotic used to treat a variety of bacterial infections. Azithromycin is a broad-spectrum macrolide antibiotic with a long half-life and a high degree of tissue penetration. It was initially approved by the FDA in 1991.

It is primarily used for the treatment of respiratory, enteric, and genitourinary

infections and may be used instead of other macrolides for some sexually transmitted and enteric infections. It is structurally related to erythromycin.

Azithromycin [9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin] is a part of the *azalide* subclass of macrolides, and contains a 15-membered ring, with methyl-substituted nitrogen instead of a carbonyl group at the 9a position on the aglycone ring, which allows for the prevention of its metabolism. This differentiates azithromycin from other types of macrolides.

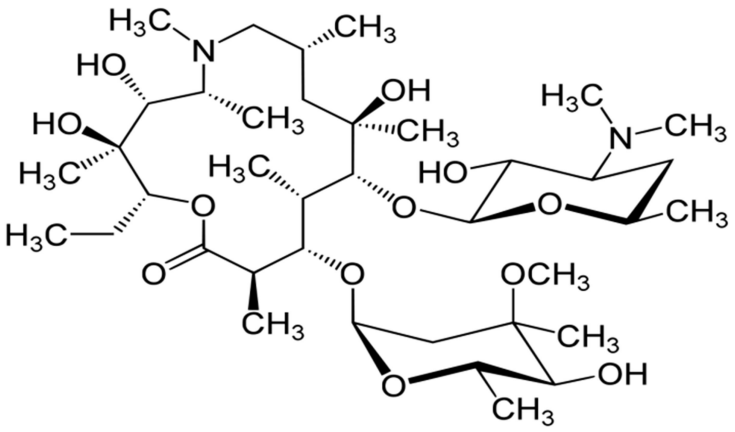


Figure 4: Structure of Azithromycin

Azithromycin

Mechanism of action:

To replicate, bacteria require a specific process of protein synthesis, enabled by ribosomal proteins. Azithromycin binds to the 23S rRNA of the bacterial 50S ribosomal subunit. It stops bacterial protein synthesis by inhibiting the transpeptidation/translocation step of protein synthesis and by inhibiting the assembly of the 50S ribosomal subunit.

This results in the control of various bacterial infections. The strong affinity of macrolides, including azithromycin, for bacterial ribosomes, is consistent with their broad-spectrum antibacterial activities.

Azithromycin is highly stable at a low pH, giving it a longer serum half-life and increasing its concentrations in tissues compared to erythromycin **[14]**.

Method development and validation for azithromycin using HPLC method:

Azithromycin is a semi-manufactured, azalide congener of erythromycin demonstrated in the treatment of respiratory parcel contaminations. Different techniques are accessible for assurance of Azithromycin, yet HPLC is the most adaptable one.

Objective: the current review depends on the turn of events also, approval of a quick, straightforward elite presentation liquid chromatography (HPLC) technique

furnished with a UV locator for quantitative examination of Azithromycin (AZN) in suspension. Material and techniques: The Method was performed by utilizing Hypersil BDS-C18 (250 mm 4.6 mm i.d.) segment MS-II, with an isocratic portable period of methanol, acetonitrile, and phosphate cradle pH 8 (60:30:10; v/v) with run time 15 minutes. The judgments were performed at a stream pace of 1.0ml/min, and the UV indicator was set at 212 nm.

The technique was viewed as explicit with a relative standard deviation (RSD) under 2.09%. The technique showed exactness with RSD under 1.34% and accuracy in repeatability with RSD under 1.42%. The technique was viewed as straight over a wide scope of fixation from 1.0 to 50.0 g/mL (R2 =995). Cutoff of identification and breaking point of measurement was viewed as 14.40 ng/mL and 43.66 ng/mL individually.

It was profitable to utilize UV locator over different techniques utilizing electrochemical, photodiode cluster, and so forth as the finder, given modest and simple accessibility. The created technique satisfied all approval boundaries according to ICH and can be effectively applied to measure percent drug content in showcased oral AZN suspension **[15]**.

Steroids:

Dexamethasone:

Dexamethasone is a glucocorticoid that is used to treat a variety of inflammatory illnesses, including bronchial asthma, as well as endocrine and rheumatic disorders. It is available in a variety of dosage forms. Dexamethasone, also known as MK-125, is a fluorinated corticosteroid used to treat a variety of disorders including endocrine, rheumatic, collagen, dermatologic, allergy, ophthalmic, gastrointestinal, pulmonary, hematologic, neoplastic, edematous, and others. It is physically related to other corticosteroids such as hydrocortisone and prednisolone and was developed in 1957.

On October 30, 1958, the FDA approved dexamethasone. Dexamethasone was suggested for use in COVID-19 patients with severe respiratory symptoms in a press release for the Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial on June 16, 2020. Dexamethasone reduced fatalities in patients who needed ventilation by about a third and by about a fifth in those who needed oxygen **[16]**.

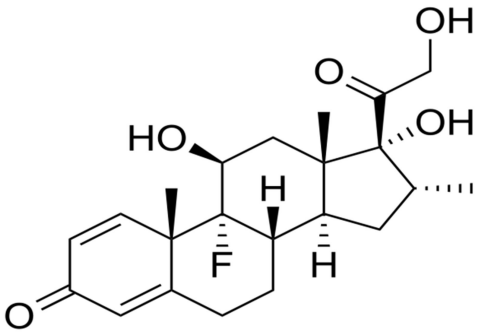


Figure 5: Structure of Dexamethasone

Mechanism of Action:

The short-term effects of corticosteroids are decreased vasodilation and permeability of capillaries, as well as decreased leukocyte migration to sites of inflammation. Corticosteroids binding to the glucocorticoid receptor mediates changes in gene expression that lead to multiple downstream effects over hours to days.

Glucocorticoids inhibit neutrophil apoptosis and demargination; they inhibit phospholipase A2, which decreases the formation of arachidonic acid derivatives; they inhibit NF-Kappa B and other inflammatory transcription factors; they promote anti-inflammatory genes like interleukin-10.

Lower doses of corticosteroids provide an anti-inflammatory effect, while higher doses are immunosuppressive. High doses of glucocorticoids for an extended period bind to the mineralocorticoid receptor, raising sodium levels and decreasing potassium levels **[17]**.

Validation for Dexamethasone Using RP-HPLC Method Development:

A new, basic, solid, and reproducible steadiness demonstrating RP-HPLC examine technique has been created for quantitative investigation of dexamethasone from dexamethasone tablets. This created technique has been approved by the ICH rule concerning framework reasonableness, particularity, accuracy, linearity, exactness, and vigor.

An isocratic state of versatile stage water (0.1% orthophosphoric acid): acetonitrile in a proportion of 60:40, v/v at a stream pace of 1.0 mL/minute over RP 2.5 Fortis C18, 100 4.6 mm, 2.5 m, the section was at 27 C kept up with. This technique is explicit and showed astounding direct reaction with relationship coefficient (R2) upsides of 0.999.

In constrained corruption, the proposed strategy has been researched with various pressure conditions as hydrolytic, oxidative, warm, and sticky as suggested by ICH rules. A precise and dependable switched stage HPLC method for the examination of dexamethasone in dexamethasone tablets was created and approved effectively **[18]**.

HYDROXYCHLOROQUINE:

Hydroxychloroquine is an antimalarial drug used to treat uncomplicated malaria patients and for chemoprophylaxis in some areas. Rheumatoid arthritis and lupus erythematosus are both treated with this disease-modifying anti-rheumatic medication (DMARD). In areas where chloroquine resistance is improbable, hydroxychloroquine is also used to prevent malaria. It was created as a quinacrine derivative with fewer adverse effects during World War II. Both chloroquine and

hydroxychloroquine are being studied as potential treatments for SARS-CoV-2.

Mechanism of action

Hydrochloroquine's exact processes are unknown. Hydroxychloroquine has been demonstrated to accumulate in the lysosomes of the malaria parasite, raising the pH of the vacuole. This function prevents the parasite from being able to proteolyze hemoglobin, inhibiting normal parasite development and reproduction. Hydroxychloroquine can also block the action of parasite heme polymerase, allowing the hazardous result beta-hematin to accumulate.

Hydroxychloroquine raises the pH of human organelles, inhibiting antigen processing, preventing the alpha and beta chains of the major histocompatibility complex (MHC) class II from dimerizing, inhibiting cell antigen presentation, and lowering the inflammatory response. The recycling of MHC complexes may be altered by elevated pH in the vesicles, resulting in only the high-affinity complexes being presented on the cell surface.

Self-peptides bind to MHC complexes with low affinity and so they will be less likely to be presented to autoimmune T cells. Hydroxychloroquine also reduces the release of cytokines like interleukin-1 and tumor necrosis factor, possibly through inhibition of Toll-like receptors.

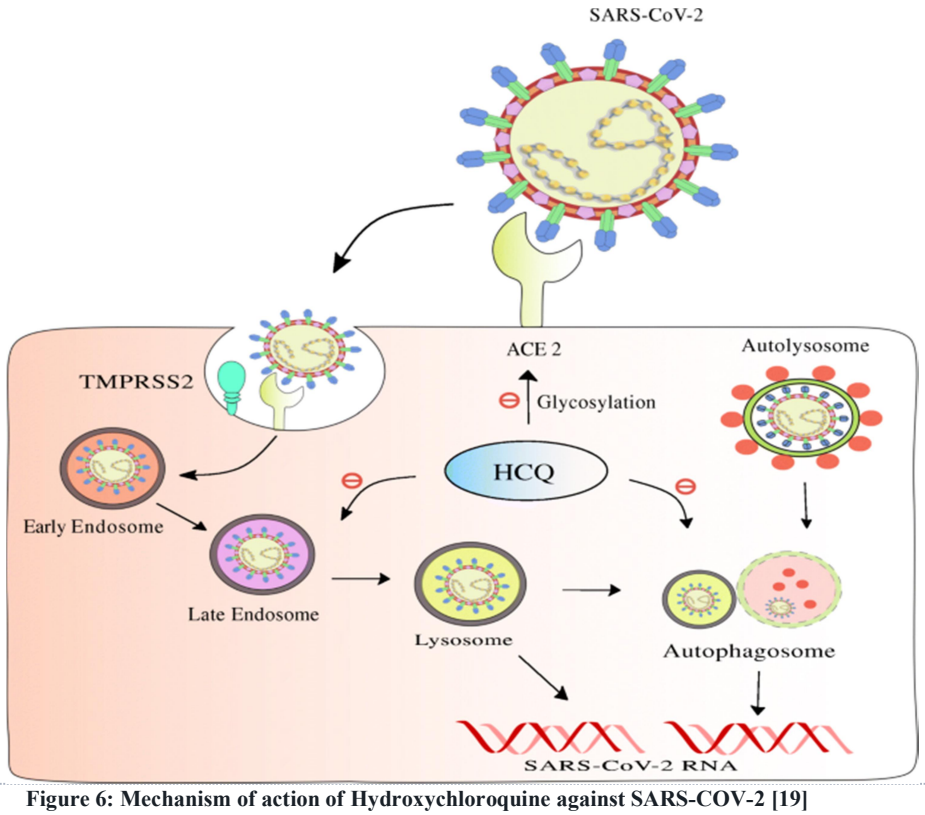


Figure 6: Mechanism of action of Hydroxychloroquine against SARS-COV-2 [19]

Development and Validation of Hydroxychloroquine Using High-Performance Liquid Chromatography:

Therapeutic drug checking of a high portion or long haul utilization of hydroxychloroquine is required. (is concentrate on plans to get an idea and approved investigation and arrangement strategy for hydroxychloroquine in volumetric absorptive microsampling (VAMS) utilizing the superior exhibition liquid chromatography–photodiode cluster identifier dependent on the Food and Drug Administration rules. Hydroxychloroquine evaluation was performed utilizing HPLC-PDA with Waters Sunfire™ C18 (5 µm; 250 × 4,6 mm) section. The mobile phase consists of acetonitrile-diethylamine 1%(65: 35, v/v) (isocratic elution) and conveyed at a stream pace of 0.8 mL/min all through the 12 minutes run. Test in VAMS is removed by fluid extraction with alkali 1% and n-hexane-ethyl acetic acid derivation (50: 50 v/v) as an extraction dissolvable. (is strategy has effectively qualified the Food and Drug Administration (2018) boundaries, with 2 ng/mL of LLOQ, the scope of adjustment bend 2–6500 ng/mL, and coefficient of relationship 0.9993–0.9997 **[20]**.

Development and Validation of an LC/MS/MS Method for Hydroxychloroquine And Azithromycin:

Hydroxychloroquine (HCQ) and azithromycin (AZM) are antimalarial medicates as of late detailed to be dynamic against extreme intense respiratory condition Covid 2 (SARS-CoV-2), which is causing the worldwide COVID-19 pandemic. In a crisis reaction to the pandemic, we expected to foster a quantitation technique for HCQ and AZM in human plasma. Liquid chromatography-mass spectrometry was utilized to foster the strategy. Samples (20 µL) are extracted by solid-phase extraction and infused onto the LC-MS/MS system outfitted with a PFP segment (2.0 × 50 mm, 3 µm). ESI+ and MRM are utilized for identification analyte **[21]**.

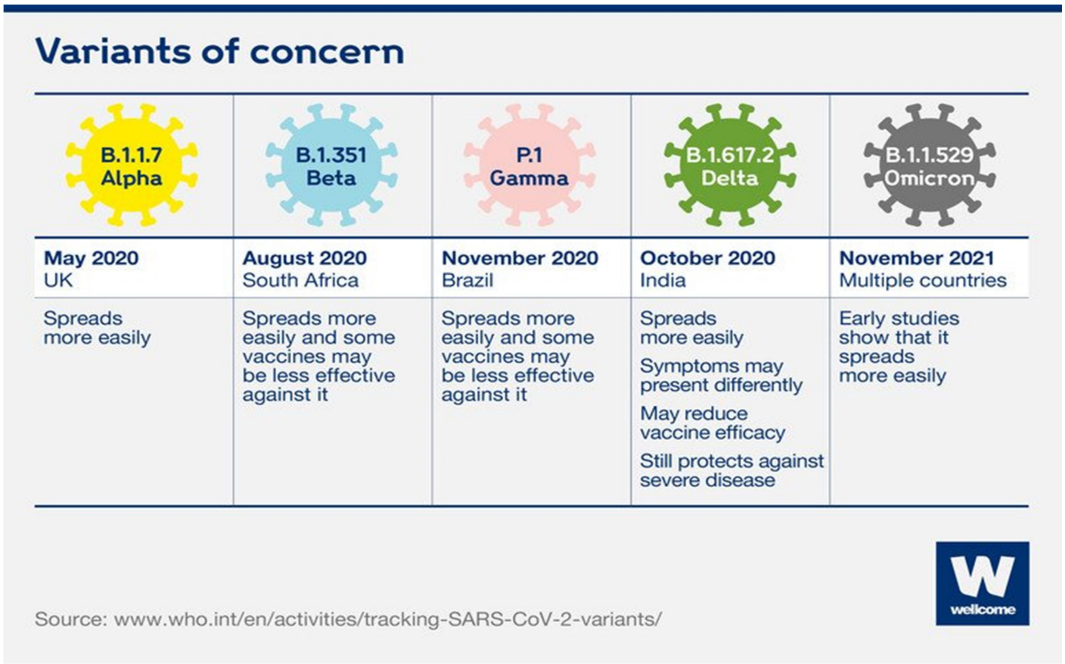
Omicron SARS-CoV-2 variant: a new chapter in the COVID-19

On Nov 25, 2021, around 23 months since the first detailed instance of Coronavirus and later a worldwide assessed 260 million cases and 5·2 million deaths, a new SARS-CoV-2 variation of concern (VoC), omicron was detailed. Omicron arose in a Coronavirus-tired world in which outrage and disappointment with the pandemic are overflowing in the midst of boundless adverse consequences on friendly, mental, and financial prosperity **[22]**.

The rise of the alpha, beta, and delta SARS-CoV-2 VOCs were related to new waves of contaminations, once in a while across the whole world **[23]**. For model, the

expanded contagiousness of the delta VoC was related with, among others, a higher viral load, the longer length of infectiousness, and high rate of reinfection, in light of its capacity to escape from regular immunity **[24]**, which brought about the delta VoC quickly turning into the around the world predominant variation. The delta VoC keeps on driving new influxes of disease also stays the predominant VOC during the fourth wave in numerous nations.

Worries about lower antibody adequacy given new variations have changed our comprehension of the Coronavirus endgame, clarifying the universe of the thought that worldwide inoculation is by itself satisfactory for controlling SARS-CoV-2 contamination **[25]**.



Variants of covid-19[26]

- **Alpha** (also known as B.1.1.7 or 20I/501Y.V1, and first discovered in the UK) has several mutations, including three on the spike protein (the protein used to enter our bodies cells). Increased transmissibility is linked to this variation.
- **'Beta'** (formerly known as B.1.351 or 501Y.V2, first identified in South Africa)has the same alterations as Alpha (increased transmissibility) and two additional mutations that make antibodies bind to and destroy it more difficult.
- **Gamma** is a type of gamma that (formerly known as P1, first identified in a group of travelers from Brazil, who were tested at an

airport in Japan) has mutations that are similar to the B.1.351 variation.

- **Delta** is a Greek word that means " (formerly known as B.1.617, first identified in India) According to preliminary research, the Delta mutation spreads more quickly than other variants, necessitating further investigation.
- **Omicron** is the smallest unit of measurement in the universe (formerly known as B.1.1.529, first identified in South Africa). There are numerous mutations in this variation, some of which are problematic. In comparison to other variants of concern, preliminary evidence suggests that this variant has a higher risk of reinfection **[26]**.

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Conflict of Interest

The authors declare that No conflict of interest for this study.

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