URF PUBLISHERS
connect with research world

Journal of Medicine and Medical Studies

https://urfpublishers.com/journal/medicine-medical-studies

Vol: 1 & Iss: 1

Research Article

Stability Indicating Development and Validation of A RP-HPLC Method for Estimation of Dolutegravir in API Form and Marketed Pharmaceutical Dosage Form

Swetha Reddy Komati Reddy

Department of Pharmaceutical Analysis, India

Citation: Swetha Reddy K, Stability Indicating Development and Validation of A RP-HPLC Method for Estimation of Dolutegravir in API Form and Marketed Pharmaceutical Dosage Form. *J M Med Stu 2024;* 1(1): 35-43.

Received: 18 November, 2024; **Accepted:** 09 December, 2024; **Published:** 12 December, 2024

*__Corresponding author:__ Swetha Reddy K, Department of Pharmaceutical Analysis, University of Teegala Krishna Reddy College of Pharmacy, India

Copyright: © 2024 Swetha Reddy K., This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1. Introduction

Analytical chemistry is used to determining the qualitative and quantitative composition of material under study. Both these aspects are necessary to understand the sample material. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components. A quantitative analysis provides numerical information as to the relative amount of one or more of these components. For analyzing the drug samples in bulk, pharmaceutical formulations and biological fluids, different analytical methods are routinely being used. In non-instrumental, the conventional and physicochemical property are used to analyze the sample. The instrumental methods of analysis are based upon the measurements of some physical property of substance using instrument to determine its chemical composition. The instrumental methods are simple, precise and reproducible as compared to classical methods. Therefore, analytical methods developed using sophisticated instruments such as spectrophotometer, HPLC, GC and HPTLC have wide applications in assuring the quality and quantity of raw materials and finished products.

1.1. Chromatography: Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation[1].

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive[2].

Introduction to chromatography, its types and classification Chromatography: Chromatography is a set of techniques in which separation of chemical substances takes place quantitatively as well as qualitatively.

1.2. Terminology used in Chromatography Mobile Phase: In chromatography the substance which is introduced with or along with the sample and causes elution of the contents of the sample. It may be liquid or gas.

1.3. Stationary phase: Stationary phase of the chromatographic system refers to that part which is present before the introduction of sample or solute in the column (as in column chromatography) or on a solid support (as in paper or similar chromatography). It may be liquid or solid.

1.4. Eluent: The substance which separates the components of the mixture in chromatographic technique. Eluent is that part which brings separation when the solution is passed either from the column or from the solid support.

1.5. Eluate: The substance which is separated as an individual component of the mixture is called eluate.

1.6. Important types of Chromatographic Techniques: Following are some important types of Chromatographic separation techniques. They are defined thoroughly by explaining their general principle, application and a brief outline of their instrumentation for a complete understanding. Following are some commonly utilized types of techniques:

- Gas Chromatography
- High Pressure Liquid Chromatography
- Supercritical fluid Chromatography
- Gel Exclusion Chromatography.

1.7. High Pressure Liquid Chromatography (HPLC): High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. HPLC has the ability to separate and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics and chemicals.

Sample retention time will vary depending on the interaction between the stationary phase, the molecules being analyzed and the solvent or solvents used. As the sample passes through the column, it interacts between the two phases at different rate, primarily due to different polarities in the analytes. Analytes that have the least amount of interaction with the stationary phase or the most amount of interaction with the mobile phase will exit the column faster.

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

HPLC has been used for manufacturing (e.g., during the production process of pharmaceutical and biological products), legal (e.g., detecting performance enhancement drugs in urine), research (e.g., separating the components of a complex biological sample or of similar synthetic chemicals from each other) and medical (e.g., detecting vitamin D levels in blood serum) purposes[1].

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

1.8. Types of HPLC: There are following variants of HPLC, depending upon the phase system (stationary) in the process:

1.8.1. Normal Phase HPLC: This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether and mixtures of these. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

1.8.2. Reverse Phase HPLC: The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

1.8.3. Size-exclusion HPLC: The column is filled with material having precisely controlled pore sizes and the particles are separated according to its their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

1.8.4. Ion-Exchange HPLC: The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

1.9. Instrumentation of HPLC

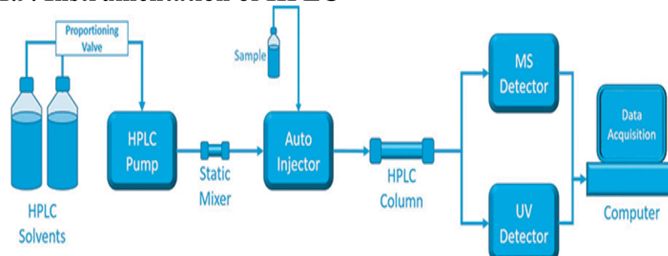

Figure1: Schematic diagram of HPLC instrumentation

As shown in the schematic diagram in Figure above, HPLC instrumentation includes a pump, injector, column, detector and integrator or acquisition and display system. The heart of the system is the column where separation occurs.

1.9.1. Solvent Reservoir: Mobile phase contents are contained in a glass reservoir. The mobile phase or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

1.9.2. Pump: A pump aspirates the mobile phase from the solvent reservoir and forces it through the system's column and detector. Depending on a number of factors including column dimensions, particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.

1.9.3. Sample Injector: The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).

1.9.4. Columns: Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm[3-6]. They are commonly filled with a stationary phase with a particle size of 3–10 µm. Columns with internal diameters of less than 2 mm are often referred to as micro bore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.

1.9.5. Detector: The HPLC detector, located at the end of the column detects the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.

1.9.6. Data Collection Devices: Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret[6-10].

1.10. Applications of HPLC: The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include:

1.10.1. Pharmaceutical Applications

- To control drug stability.
- Tablet dissolution study of pharmaceutical dosages form.
- Pharmaceutical quality control.

1.10.2. Environmental Applications

- Detection of phenolic compounds in drinking water.
- Bio-monitoring of pollutants.
- Applications in Forensics
- Quantification of drugs in biological samples.
- Identification of steroids in blood, urine etc.
- Forensic analysis of textile dyes.
- Determination of cocaine and other drugs of abuse in blood, urine etc.

1.10.3. Food and Flavour

- Measurement of Quality of soft drinks and water.
- Sugar analysis in fruit juices.
- Analysis of polycyclic compounds in vegetables.
- Preservative analysis.

1.10.4. Applications in Clinical Tests

- Urine analysis, antibiotics analysis in blood.
- Analysis of bilirubin, biliverdin in hepatic disorders.
- Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

2. Method Development

Steps for HPLC Method Development: Analytical method development is considered as a critical process in pharmaceuticals. Availability of the different types of columns, operating parameters and mobile phase composition, diluent and pH values make it critical to develop an analytical method. A good analytical method should be simple, used column, mobile phase and buffer should be common. It can be done easily step by step[10-13].

Following is the common HPLC method development steps.

1. Selection of HPLC Analytical Method
2. Selection of Chromatographic Conditions
3. Parameter Optimization

2.1. Selection of HPLC Analytical Method: First of all, consult the literature that is available on the product. It will help you to understand the nature of the product that will help to select the different parameters.

A. Sample Preparation: Select method to prepare the sample according to its solubility, filtration requirements, extraction requirements or other special requirements to make a clear solution of HPLC analysis.

B. Chromatography: Reverse phase chromatography is used for most of the samples but when acidic or basic molecules are present in the sample then reverse phase ion suppression (for weak acid or base) or reverse phase ion pairing (for strong acid or base) should be used. The stationary phase should be C18 bonded. Normal phase is used for low or medium polarity analyte especially when it is required to separate the product isomers. Choose cyano bonded phase for normal phase separations. Ion exchange chromatography is best to use for inorganic anion or Cation analysis. If analyte has higher molecular weight than size exclusion chromatography is the best to use[13-17].

C. Gradient/Isotonic HPLC: Gradient HPLC is helpful in the analysis of complex samples having a number of components. It will help to get higher resolution than isotonic HPLC having constant peak width while in isotonic HPLC peak width increases with the retention time. Gradient HPLC has great sensitivity, especially for the products having longer retention time.

D. Column Size: 100-150 mm columns are used for most of the samples. It reduces the method development and analysis time for the sample. Bigger columns are used for complex samples those take more time in separation. Initially, a flow rate should be kept between 1 and 1.5 ml/min and column particle size should be between 3 and 5 µm[17-20].

E. HPLC Detectors: If the analyte has chromophores that enable the compound to be detected by UV than it is better to use UV detector. It is always better to use a UV detector than others. Fluorescence and electrochemical detectors should be used for trace analysis. Samples having high concentration should be analyzed using refractive index detectors.

F. Wavelength: λmax of the sample has the greatest sensitivity to the UV light. It detects the sample components that have chromophores. A wavelength above 200 nm gives greater sensitivity than the lower wavelengths. Wavelengths lower than 200 nm gives more noise, therefore, it should be avoided.

2.2. Selection of Chromatographic Conditions: After selection of analytical method, different chromatographic conditions are selected. The flow of the analytes through the column depends upon the concentration of the solvent in the mobile phase. The concentration of solvent is generally used to control the

retention time. Mobile phase pH and ion pairing reagents also affect the retention time of the sample. Samples having a large number of components are analyzed using the gradient to avoid the large retention time while the samples containing one or two components are analyzed on an isotonic system.

2.3. Parameter Optimization: After taking the same sample runs some parameters including column dimensions, particle size, run time and flow rate are optimized. It is done to get the best resolution and minimum run time. After proper optimization of the analysis method, it is validated to ensure the consistency of the analytical method. Analytical method validation is now done mandatory by all regulatory authorities.

3. Parameters For HPLC

3.1. Performance Calculations: Calculating the following values (which can be included in a custom report) used to access overall system performance.

- Relative retention
- Theoretical plates
- Capacity factor
- Resolution
- Peak asymmetry
- Plates per meter

The following information furnishes the parameters used to calculate these system performance values for the separation of two chromatographic components[21-24]. (Note: where the terms w and t both appear in the same equation, they must be expressed the same units).

3.2. System suitability parameters: The theory of chromatography has been used as the basis for system-suitability tests, which are set of quantitative criteria that test the suitability of the chromatographic system to identify and quantify drug related samples by HPLC at any step of the pharmaceutical analysis.

3.2.1. Relative retention: The time elapsed between the injection of the sample components in to the column and their detection is known as the retention time (Rt).

$\alpha = (t_2-t_a) / (t_1-t_a)$

Were,

α =Relative retention.

t_1 = Retention time of the peak measured from point of injection.

t_2 = Retention time of the second measured from point of injection.

t_a = Retention time of an inert peak not retained by the column, measured from point of injection.

2. Theoretical plates:

$n = 16 (t_R / w)^2$

Were,

n =Theoretical plates.

t_R = Retention time of the component.

W = Width of the base of the component peak using tangent method.

3.3. Capacity factor: The capacity factor describes the thermodynamic basis of the separation and its definition is the ratio of the amounts of the solute at the stationary and mobile phases within the analyte band inside the chromatographic column.

$K^1 = (t_2/t_a)-1$

Were,

K^1 = Capacity factor.

t_a = Retention time of an inert peak not retained by the column, measured from point of injection.

4. Resolution: the gap between two peaks

$R = 2 (t_2 - t_1) / (w_2-w_1)$

Were,

R =Resolution between a peak of interest (peak 2) and the peak preceding it (peak 1).

W_2 = Width of the base of component peak 2.

W_1 = Width of the base of component peak 1.

5. Peak Asymmetry

$T = W_{0.05} / 2f$

Were,

T = Peak asymmetry or tailing factor.

$W_{0.05}$ = Distance from the leading edge to the tailing edge of the peak, measured at a point 5 % of the peak height from the baseline.

f= Distance from the peak maximum to the leading edge of the peak.

6. Plate per Meter:

N =n/L

Were,

N = plates per meter.

L = column length in meters.

3.4. Advantages:

- HPLC separations can be accomplished in a minute, in some cases even in seconds.
- High resolution of complex sample mixture into individual components.
- Rapid growth of HPLC is also because of its ability to analyze substances that are unsuitable for gas liquid chromatographic (GLC) analysis due to non-volatility or thermal-instability.
- Quantitative analysis is easily and accurately performed and errors of less than 1 % are common to most HPLC methods.
- Depending on sample type and detector used, it is frequently possible to measure 10^{-9} g or 1 ng of sample. With special detectors, analysis down to 10-12 pg has been reported.
- As HPLC is versatile, it can be applied to wide variety of samples like organic, inorganic, high molecular weight liquids, solids and ionic-nonionic compounds.

3.5. Disadvantages:

- HPLC instrumentation is expensive and represents a major investment for many laboratories.
- HPLC cannot handle gas samples.
- HPLC is poor identifier. It provides superior resolution but it does not provide the information that identifies each peak.
- Only one sample can be analyzed at a time.
- Finally, at present there is no universal and sensitive detector.

4. Method Validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Analytical methods need to be validated or revalidated.

- Before their introduction into routine use;
- Whenever the conditions change for which, the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and
- Whenever the method is changed and the change is outside the original scope of the method.

4.1. Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s)[25-28]. This definition has the following implications: Identification: to ensure the identity of an analyte. Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc. Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

4.2. Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

4.3. Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

4.3.1. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

4.3.2. Intermediate precision: Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

4.3.3. Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

4.4. Detection Limit: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

4.5. Quantitation Limit: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products.

4.6. Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

4.7. Range: The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

4.8. Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage[29-32].

5. Drug Profile

Drug Profile of Dolutegravir

Name of the Drug: Dolutegravir

Description: Dolutegravir is a HIV-1 intergrase inhibitor that blocks the strand transfer step of the integration of the viral genome into the host cell (INSTI).1 The effect of this drug has no homology in human host cells which gives it an excellent tolerability and minimal toxicity.11 Dolutegravir was developed by ViiV Healthcare and FDA approved on August 12, 2013.15 On November 21, 2017, Dolutegravir, in combination with Rilpivirine, was approved as part of the first complete treatment regimen with only two drugs for the treatment of adults with HIV-1 named Juluca.

Synonyms: Dolutegravir, 1051375-16-6, GSK1349572, S/GSK1349572, Tivicay, Dolutegravir (GSK1349572), GSK 1349572, GSK-1349572, S-349572, UNII-DKO1W9H7M1.

Chemical Structure:

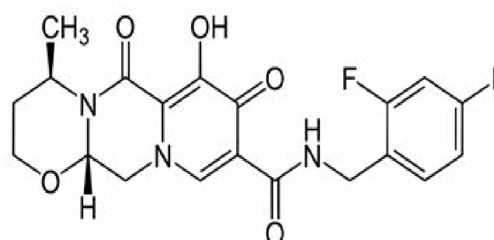

Figure 1: IUPAC Name: (3S, 7R)-N-[(2,4-difluorophenyl) methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo[8.4.0.03,8]tetradeca-10,13-diene-13-carboxamide

Molecular formula: $C_{20}H_{19}F_2N_3O_5$

Molecular weight: 419.3788g/mol

Pka value: 8.2

Melting Point: 190-193ºC

Log P: 2.2

Bioavailability: 98.9%

Physical Appearance: White to light yellow powder.

Solubility: Dolutegravir was found to be freely soluble in methanol, acetonitrile, slightly soluble in water.

5.1. Indication: Dolutegravir is indicated in combination with other antiretroviral agents for the treatment of patients with HIV-1 infection that comply with the characteristics of being adults or children aged 12 years and older and present at least a weight of 40 kg.7 The FDA combination therapy approval of Dolutegravir and Rilpivirine is indicated for adults with HIV-1 infections whose virus is currently suppressed (< 50 copies/ml) on a stable regimen for at least six months, without history of treatment failure and no known substitutions associated to resistance to any of the two components of the therapy.

5.2. Pharmacokinetics

5.2.1. Absorption: When 50 mg of Dolutegravir once daily was orally administered to HIV-1 infected adults, the AUC, Cmax and Cmin is 53.6 mcg h/mL, 3.67mcg/mL and 1.11mcg/mL, respectively. The peak plasma concentration was observed 2 to 3 hours post-dose. Steady state is achieved within approximately 5 days with average accumulation ratios for AUC, Cmax and C24h ranging from 1.2 to 1.5. When 50 mg once daily is given to pediatric patients (12 to < 18 years and weighing ≥40 kg) the Cmax, AUC and C24 is 3.49mcg/mL, 46mcg.h/mL and 0.90mcg/mL respectively.

5.2.2. Volume of distribution: The administration of a dose of 50 mg of Dolutegravir presents an apparent volume of distribution of 17.4 L. The median Dolutegravir concentration in CSF was 18ng/mL after 2 weeks of treatment.

5.2.3. Protein binding: Dolutegravir is highly protein bound to human plasma proteins reaching a percentage 98.9% of the administered dose.

5.2.4. Metabolism: Dolutegravir is highly metabolized through three main pathways and it forms no long-lived metabolites. The first pathway is defined by the glucuronidation by UGT1A1, the second pathway by carbon oxidation by CYP3A4 and the third pathway is what appears to be a sequential oxidative defluorination and glutathione conjugation. The main metabolite found in blood plasma is the ether glucuronide form (M2) and its chemical properties disrupt its ability to bind metal ions, therefore, it is inactive.

5.2.5. Route of elimination: When a single oral dose of Dolutegravir is given, nearly all complete dose is recovered in a proportion of 53% excreted unchanged in the feces and 31% excreted in urine. The renal eliminated recovered dose consists of ether glucuronide of Dolutegravir (18.9%), a metabolite formed by oxidation at the benzylic carbon (3.0%), a hydrolytic N-dealkylation product (3.6%) and unchanged drug (< 1%).

5.2.6. Pharmacodynamics: HIV-1 infected subjects on Dolutegravir monotherapy demonstrated rapid and dose-dependent reduction of antiviral activity with declines of HIV-1 RNA copies per ml. The antiviral response was maintained for 3 to 4 days after the last dose.3 The sustained response obtained in clinical trials indicates that Dolutegravir has a tight binding and longer dissociative half-life providing it a high barrier to resistance. The combination therapy (Rilpivirine and Dolutegravir) presented the same viral suppression found in previous three-drug therapies without integrase strand transfer inhibitor mutations or Rilpivirine resistance.

5.2.7. Mechanism of action: Dolutegravir is an HIV-1 antiviral agent. It inhibits HIV integrase by binding to the active site and blocking the strand transfer step of retroviral DNA integration in the host cell. The strand transfer step is essential in the HIV replication cycle and results in the inhibition of viral activity. Dolutegravir has a mean EC50 value of 0.5nM (0.21ng/mL) to 2.1nM (0.85ng/mL) in peripheral blood mononuclear cells (PBMCs) and MT-4 cells.

5.3. Half Life: The half-life of Dolutegravir is 14 hours.

5.3.1. Drug interactions:

Afatinib: The serum concentration of Dolutegravir can be increased when it is combined with Afatinib.

Alectinib: Alectinib may decrease the excretion rate of Dolutegravir which could result in a higher serum level.

Allopurinol: The excretion of Allopurinol can be decreased when combined with Dolutegravir.

Alprostadil: The excretion of Alprostadil can be decreased when combined with Dolutegravir.

Adenine: The metabolism of Dolutegravir can be decreased when combined with Adenine.

5.3.2. Drug-Food Interactions:

- Avoid multivalent ions. Cations should be separated from Dolutegravir administration by 2 hours before and 6 hours after Cation administration. Cations can be administered with Dolutegravir if given with food.
- Avoid St. John's Wort.
- Take with or without food. Food, particularly high-fat meals, may increase the AUC, Cmax and Tmax of Dolutegravir.

5.3.3. Contraindications:

- Human immunodeficiency virus (HIV) infection resistance.
- Autoimmune disease, Graves' disease, Guillain-Barre syndrome, immune reconstitution syndrome.
- Serious rash.
- Hepatic disease, hepatitis B and HIV coinfection, hepatitis C and HIV coinfection.
- Geriatric, renal failure.

5.3.4. Adverse effects:

- Changes in your immune system (called immune reconstitution inflammatory syndrome or IRIS).
- Trouble sleeping, tiredness and headache, which are among the most common side effects of Dolutegravir.

5.3.5. Medical Uses: Dolutegravir is used with other HIV medications to help control HIV infection. It helps to decrease the amount of HIV in your body so your immune system can work better[33-37]. This lowers your chance of getting HIV complications (such as new infections, cancer) and improves your quality of life.

6. Marketed Formulation

S. No.	Drug Name	Label Claim	Brand Name	Company
1	Dolutegravir	50mg	Instgra	E m c u r e Pharmaceuticals

7. Literature Review

Venkatnarayana M, et al. (2020): A simple, rapid and robust reverse phase HPLC method was developed and validated for the determination of impurities in Dolutegravir drug substance. The main aim of this study is to reduce the time consumption and to develop and validate a less expensive method by using HPLC. The chromatographic separation of Dolutegravir and its related impurities is carried out by using C8 column (150 × 4.6 mm), 5µm with 0.1% trifluoroacetic acid in water as mobile phase A, methanol as mobile phase B. The flow rate is 1.0 mL/min with gradient elution mode and the wave length for detection is 240 nm (UV detector). The developed method was validated and proved that the method was specific, accurate and precise as per ICH. The system suitability criteria found to be within the limits. The limit of detection and limit of quantification demonstrate that the method is sensitive. The linearity curve was found to be linear and the correlation coefficient obtained is not less than 0.998. The average percentage recoveries of impurities were in the range of 97 to 101%. The proposed method was found to be suitable and accurate for quantitative determination of impurities in Dolutegravir drug substance.

Dr. P. T. S. R. K. Prasada Rao, et al. (2018): The aim of the method was to develop and validate a rapid, sensitive and accurate method for simultaneous estimation of Lamivudine and Dolutegravir in drug product by liquid chromatography. The chromatographic separation was achieved on the C18 column (Inertsil ODS 3V 250*4.6, 5um) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile (35:65). The flow rate was 0.8ml/ minute and ultraviolet detector at 260nm. The average retention time for Lamivudine and Dolutegravir found to be 2.984 min and 4.340 min. The proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 300.0 – 900.0µg/mL for Lamivudine and 50.0 - 150.0µg/mL of Dolutegravir.

Sapna M Rathod, et al. (2020): The present study was aimed to develop and validate simple, sensitive, precise, economic and accurate method for simultaneous estimation of Lamivudine and Dolutegravir Sodium in synthetic mixture. The RP-HPLC was performed using Inertsil C18 column (150mm*3mm, 3µm particle size), a binary mixture of methanol and water at a flow rate of 1.0 ml/min. Both the drugs were analyzed at 260 nm using PDA Detector. The Lamivudine and Dolutegravir Sodium show linearity in the range of 7.5–45 µg/ml and 1.3–7.8 µg/ml respectively. The retention times of Lamivudine and Dolutegravir Sodium were found to be 2.870 min and 5.637 min respectively. The developed method was evaluated for precision, accuracy and robustness parameters also. The intraday and inter day precision data found to be less than 2 % RSD showing the method is precise. The accuracy study was performed using standard addition technique and found between 98–102 %. The Limit of Detection was found to be 2.10µg/ml and 0.37µg/ml for Lamivudine and Dolutegravir Sodium respectively. The limit of Quantitation was found to be 6.38µg/ml and 1.14µg/ml for Lamivudine and Dolutegravir Sodium respectively. The % RSD for robustness study was also found below 2%, indicates that the method is robust.

Ramreddy Godela, et al. (2020): Background: A Simple, sensitive and specific stability indicating reverse phase HPLC method was developed for simultaneous estimation of Lamivudine and Dolutegravir in bulk and tablet dosage form. Effective separation was achieved by injecting10 µL of the standard solution into Xbridge Phenyl (250 × 4.6 mm, 5 µ, 100 A0) column, using a mobile phase composition of methanol: buffer (0.1% v/v Trifluoroacetic acid in water) (85:15 v/v) and isocratic elution programming have been done at a flow rate of 0.8 mL/min. The eluted analytes detected at 258 nm wavelength. The stress conditions such as acid, base, oxidative, thermal and photo stability were applied as per ICH guidelines to determine the stability of the drugs in different environmental conditions. Results: The retention times of Lamivudine and Dolutegravir were found to be 3.4 and 5.0 min respectively. The developed method was linear in the concentration range of 5–15µg/mL and 30–90µg/mL for Dolutegravir and Lamivudine respectively. Detection and quantification limits were observed at 3.6 and 11µg/mL for Lamivudine and 0.50 and 1.5µg/mL for Dolutegravir. Method validation parameters were within the acceptance criteria of ICH guidelines and the degradation products were well resolved from Dolutegravir and Lamivudine peaks, which indicate the stability of the method. Conclusion: The developed RP-HPLC method was highly precise, specific, sensitive and stability indicating. Hence, the method has the ability to use in quality control department for regular analysis for the estimation of Lamivudine and Dolutegravir.

Khaleel Noorbasha, et al. (2020): Background: A fresh selective, rapid, accurate, precise and RP-HPLC stability-indicating method was developed and validated for the quantitative simultaneous determination of Dolutegravir and lamivudine in the bulk as well as pharmaceutical dosage form. A chromatographic separation was done by using Inertsil ODS 3V (250 × 4.6 mm, 5µm) column and mobile phase composed of phosphate buffer, pH 3.0: acetonitrile: methanol (50:20:30% v/v/v) with flow rate of 1.0 mL/min and the detection of eluents was carried out at a wavelength of 257 nm utilizing a PDA detector. The drugs, Dolutegravir and lamivudine, were subjected to varied conditions like base hydrolysis, acid hydrolysis, oxidation, thermal, photochemical and UV. The suggested method was analyzed statistically and validated to fulfill requirements of International Conference on Harmonization (ICH) and the validation covered accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness, ruggedness and specificity. Results: The retention time of Dolutegravir and lamivudine were observed to be 6.36 and 2.16 min, respectively. The method was found to be linear within the range of 14.98 to 91.25µg/mL for lamivudine and 2.54 to 15.35µg/mL for Dolutegravir. The percentage recoveries (accuracy) for Dolutegravir and lamivudine were in the range of 98.35 to 102.14% and 98.01 to 101.5%. The computed relative variance (%RSD) was within the suitable criterion of less than 2.0. Conclusion: The suggested method was set to be precise as well as stability-indicating since no interfering degradants peaks and excipients were evident. All the peaks of degradation were successfully resolved by the use of the developed analytical method with altered retention times. Results obtained were analyzed statistically and found to be acceptable in line with

the ICH guidelines. Hence, such method is often employed successfully for routine analysis of active analytes in the bulk as well as pharmaceutical dosage form. It is going to be extended to review for its estimation in plasma and other biological fluids and may even be employed for quality control stability sample estimation and in cleaning method analysis during cleaning validation.

8. Aim and Objectives

- Review of literature for Dolutegravir gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other drugs.
- Literature survey reveals that certain spectrophotometric methods were reported for estimation of Dolutegravir and there is a method is available for such estimation by RP-HPLC.
- In view of the need for a suitable RP-HPLC method for routine analysis of Dolutegravir in formulations, attempts were made to develop simple, precise and accurate analytical method for estimation of Dolutegravir and extend it for their determination in formulation.
- Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.
- The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient.
- The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

The primary objective of proposed work is:

- To develop new simple, sensitive, accurate and economical analytical method for the estimation of Dolutegravir in bulk form and marketed pharmaceutical dosage form.
- To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Dolutegravir in bulk and its dosage form.

9. Plan of Work

I. Collection of literature for the selected drug.

II. Extensive literature survey for selection of appropriate solvents to dissolve respective selected drug and preparation of stock solution.

III. Study of drug profile

IV. Procurement of samples, standards and other chemicals.

V. Selection of chromatographic conditions

VI. Selection of mobile phase

VII. Method trials on HPLC by using different solvents and columns.

VIII. Development of RP-HPLC method which is different from the finished articles.

IX. Optimization of the developed method by varying mobile phase conditions, temperature.

X. Validation of the developed method for the following parameters:

- Accuracy
- Precision
- Specificity
- Limit of detection
- limit of quantitation
- Linearity
- Robustness
- System Suitability

10. Materials and Methods

- Pure samples of Dolutegravir will be procured from industries involved in bulk manufacture of this drug.
- Dosage formulation will be procured from local market.
- The methods will be developed and validated in Analytical R & D of Syncorp Clincare Technologies Pvt. Ltd. Dilsuknagar, Hyderabad.
- The methods will be first developed, then validated as per ICH guidelines, then the method will be applied to the formulations.

11. Equipment's

- A suitable HPLC having isocratic system equipped with manual injector with UV detector.
- LABINDIAT-60 UV - Vis spectrophotometer.
- Analytical Balance, capable of measuring the 0.01mg.
- Ultra Sonicator
- Vaccum filtration kit
- Usual laboratory glass ware of class-A

12. Chemicals And Reagents

- HPLC grade water, Methanol, Acetonitrile
- Di-potassium hydrogen orthophosphate
- Potassium dihydrogen orthophosphate
- Ortho phosphoric acid
- 0.1N Hydrochloric acid
- 0.1N Sodium Hydroxide
- 3% Hydrogen Peroxide

7. References

1. “HPLC in Pharmaceutical Industry”, Fong and Long. Marcel Dekker Series.
2. “Instrumental Method of Chemical Analysis” by Chatwal Anand, Himalaya Publishing House, 615-623.
3. “Practical Pharmaceutical Chemistry”, 4th edition, Part 2, by Beckett and Stenlake, CBS Publishers and Distributors, 157-174.
4. Settle FA. In: Handbook of Instrumental Techniques for Analytical Chemistry. 1st Ed, Singapore, Pearson Education Inc, 2004.
5. Willard HH and Dean AJ. Instrumental Methods of Analysis. CBS Publishers and distributors, 7th Ed, 1986;513-515.
6. Connors AK. In: A Text Book of Pharmaceutical Analysis. A Wiley Interscience Publication, 3rd ed, 2005:373-400.

7. Ahuja S. In: High Pressure Liquid Chromatography of Comprehensive Analytical Chemistry. Elsevier Publications, 2006.
8. Principles and Methods. In: Amesham Biosciences of Reversed Phase Chromatography, 6-8.
9. Synder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development, 2nd Ed, John Wiley and Sons Inc. Canada, 1997.
10. Snyder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development. 2nd ed, 2001.
11. “Principles of Instrumental Analysis”, 5th edition, Harcourt Publishes Int Company, Skoog, Holler and Nieman, Chapter 28, 726-766.
12. Uwe D. Neue, Wiley-VC. “HPLC Columns” Theory, Technology and Practice.
13. Elena Katz, et al. Handbook of HPLC. Marcel Dekker, 78.
14. Galen W, Ewing. “Instrumental Methods of Chemical Analysis”, 5th Edition, McGraw Hill Book Company, 1988.
15. Govt. of India, Ministry of Health and Family Welfare. Delhi: Publication by Controller of Publication. Indian Pharmacopoeia, 2007;2:484-554.
16. Mohammad T, et al., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. International Pharmaceutica Sciencia, 2012;2:14.
17. British Pharmacopoeia. (International Ed.) 1993; Vol. 1:429, 483. Published on the Recommendation of the Medicines Commissions Pursuant to Medicines Act 1968, 1993.
18. United States Pharmacopoeia 29 NF 24, Published on the Recommendation of the Medicines Commissions Pursuant to Medicines, 587.
19. Kealey D and Haines PJ. Analytical Chemistry, 1stedition, Bios Publisher, 2002:1-7.
20. Skoog, West, Holler, Crouch, “Fundamentals of analytical chemistry”, eighth edition, 2009 (Indian edition), Cengage learning India Pvt ltd, New Delhi, 271-280.
21. A.V Kasture KR Mahadik SG, Wadodkar HN. More, “A textbook of pharmaceutical analysis, Instrumental methods”, Nirali Prakashan, vol.2, 9th edition, page no. 5-7, 28-30.
22. Vibha G, et al. Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences, 2012;2:22-23.
23. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc, 2006:88-92.
24. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva, 1996;11.
25. Vibha Gupta, et al. Development and validation of HPLC method - A Review, International Research Journal of Pharmaceutical and Applied Sciences, 2012;2:17-25.
26. Santosh Kumar Bhardwaj, et al. A Review: HPLC Method Development and Validation. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
27. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
28. Lalit V Sonawane. Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
29. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
30. https://go.drugbank.com/drugs/DB08930
31. https://pubchem.ncbi.nlm.nih.gov/compound/Dolutegravir
32. https://en.wikipedia.org/wiki/Dolutegravir
33. Venkatnarayana M and Siva Jyothi N. Development of Validation and Stability Indicating Method of Anti-HIV Dolutegravir Drug and its Related Impurities by Using RP-HPLC, Journal of Chromatography & Separation Techniques, Short Communication, 2020;11.
34. Dr. Prasada Rao PTSRK. RP-HPLC Method Development and Validation for the Simultaneous Estimation of Dolutegravir and Lamivudine in Drug Product, European J of Biomedical AND Pharmaceutical sciences, ejbps, 2018;5:538-543.
35. Sapna M Rathod, Paresh U Patel. Development and Validation of RP – HPLC Method for Estimation of Lamivudine and Dolutegravir Sodium in Synthetic Mixture, Research J. Pharm. and Tech, 2020;13:2864-2868.
36. https://fjps.springeropen.com/articles/10.1186/s43094-020-00026-0
37. Khaleel Noorbasha and Sharmila Nurbhasha, A new validated stability-indicating RP-HPLC method for simultaneous quantification of Dolutegravir and lamivudine in bulk and pharmaceutical dosage form, Future Journal of Pharmaceutical Sciences, 2020;6.