IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
June 2021 Vol.:21, Issue:3

A Systematic Review on Method Development and Validation of Few Antiviral Drugs by Using RP-HPLC

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INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
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ISSN 2349-7203
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Submitted: 25 May 2021
Accepted: 02 June 2021
Published: 30 June 2021

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Antiviral Drugs, method development, validation, pharmaceuticals

ABSTRACT

In our fight between humans and viruses, both sides are continually evolving and perfecting their offensive and defensive techniques. A systematic study of the genetic and molecular mechanisms that lead to disease progression. A multitude of novel pharmaceuticals have recently emerged, and an additional number are in the process. In product development, the RP-HPLC is utilized to design and validate development parameters, such as accuracy, precision, linearity, robustness, softness, suitability, and so on. There are various mobile phases and columns employed in the development of a method and this limits detection and quantification ability.

INTRODUCTION

For centuries, human civilizations have understood the danger of infectious diseases. Although different microbes cause infectious illness (bacteria, viruses, and fungi).[1] The viral structure is simpler, as it is just made up of a protein coat, nucleic acid, viral enzymes, and sometimes a lipid envelope. Viruses also utilize the cellular machinery within the host to replicate, so they are obligatory intracellular parasites. Problems like these make it difficult to develop medications that selectively target viruses.[2] The two viruses known to produce disease in humans, animals, and plants are ultra-microscopic and are composed of either DNA or RNA. Human and viral conflict is a constant struggle because both groups will change tactics as they face off against each other. Target discovery and screening, lead generation and optimization, clinical investigations, and drug registration are all crucial stages in the development of antiviral medicines.[3] Because many human beings have lost their lives due to viral infections in human history, there is a compelling need for antiviral medicine development. The first antiviral medicine to receive U.S. Food and Medicine Administration (FDA) approval, "idoxuridine," was launched in June 1963 to herald the start of a new era in antiviral drug development. There have been many antiviral medications created for human use that treat millions of people globally.[4] Viral infections are best treated with antiviral medications of a particular class. As with antibiotics, particular antiviral medicines are used to treat certain viruses. Instead of attacking the infection directly, antiviral medications instead hinder the development of the disease. This makes it challenging to build a safe and effective antiviral medicine because the viruses proliferate within the host's cells. Also, as it is difficult to locate virus targets that will block the virus while not hurting the host cells, it is a challenge to find therapeutic targets that will do this. Another issue for the development of antiviral medications and vaccines is viral variation. [5] A novel computer-assisted methodology, known as drug discovery, led to the development of Nelfinavir, which was shown to be effective in treating human immunodeficiency virus (HIV) infection in the 1990s.[6] Only a few antiviral medicines are being licensed for human use due to side effects of medication resistance. More people are aware of the viruses, the way they enter your system, and the pace of their evolution, and this will expedite the creation of newer antiviral drugs. [7] It appears that the emergence of microbiological dangers, due to increasing climate change and globalization, is accelerating continuously over the planet. [8] cellular DNA virus Single-digit DNA is a common property of viruses containing double-stranded DNA, including those known as poxviruses, herpes viruses, adenoviruses, and papillomaviruses.

New viruses are born when a DNA virus invades the cell's core. Infectious RNA virus Single descriptor RNA viruses include the common cold, measles, mumps, and tuberculosis, which are all viral infections (ssRNA). Because RNA viruses are unable to enter the cell nucleus, they do not affect the body (in addition to the cold virus contamination this season). Once viral RNA is made, it is used to generate a DNA copy of the viral RNA that is then organized by the host genome, after which a retrovirus synthesizes an RNA copy of the viral RNA.[9]

INSTRUMENTATION

One of the most powerful analytical techniques in chemistry today is liquid chromatography. It can identify, sort, and quantify dissolved compounds in any given fluid. High-performance liquid chromatography (HPLC) is an accurate and highly utilized method of analysis for both quantitative and qualitative testing of pharmaceuticals.[10] To demonstrate the idea, a fluid (mobile phase) is pumped through a column (stationary phase) at high pressures. Changes in migration rates between the stationary and mobile phases are a cause of the varying sample divisions used in this example. The rate of elution is highly dependent on component partitioning activity. [11] While the sample compound with a higher affinity travels slower than the one with a lower affinity, it does so further and at a slower rate. open-mindedness The benefits of high-performance liquid chromatography are supported by the fact that it can use all mobile and stationary phases that are liquid and thermally stable, as well as all fluid and thermally stable materials. [12,13]

METHOD DEVELOPMENT [14,15]

- Sample preparation
- Method optimization
- Method validation

Sample preparation; The analyst must complete the sample preparation process as part of the production process. For each analysis technique used for a specific in-process sample or dosage type for subsequent HPLC analysis, the sample preparation method should be properly defined. The manufacturer, filter type, and pore size of the filter media must be calculated for the analytical process. [16] sample preparation aims to establish a processed sample that, when combined with the original sample, yields more accurate analysis results.

Aliquots should be prepared using as few HPLC-compatible interfaces as possible and without causing column damage. [17–19]

Method optimization. The majority of optimizations in the development of HPLC methods have focused on optimizing HPLC conditions. In the liquid chromatography (LC) optimization procedure, the primary control variables are the various components of acidity, solvent, gradient, fluctuating temperature, sample volumes, and diluents solvent type determination in the mobile phase. This is used to evaluate the optimal combination of resolve and analytical time following efficient selection. We considered column size, particle size, and column packing based on flow rate. These parameters are adjustable regardless of ability level or range.

Method Validation. Every new or changed technique must be validated to ensure reproducible and consistent outcomes when conducted in the same or different laboratories by different operators using the same equipment. The validation method that is needed is entirely dependent on the process that is being validated and the applications that are being proposed. Method validation results may be used to ascertain the precision, reliability, and consistency of study findings; these are essential components of any successful analysis. The method validation process necessitates the use of properly balanced and specification-based equipment. Methods of analysis must be tested or revalidated. [20–22]

Specificity: Selectivity in analytical methods is described as the degree to which an analytical method can measure the analyte, when interferences are present, with absolute accuracy. [23,]

Linearity and range: the ability of an analytical method to obtain test results that are directly proportional to the concentration of the sample analyte is referred to as linearity (within a defined range). A linear relationship can be evaluated across the entire empirical spectrum. Typically, linearity is expressed as the slope of the regression line. [24 – 26] For linearity, the ICH recommends a minimum of five concentrations. [27]

Precision: The degree of agreement (degree of scattering) between a series of measurements made under defined conditions with multiple samples from the same homogeneous specimen is referred to as the precision of a process. Repeatability, moderate precision, and reproductivity are three distinct levels of precision [27]. Usually, research precision is expressed in terms of the standard deviation or relative standard deviation of the

measurement sequence. Precision may refer to an analytical process's reproducibility or recurrence under normal operating conditions. The word "medium accuracy" (i.e. "roughness") refers to differences between laboratories on different days or between analysts or equipment within a single laboratory.

Accuracy (Recovery): The degree of correspondence between a value known as a standard true value or an agreed-upon reference value and the value discovered indicates the analytical method's accuracy. lt is calculated using the same sampling technique as the analyte concentrations. These can be examined using normal and blank solutions to ensure that there is no need for intervention. The accuracy is then expressed as a percentage of analytes fully recovered from the test results. Additionally, it can be expressed as a recovery by conducting tests on additional analyte concentrations that are already present. [24,27]

Solution stability. When conducting validation and storage tests under normal conditions and storage conditions, the standards and samples' stability are determined as well as when, in certain cases, they are measured on the instrument to determine whether additional measures, such as climate control or light safety, are needed.

Limit of detection (LOD) However, a very limited quantity of measurement (not an exact number) is done on a sample. The signal-to-noise (S/N) ratio used in an analytical technique, such as an analysis of the concentration of an analyte in a sample, can be between 3:1 (it is calculated using the amount of the analyte present in the sample). A maximum height of a component, or part's maximum height, is called "H." This is also known as the "signal-to-noise ratio." h = the absolute value of the largest difference between the chromatogram's baseline and the sound used to collect data. [25-27]

Limit of Quantification (LOQ): A quantitation limit is an analysis method that is defined as the smallest amount of analysis in a sample that can be quantified accurately and precisely. In analytic procedures, like HPLC, with base noise, the LOQ is usually by calculating the S/N ratio (10:1) and is then checked by injection criteria and provides an appropriate relative percentage defect. [26,27]

Robustness A system's capability to keep its steady, stable characteristics despite small but deliberate parameter alterations (e.g. pH, mobile phase composition, temperature, and instrumental adjustments). [26,27]

System Suitability. The device was calibrated before beginning the study to ensure that its detection sensitivity, resolution, and reproducibility were optimized. Since it is assumed that all of the instruments, electronics, analytical processes, and samples to be tested are all integrated into a single device, which can be measured, it follows that every instrument, electronics, analytical process, and sample has been integrated into the test device. Applying the approach involves determining a variety of test parameters, including peak resolution, theoretical plate numbers, peak tailing, and applicability. [24-27]

Table No. 1: Overall representation of antiviral drugs by using RP-HPLC

Sno	Title	Uses	Column	Mobile Phase	Flow Rate	Injection Volume	Detection Wavelength	reference
1.	Stability Indicating Rp-Hplc Method For Simultaneous Determination Of GlecaprevirAndPibrentasvir In Bulk And Pharmaceutical Dosage Form	Chronic hepatic virus	Cosmicsil C18 Column (250 mm x 4.6 mm, 5µm)	0.1M Phosphate buffer: Methanol	1.0ml/min	10 µL	225nm.	28
2.	Development & Validation of a Stability – indicating Method for the Simultaneous Estimation of Sofosbuvir & Ledipasvir by RP-HPLC	Hepatic C virus	C18 (250×4.6 mm, 5 µ particle size)	0.1 % orthophosphoric acid and acetonitrile 45:55	1.0ml/min	10 µL	270nm	29
3.	RP-HPLC Method For Simultaneous Estimation Of Impurities From Emtricitabine And Tenofovir Disoproxil Fumarate Tablet	Hepatic B virus	ACE C18 (250 x 4.6, 5µ).	0.01M potassium dihydrogen phosphate buffer with pH4.0	1.0ml/min	20 µL	270nm	30

				adjusted using diluted ortho-32phospho ric aci33d & meth34an ol				
4.	**Validated Stability Indicating RP-HPLC Method For The Simultaneous Estimation Of Rilpivirine And Dolutegravir In Bulk Form**	HIV	Hypersil ODS (250mm × 4.6mm i.d., 5µm)	Methanol and water	1.0ml/ min	20 µL	282nm	31
5.	Method Development and Validation for Simultaneous Estimation of Emtricitabine, Bictegravir And Tenofovir Alafenamide by RP-HPLC	HIV-1 and HIV-2	BDS (C8 150x4.6 mm, 5m)	0.01N KH2PO4 buffer at pH adjusted to 3.47 with dil. Ortho-phosphori c acid solution & Acetonitril e	1ml/m in	10µL	272nm	32
6.	**A Novel Stability Indicating RP-HPLC Method For Simultaneous Estimation Of Anti-Viral Class Of Elbasvir And**	Hepatic C virus	Luna C18 (150 mm × 4.6 mm, 5 µm)	OPA buffer (0.1%) and acetonitril	1.0ml/ min	10µL	258nm	33

	Grazoprevir In Bulk And Pharmaceutical Dosage Form			e				
7.	Simultaneous Estimation of Daclatasvir and Sofosbuvir in Tablet Dosage form by Reverse Phase High-Performance Liquid Chromatography	Hepatic C virus	Inertsil ODS-C18 column (250 x 4.6 mm, 5µ)	Acetonitrile: Methanol: 0.1% Triethylamine buffer (pH-3.0)	1.0ml/min	-----	250 nm	34
8.	RP-HPLC Method For Simultaneous Estimation Of Ritonavir, Ombitasvir And Paritaprevir In Tablet Dosage Forms And Their Stress Degradation Studies	HIV infection and AIDS & Hepatic C virus	Hypersil BDS C18 column (250 mm X 4.6 mm i.e., 5 µm particle size)	(0.01N % w/v potassium di-hydrogen orthophosphate buffer, pH 3.0 adjusted with dilute orthophosphoric acid &acetonitrile	1.0ml/min	10µL	254 nm	35
9.	Development And Validation Of Rp-Hplc Method For The Estimation Of Dolutegravir And RilpivirineIn Bulk And	HIV	Phenomenex C18 (150x4.6 mm, 5µm).	0.1% Ortho phosphoric acid and acetonitrile	1.0ml/min	10µL	262nm	36

	Pharmaceutical Dosage Form And Its Application To Rat Plasma							
10.	RP-HPLC method development and validation for the estimation of Emtricitabine, Bictegravir and Tenofovir alafenamide in bulk and pharmaceutical dosage form	HIV-1 and HIV-2	Denali C18 column (150 mm × 4.6 mm, 5 µm)	Buffer and acetonitrile	1.0ml/min	10µL	272 nm	37
11.	Analytical Method Development and Validation of Elbasvir and Grazoprevir in Bulk and Tablet Formulations by Rp-HPLC	Hepatitis C Virus	Inertsil ODS, 5µm C18(150 x4.6 ID)	Methanol & water	1ml/min	20µl	260 nm	38
12.	Assay and Dissolution Methods Development and Validation for Simultaneous Determination of Sofosbuvir and Ledipasvir by RP-HPLC Method in Tablet Dosage Forms	Hepatitis C Virus	Eclipse XDB C18 column (250 mm X 4.6 mm, 5 µm	buffer solution of pH 3.0 containing 0.02 M potassium dihydrogen phosphate and 5.7 mM hexane sulfonate: acetonitrile	1.5 ml/min	10 µL	254 nm	39

CONCLUSION:

I conclude that various methods are applied for the qualitative evaluation of antiviral drugs. This review will include a comprehensive review of the literature on the method development and validation of few antiviral drugs. This will provide a foundation for researchers working in the areas of product creation and product testing'.

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