Title: Analytical method development and validation for Assay, Related Compound and degradants of Acyclovir and Valacyclovir antiviral drug products.

1. INTRODUCTION:

Development of analytical methods for bulk drug and their formulations is an important aspect in the drug product development as it helps to maintain the quality and efficacy of the drug product right from the product development process till its ultimate therapeutic use. Quality can be defined as the character, which defines the grade of excellence. The quality drug is something, which will meet the established product specifications, can be safely bought and confidently used for the purpose for which it is intended. There is no fear of adulteration or unpredictable side effects with such a quality drug. Quality is important in every aspect of life and when it comes to life it is crucial. Quality issues such as the studies of impurity, stability, degradation and analysis of drug product would be the research work to stress upon. These demands analytical development and standardization of sensitive and specific instrumental methods for testing of simultaneous, product study and analysis of drug product. Quality assurance plays a central role in determining the safety and efficacy of medicines. Highly specific and sensitive analytical technique holds the key to the design, development, standardization and quality control of medicinal products.\(^1\)

The efficacy and safety of a medicinal product can be assured by analytical monitoring of its quality. It is important that analytical procedure proposed of a particular active ingredient or its dosage form should be systematically sound under the condition in which it is to be applied.

**Antiviral drugs:**

In the mid- to late-20th century, medical science and practice included an array of effective tools, ranging from antiseptics to vaccines and antibiotics, but no drugs to treat viral infections. While vaccines were effective in preventing many viral diseases, they could not help once a viral infection set in. Prior to the development of antivirals, when someone contracted a virus, there was little that could be done other than treating the symptoms and waiting for the disease to run its course. It was not until the 1980s, when the full genetic sequences of viruses began to be unraveled, that researchers began to learn how viruses worked in detail, and exactly what
chemicals were needed to thwart their reproductive cycle. Dozens of antiviral treatments are now available, and medical research is rapidly exploiting new knowledge and technology to develop more.

**Virus life cycle**

Viruses consist of a genome and sometimes a few enzymes stored in a capsule made of protein (called a capsid), and sometimes covered with a lipid layer (sometimes called an 'envelope'). Viruses cannot reproduce on their own, and instead propagate by subjugating a host cell to produce copies of themselves, thus producing the next generation.

Researchers working on such "rational drug design" strategies for developing antivirals have tried to attack viruses at every stage of their life cycles. Some species of mushrooms have been found to contain multiple antiviral chemicals with similar synergistic effects. Viral life cycles vary in their precise details depending on the species of virus, but they all share a general pattern:

- Attachment to a host cell.
- Release of viral genes and possibly enzymes into the host cell.
- Replication of viral components using host-cell machinery.
- Assembly of viral components into complete viral particles.
- Release of viral particles to infect new host cells.

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics for bacteria, specific antivirals are used for specific viruses. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development. Most of the antivirals now available are designed to help deal with HIV, herpes viruses (best known for causing cold sores and genital herpes, but actually causing a wide range of diseases), the hepatitis B and C viruses, which can cause liver cancer, and influenza A and B viruses. Designing safe and effective antiviral drugs is difficult, because viruses use the host's cells to replicate. This makes it difficult to find targets for the drug that would interfere with the virus without also harming the host organism's cells. [2,3]
The emergence of antivirals is the product of a greatly expanded knowledge of the genetic and molecular function of organisms, allowing biomedical researchers to understand the structure and function of viruses, major advances in the techniques for finding new drugs, and the intense pressure placed on the medical profession to deal with the human immunodeficiency virus (HIV), the cause of the deadly acquired immunodeficiency syndrome (AIDS) pandemic.

Almost all anti-microbials, including anti-virals, are subject to drug resistance as the pathogens mutate over time, becoming less susceptible to the treatment. For instance, a recent study published in Nature Biotechnology emphasized the urgent need for augmentation of oseltamivir (Tamiflu) stockpiles with additional antiviral drugs including zanamivir (Relenza) based on an evaluation of the performance of these drugs in the scenario that the 2009 H1N1 'Swine Flu' neuraminidase (NA) were to acquire the tamiflu-resistance (His274Tyr) mutation which is currently widespread in seasonal H1N1 strains.[4]

Antiviral drugs are medicines that cure or control virus infections. Antivirals are used to treat infections caused by viruses. Unlike antibacterial drugs, which may cover a wide range of pathogens, antiviral agents tend to be narrow in spectrum, and have limited efficacy. Exclusive of the antiretroviral agents used in HIV (AIDS) therapy, there are currently only 11 antiviral drugs available, covering four types of virus. Acyclovir (Zovirax), famciclovir (Famvir), and valacyclovir (Valtrex) are effective against herpes virus, including herpes zoster and herpes genitalis. They may also be of value in either conditions caused by herpes, such as chickenpox and shingles. These drugs are not curative, but may reduce the pain of a herpes outbreak and shorten the period of viral shedding. Amantadine (Symmetrel), oseltamivir (Tamiflu), rimantidine (Flumadine), and zanamivir (Relenza) are useful in treatment of influenza virus. Amantadine, rimantadine, and oseltamivir may be administered throughout the flu season as preventatives for patients who cannot take influenza virus vaccine.
Acyclovir

Chemical Name: 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one

Molecular Formula: C$_8$H$_{11}$N$_5$O$_3$

Acyclovir is a white, crystalline powder

Molecular weight: 225.

The maximum solubility in water at 37°C is 2.5 mg/mL.

The pKas of acyclovir are 2.27 and 9.25.

Acyclovir is an antiviral drug. It slows the growth and spread of the herpes virus so that the body can fight off the infection. Acyclovir will not cure herpes, but it can lessen the symptoms of the infection. Acyclovir is used to treat infections caused by herpes viruses. Illnesses caused by herpes viruses include genital herpes, cold sores, shingles, and chicken pox. Acyclovir may also be used for purposes other than those listed in medication guide.

Acyclovir [9-[(2-hydroxyethoxy)methyl] guanine, zovirax] is a guanosine analogue with an acyclic side chain at the 9 position. It is a prototype of the group of viral agents that are activated by viral thymidine kinases (Tk) to become inhibitors of viral DNA polymerases and block viral DNA synthesis. Acyclovir uptake and intracellular phosphorylation to monophosphate is mediated by viral thymidine$^{[5,6]}$
Valacyclovir

[Chemical structure of Valacyclovir]

Chemical Name: (S)-2-[(2-amino-6-oxo-6,9-dihydro-3H-purin-9-yl)methoxy]ethyl-2-amino-3-methylbutanoate
Molecular Formula: C₁₃H₂₀N₆O₄
Molecular weight: 360.80

The maximum solubility in water at 25°C is 174 mg/mL
The pKa's of valacyclovir are 1.90, 7.47, and 9.43.

Valacyclovir is an antiviral drug used in the management of herpes simplex and herpes zoster (shingles). It is a prodrug, being converted *in vivo* to acyclovir. Valaciclovir is prepared starting from the natural proteinogenic amino acid (L)-valine. Valacyclovir is a prodrug, an esterified version of acyclovir that has greater oral bioavailability (about 55%) than acyclovir (10–20%). It is converted by esterases to the active drug acyclovir, as well as the amino acid valine, via hepatic first-pass metabolism.

Subsequently, the mono-phosphate form is further phosphorylated into the active triphosphate form, aciclo-GTP, by cellular kinases. Aciclo-GTP is a very potent inhibitor of viral DNA polymerase; it has approximately 100 times higher affinity to viral than cellular polymerase. Its monophosphate form also incorporates into the viral DNA, resulting in chain termination. It has also been shown that the viral enzymes cannot remove aciclo-GMP from the chain, which results in inhibition of further activity of DNA polymerase. Aciclo-GTP is fairly rapidly metabolised within the cell, possibly by cellular phosphatases.