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A Comprehensive Review On Analytical Method Development And Validation Of Antiviral Drug By Rp Hplc

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ABSTRACT

Antiviral drugs play a crucial role in treating viral infections that affect humans, animals, and plants. These medications follow two main strategies: targeting the viruses directly or the host cell factors. The development of antiviral drugs is a meticulous process, involving stages such as target identification, lead generation, clinical studies, and drug registration. In the pharmaceutical industry, analytical method development is essential to meet regulatory requirements. Testing procedures, validated according to standards like ICH, WHO, or GMP, are used to identify pharmaceutical active ingredients (API) and excipients. High-Performance Liquid Chromatography (HPLC) is a key analytical technique for sorting, identifying, and measuring complex mixtures. This review explores the basics of RP-HPLC, including the separation principle, types of stationary and mobile phases, and factors affecting separation. Recent advancements in RP-HPLC, such as new phases and downsizing, are discussed, offering a valuable guide for method development and validation. In simple terms, the review provides a tool for designing and testing RP-HPLC methods, showing its significance across industries.

Keywords: Antiviral drug, HPLC, Method development, validation, analytical method, ICH Q2(R1)

I. INTRODUCTION

Infectious diseases have been present throughout human history, caused by various microorganisms like bacteria, viruses, and fungi. These diseases can spread through air, water, food, or vectors such as insects [1]. The World Health Organization reported that since the pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was declared in March 2020, there have been over 5 million deaths and about 300 million confirmed cases worldwide[2]. The virus is still posing a major threat to public health. Drug manufacturers have been working feverishly to create novel vaccines and antiviral compounds against SARS-CoV-2, the causative virion, since the beginning of the epidemic. Nevertheless, this has not been an easy task [3]. Antiviral drugs are essential in treating viral infections, focusing on specific viruses or having a broad-spectrum effect. Unlike virucides, which deactivate viruses, antivirals are safe for the host and used to treat infections[4].Treatingviral infections requires specific antiviral medications tailored to the virus class. Antivirals impede the virus's ability to replicate, unlike antibiotics that directly attack bacteria. Developing safe and effective antiviral medications is challenging due to the delicate balance required to inhibit viral growth without harming host cells. Identifying therapeutic targets that achieve this balance is a significant challenge, complicated further by the rapid viral mutation, leading to different strains[5].The structure of viruses is relatively simple, consisting of a protein coat, nucleic acid (DNA or RNA), viral enzymes, and sometimes a lipid envelope. Viruses rely on the host's cellular machinery for replication, making them obligatory intracellular parasites. The ongoing conflict between humans and viruses highlights the difficulty in developing effective antiviral medications [6]. Two types of viruses, DNA and RNA, cause diseases in humans, animals, and plants. The constant struggle involves a dynamic interplay where both entities adapt and change tactics in response to each other,

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underscoring the difficulty in developing effective antiviral medications[7]. The development of antiviral medicines involves crucial stages such as target discovery, lead generation, clinical investigations, and drug registration. Given the historical impact of viral infections, there is a continuous need for antiviral medicine development. The journey began with the approval of the first antiviral medicine, "idoxuridine," by the U.S. FDA in 1963, marking the start of a new era [8].

Antiviral drugs play a crucial role in treating viral infections by inhibiting specific viruses' development. Unlike antibiotics, they impede replication without destroying the target pathogens, posing challenges in finding targets without harming host cells. Viral variation complicates drug and vaccine development, and computer-based drug discovery has emerged as a valuable approach, exemplified by the discovery of nelfinavir for HIV treatment[9].

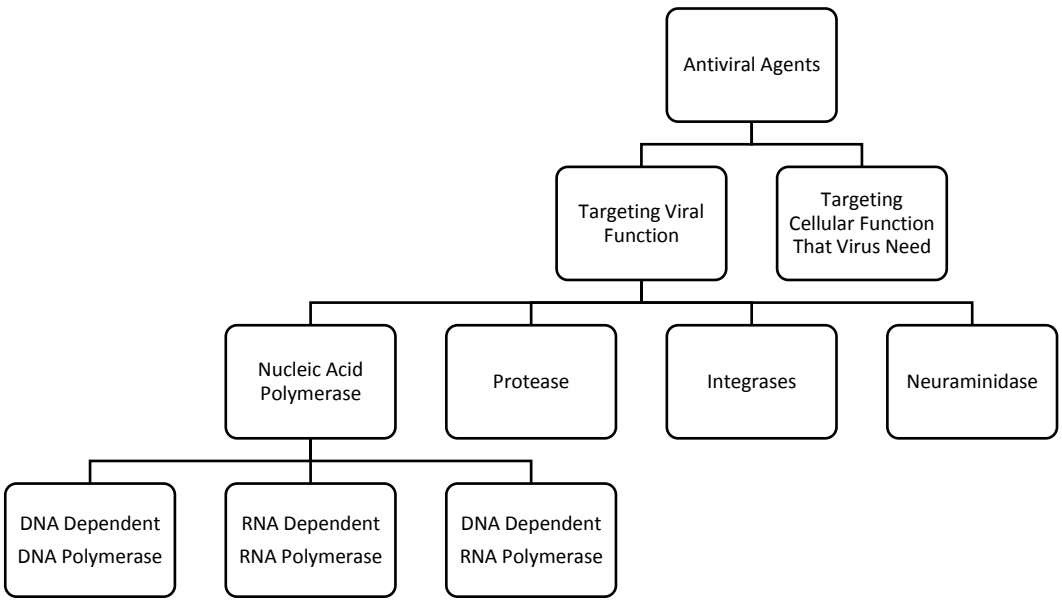
DNA viruses, like poxviruses and herpes, have a double-stranded DNA structure and use the host cell's machinery for replication. RNA viruses, including influenza and retroviruses like HIV, have diverse classifications. RNA viruses typically replicate within the cytoplasm of the host cell, and retroviruses integrate their RNA into the host genome, contributing to treatment challenges [10].

1.1 STEPS OF VIRAL INFECTIONS

The process of viral infection can be simplified into several key steps: -

1. **Attachment**: The virus first attaches to specific host cells using viral surface proteins that bind to receptors on the host cell surface.
2. **Entry**:The virus enters the host cell, either through direct fusion with the cell membrane or by endocytosis, where the cell engulfs the virus in a membrane-bound vesicle.
3. **Uncoating**: Once inside the host cell, the viral genetic material (either DNA or RNA) is released from its protective protein coat.
4. **Replicationand Transcription:**The viral genetic material takes control of the host cell's machinery to replicate its own genetic material and produce viral components. This often involves transcription of the viral genome to generate RNA or DNA for the synthesis of new viral components.
5. **Translation and Protein Synthesis:** The host cell's ribosomes are hijacked to translate viral RNA or DNA into viral proteins.
6. **Assembly:** Newly synthesized viral components are assembled into complete viral particles, often in the host cell's cytoplasm.
7. **Release:**New viral particles are released from the host cell, either through cell lysis (cell bursting) or budding, where the virus exits the cell without destroying it.
8. **Spread:**The released viruses can then infect neighboring cells, starting the cycle anew[11]

Fig No 1: Common inhibitory action of Antiviral drug



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1.2 INSTRUMENTATION

High-Performance Liquid Chromatography (HPLC) is a vital tool for analysing and understanding chemical compounds.One popular technique within HPLC is Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC), which excels in separating, identifying, and quantifying a wide range of analytes. RP-HPLC is especially crucial in pharmaceutical analysis.RP-HPLC is excellent at separating compounds based on their hydrophobic or lipophilic properties. This technique is essential for separating and quantifying Active Pharmaceutical Ingredients (APIs) and contaminants in pharmaceuticals. Reliable and accurate RP-HPLC processes are necessary to evaluate the quality, safety, and effectiveness of drugs[12].

In developing RP-HPLC methodologies, it's crucial to carefully choose the stationary and mobile phases, optimize separation conditions, and select appropriate chromatographic parameters. This review discusses the foundations of RP-HPLC, including separation principles, choices for stationary and mobile phases, and factors influencing RP-HPLC separation.It emphasizes the diverse applications of RP-HPLC, such as pharmaceutical, food, and environmental analysis. Recent advancements in RP-HPLC, including new stationary and mobile phases, downsizing, and hyphenated techniques like LC-MS, are also addressed.

RP-HPLC plays a crucial role in the pharmaceutical sector by enabling the separation and quantification of impurities and APIs. Recent improvements in both stationary and mobile phases have enhanced the effectiveness and selectivity of RP-HPLC. Advances in miniaturization allow for the analysis of smaller samples at a lower cost and in less time. The integration of hybrid techniques like LC-MS has increased sensitivity and selectivity in RP-HPLC analysis[13].

1.2.1 High-Performance Liquid Chromatography (HPLC)

Principle:

HPLC is based on the principle of separating components of a mixture through their differential interaction with a stationary phase and a mobile phase. In this method, a liquid sample (mobile phase) is pumped through a column packed with a stationary phase at high pressures. The stationary phase can be a solid or a liquid that Interacts differently with the components of the sample[14].

Process:

1. **MobilePhase:** The liquid sample, or mobile phase, is pumped through the column under high pressure. It carries the sample components through the stationary phase.
2. **Stationary Phase:** The stationary phase, typically packed into a column, interacts with the sample components based on their chemical properties.
3. **Separation:** Components with different affinities for the stationary phase will move through the column at different rates, leading to separation.
4. **Detection:** As the components elute from the column, they are detected by a detector, often a UV-Vis spectrophotometer. The signal is then recorded as a chromatogram [15].

Type of HPLC

HPLC, depending on the substrate or stationary phase used, is categorized into different types:

1. Normal Phase HPLC:

In Normal Phase separation is based on the polarity of the compounds. The stationary phase typically consists of polar materials such as silica, while non-polar solvents like hexane, chloroform, or diethyl ether are used as the mobile phase. Polar samples are retained on the column longer due to their interaction with the stationary phase.

2. Reverse Phase HPLC:

Reverse Phase is essentially the opposite of normal phase HPLC. Here, the mobile phase is polar, while the stationary phase is non-polar or hydrophobic. This setup results in increased retention for compounds with greater non-polar characteristics.

3. Size-Exclusion HPLC:

Size-Exclusion relies on a column containing substrate molecules with precise sizes. Separation occurs based on differences in molecular sizes, with smaller molecules eluting through the column faster than larger ones.

4. Ion-Exchange HPLC:

Ion-Exchange employs a stationary phase with an ionically charged surface opposite to the charge of the sample ions. Aqueous buffers are used as the mobile phase to control pH and ionic strength, allowing for separation based on

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differences in the ionic interactions between the stationary and mobile phases.

Benefits of HPLC

1. Versatility:
HPLC is versatile and can handle various types of samples, making it suitable for a wide range of applications, especially in pharmaceutical analysis.

2. Materials Compatibility:
It can use liquid and thermally stable mobile and stationary phases, offering flexibility in method development.

3. Quantitative and Qualitative Analysis:
HPLC is capable of both quantitative and qualitative analysis. It allows for the determination of the concentration of specific components in a mixture.

4. Sensitivity:
HPLC is a highly sensitive technique, allowing for the detection of trace amounts of compounds in a sample.

5. Wide Applicability:
It finds applications in pharmaceuticals, environmental analysis, food industry, and many other fields due to its precision and accuracy[17].

II. ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

Analytical techniques are crucial in pharmaceutical discovery, development, and manufacturing. Every year, new drugs are introduced, some with entirely new compounds or modified structures. Detecting contaminants, validating methods, and characterizing these drugs are essential steps. Some drugs, initially considered improvements, might face constant administration due to reported toxicities. However, widely accepted pharmacopoeias may lack the necessary analytical methods and standards for these new drugs and impurities, making identification challenging as the market continues to grow.

The pharmaceutical industry relies heavily on creating new ways to analyse,identify, and validate drugs, including characterizing contaminants. Each year, new medications hit the market, some with entirely new compounds or minor structural changes. Reports of their effects may lead to more frequent use. While initially considered better options, these drugs might eventually be included in widely accepted pharmacopoeias. Despite the increasing number of drugs introduced yearly, identifying them becomes challenging if the relevant pharmacopoeia lacks necessary analytical methods.In response, it's crucial to develop new analysis techniques. Studying a drug involves examining its structure, chemical composition, production, physical and chemical qualities, quality control techniques, and storage requirements. Research also delves into the drug's effects on organisms, part of pharmaceutical chemistry. India's pharmacy sector thrives, and the country capitalizes on this by promoting its products, meeting the demand in developed nations like the USA, Australia, and Britain. While India supplies formulations to these countries, they are now enforcing stricter regulatory standards for market acceptance[18], [19].

2.1 Need forDeveloping an Analytical Method

Developing methods, techniques, and methodologies is crucial to ensure the quality of products and analyse active drug-medicinal compounds, chemicals, and drug products. This is especially important for recently licensed phytochemical therapeutic molecules found in herbal products.

Method development plays a key role in impurity profiling of dosage forms, residual microanalysis, and identification of specific components in matrices. These methods adhere to test metrics outlined in accordance with ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guideline Q2-R1 criteria, such as

Accuracy
Precision
- Repeatability
- Intraday precision
- Interday precision

Linearity
Range
Specificity
Recovery
Robustness
LOD (limit of Detection)
LOQ (limit of Quantification)
System suitability

2.2 Steps Involved in Method development

- Collect data; specify division objectives.
- Material pre-treatment and a unique HPLC process are required
- Choosing a detector and configuring a detector.
- Choosing the LC technique, running a test run, and determining the ideal separation parameters.
- Improve separation circumstances.

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- Look for issues or the need for a specific process.

Sample information

- Quantity of compounds found.
- Compounds chemical composition.
- Chemical nature.
- The molecular weight of substances.
- Pka Value(s).
- Solubility of the sample.
- Stability of sample and storage[20].

2.3 Mobile Phase Selection

Choosing the appropriate composition and type of mobile phase is crucial as it significantly influences the separation process in chromatography. However, the options are limited based on the stationary phase employed in the column. The primary distinction lies between reversed-phase and normal chromatography.

In a normal phase system, nonpolar solvents like hexane or iso-octane are utilized. In contrast, reversed-phase chromatography involves the use of polar solvents such as water, acetonitrile, or methanol. The decision on the mobile phase is guided by the physical properties of the solvent, considering factors such as polarity, miscibility with other solvents, chemical inertness, UV cut-off wavelength, and toxicity. In summary, the choice between normal and reversed-phase chromatography determines whether nonpolar or polar solvents are employed in the mobile phase, respectively. This decision is crucial for achieving optimal separation in chromatographic processes [21].

A-Normal Phase		
Solvent	**Polarity index**	**UV cut off (NM)**
Hexane	0.1	210
Isooctane	0.1	205
Diethyl ether	2.8	218
Dichloromethane	3.1	205
B-Reverse Phase		
Water	10.2	200
Methanol	5.1	210
Acetonitrile	8.8	210
Tetrahydrofuran	4	280

Table No. 1- Typical Solvent for HPLC Mobile Phase [22]

2.4 Method Optimization

In the development of HPLC methods, the emphasis has predominantly been on optimizing HPLC conditions. Liquid chromatography (LC) optimization involves manipulating key variables such as acidity components, solvent composition, gradient settings, temperature fluctuations, sample volumes, and the choice of diluent solvent in the mobile phase. The goal is to assess the ideal combination that balances effective resolution and analytical time. Factors like column size, particle size, and column packing are also considered in relation to the flow rate. These parameters are adaptable across different skill levels and within a specified range[23].

III. ICH Q2(R1) – METHOD VALIDATION

Validating an analysis method is necessary to show that it is appropriate for the intended use. An overview of the qualities crucial for impurity management is given in a table. Test methods as well as identity. In the future, this content might be modified to reflect new techniques for analysis.

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1. Analytical Procedure:

The research methodology is referred to as the analytical process. The specifications needed to carry out each chemical test should be provided in detail. The sample's preparation. Reference standard, and reagents; using the equipment, creating the calibration curve, applying the computation formulae, etc., may all be necessary, but they are not the only ones.

2. Specificity:

The ability to evaluate a substance in the presence of ingredients that are reasonably expected to be present is known as specificity. These usually include pollutants, degradants, chemicals, etc.There are numerous supporting analytical methods that can make up for a particular analytical method's lack of specificity.This description has the following implications:

i. Recognition:

To verify the identification of an analyte.

ii. Tests for Purity:

Ensuring that every analytical process provides an accurate description of the contaminants contained in an analyte, including the assessment of related compounds, heavy metals, residual solvent content, etc.

iii. Content or Potency of Assay:

To achieve an accurate result that enables the determination of the concentration or potency of the analyte in a sample.

3. Accuracy:

The degree of agreement between the value agreed upon as either a traditional real value or an authorized reference value and the value revealed is how the accuracy of the analytical technique is communicated.

4. Precision:

An analytical procedure's precision expresses the degree of agreement (degree of scatter) between a set of measurements acquired from multiple sampling of the same homogenous sample under the defined conditions.

Precision can be divided into three categories: repeatability, intermediate precision, and reproducibility.Precision should be assessed using true, homogeneous samples. If a homogenous sample cannot be obtained, it may be studied using artificially generated samples or a sample solution.

i. Repeatability:

Repeatability expresses precision across a short time interval under the same operating conditions. Intra-assay precision is another synonym for repeatability.

ii. Intermediate precision:

Intermediate precision expresses variability within laboratories: various days, different analysers, different equipment, and so on.

iii. Reproducibility:

The reproducibility between laboratories (collaborative investigations, usually used to standardize methodology) is expressed by reproducibility.

5. Limit of Detection (LOD):

The lowest concentration of analyte in a sample that can be identified but may not always be quantified as an exact number is known as the detection limit of a particular analytical method.

6. Limit of Quantification (LOQ):

The lowest concentration of analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the quantitation limit of a particular analytical process. A parameter of quantitative assays for low concentrations of chemicals in sample matrices is the quantitation limit, which is especially useful for identifying contaminants and/or degradation products.

7. Linearity:

The capacity of an analytical method to produce test findings that are exactly proportionate to the concentration (amount) of analyte in the sample, within a specified range, is known as linearity.

8. Range:

The range of an analytical technique is the range of concentrations (amounts) of analyte in the sample, including these concentrations, for which the analytical procedure has been shown to have an appropriate degree of linearity, accuracy, and precision.

9. Robustness:

The robustness of an analytical procedure is a measure of its ability to remain unaffected by minor but deliberate modifications in method parameters, and it provides an indication of its dependability under normal conditions.

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10. System suitability:

System Suitability (SS) plays a crucial role in the validation of analytical procedures. This testing is grounded in the idea that the equipment, electronics, analytical processes, and the samples under analysis collectively form an interconnected system that can be assessed as a whole. The specific parameters for system suitability tests vary depending on the type of procedure undergoing validation. Additional information and guidelines can be found in pharmacopoeias[24].

11. Stability Testing

a. Light Exposure:

Drugs can break down when exposed to light, like sunlight or UV light. This can create new substances called degradation products. To test this, we put the drug solution under different light sources and see if its concentration changes or if new substances form over time.

b. Oxidation:

When drugs react with oxygen, they can change into different substances called oxidized products. To check for this, we expose the drug solution to things that cause oxidation, like hydrogen peroxide or certain metals, and watch for any changes in the drug's concentration or the appearance of new substances.

c. Acidic Conditions:

If drugs are exposed to acidic environments, they can break apart through a process called hydrolysis. This means the drug molecule falls apart. To test this, we expose the drug solution to acid, like hydrochloric acid, and see if the drug breaks down over time.

d. Alkaline Conditions:

Similarly, exposing drugs to alkaline (basic) environments can also make them break apart through hydrolysis. To test this, we expose the drug solution to a base, like sodium hydroxide, and observe if the drug degrades or forms new substances.

e. Heat:

High temperatures can speed up the breakdown of drugs, causing reactions like hydrolysis or oxidation to happen faster. To test this, we heat the drug solution to different temperatures, like 40°C or 50°C, and see if the drug changes or if new substances are formed[25,26].

V. CONCLUSION

In conclusion, RP-HPLC is an important analytical approach in the research of pharmaceuticals. Method development using RP-HPLC involves selecting Stationary phase and Mobile phases, maximizing separation condition and figuring Chromatographic. This article explains what validation is, what it is used for, why it is important, how to develop a method, and how to carry out the validation procedure to demonstrate that the technique can serve the intended goal. All validation measures (LOD, LOQ, linearity, range, selectivity, specificity, robustness, ruggedness, accuracy, and precision) are described. This review also covers multiple validation approaches that are employed at different phases of the process. Various stages of pharmaceutical processing. Because a validated method or procedure is required to create a safe and high-quality product.Toconclusion that many approaches are utilized to evaluate the quality of antiviral medications. This study will conduct a thorough review of the literature on the technique development and validation of several antiviral medications. This will provide a framework for researchers working on product development and testing.

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