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A REVIEW- ON HPLC METHOD FOR ESTIMATION OF RALTEGRAVIR

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ABSTRACT

The objective of the present review article was to develop an innovative, simple, and economic method for estimation of RALTEGRAVIR by HPLC. The chromatographic conditions were performed on Symmetry Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. as stationary phase and mobile phase was prepared with a mixture of Phosphate buffer(pH=3.0): Methanol with 30:70, flow 1.0 ml/min, with Injection Volume 10µl, at detection wavelength 246 nm and run time at 5.0 min. The analytical method is valid for estimation of Raltegravir over a range of 20 µg/ml–70 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range. A specific, sensitive, economic method estimation of Raltegravir has been developed based on ICH Guidelines with bulk and dosage forms.

Keywords: Raltegravir, HPLC, Method Development, ICH, Validation, Accuracy, Precision.

INTRODUCTION

Raltegravir Potassium, [N-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-2-{2-[(5-methyl-1,3,4-adiazol-2-yl)formamido]propan-2-yl}-6-oxo-1,6-dihydropyrimidine-4-carboxamide, is involves integrase which is an enzyme necessary for the HIV virus to successfully insert its viral DNA into a human host's DNA. The virus must be able to carry out this process in order to use the host's cellular machinery to make copies of its viral DNA in order to successfully spread the HIV infection. Integrase inhibitors, like Raltegravir, block the action of integrase and prevent the HIV virus from successfully inserting its DNA into the host DNA.[1,2]Raltegravir is absorbed with a T max of approximately 3 hours post dose in the fasted state. It is approximately 83% bound to human plasma protein over the concentration range of 2 to 10 µM. The apparent terminal half-life of Raltegravir is approximately 9 hours, with a shorter α-phase half-life (~1 hour) accounting for much of the AUC.[3,4]

Raltegravir (Isentress) is an integrase strand transfer inhibitor (INSTI) used in the treatment of HIV/AIDS. Since its approval in 2007, numerous studies have evaluated its efficacy and safety1. Clinical trials have demonstrated that raltegravir effectively reduces HIV-1 RNA levels and increases CD4+ cell counts in both treatment-naïve and treatment-experienced patients. It is often used in combination with other antiretroviral drugs to enhance its efficacy and reduce the risk of resistance. Research has also focused on the pharmacokinetics and pharmacodynamics of raltegravir, showing that it has a rapid onset of action and a favorable safety profile. Studies have highlighted its potential in pediatric populations, with formulations available for children and adolescents aged 2–18 years.

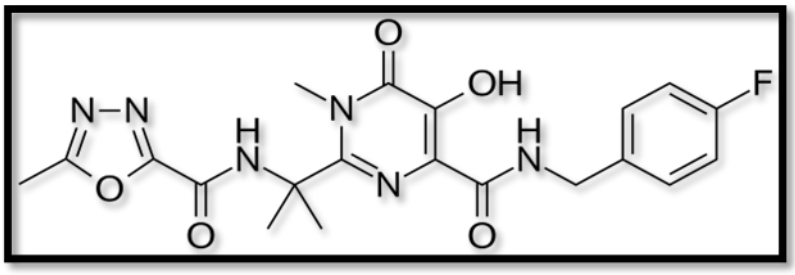

Fig-1: Structure of Raltegravir

Side effects are generally mild, with common ones including trouble sleeping, fatigue, nausea, high blood sugar, and headaches. Severe side effects, such as allergic reactions, muscle breakdown, and liver problems, are rare but require monitoring. Ongoing research continues to explore the long-term effects of raltegravir, strategies to overcome resistance, and its use in various patient populations. The drug remains a key component of highly active antiretroviral therapy (HAART) regimens, contributing significantly to the management of HIV/AIDS.

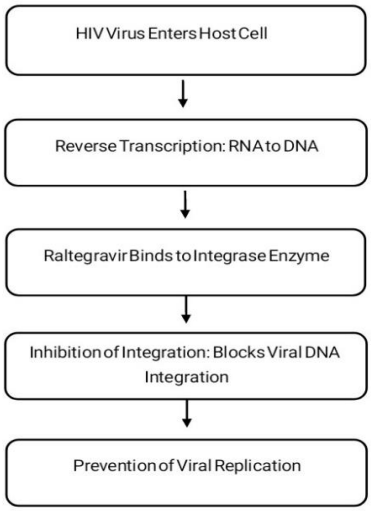

Fig-2: Mechanism of Action of Raltegavir

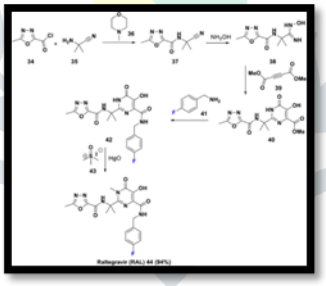

Fig-3: Synthesis of Raltegravir

High Performance Liquid Chromatography

High Performance Liquid Chromatography, which is also known as High Pressure Liquid Chromatography. It is a popular analytical technique used for the separation, identification and quantification of each constituent of mixture. HPLC is an advanced technique of column liquid chromatography. The solvent usually flows through Column with the help of gravity but in HPLC technique the solvent will be forced under high pressures up to 400atm so that sample can be separated into different constituents with the help of difference in relative affinities.

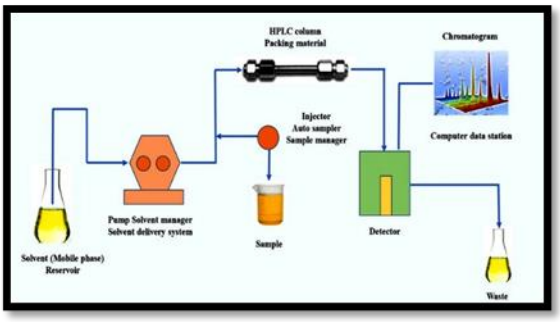

Fig-4: Flow diagram of HPLC

Types of HPLC

Depending on the substrate used i.e. stationary phase used, the HPLC was divided into following types:

Normal Phase HPLC: In this method the separation is based on polarity. The stationary phase is polar, mostly Silica is used and the non-polar phase used is hexane, chloroform and diethyl ether. The polar samples are retained on column.

Reverse Phase HPLC: It is reverse to normal phase HPLC. The mobile phase is polar and the stationary phase is Non-polar or hydrophobic. The more is the non-polar nature the more it will be retained.

Size-exclusion HPLC: The column will be incorporating with precisely controlled substrate molecules. Based on The difference in molecular sizes the separation of constituents will occur.

Ion exchange HPLC: The stationary phase is having ionically charged surface opposite to the sample charge. The mobile phase used is aqueous buffer, which will control pH and ionic strength[5]

Instrumentation

High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for separating, identifying, and quantifying compounds in a mixture. The instrumentation of an HPLC system consists of several key components that work together to perform the analysis. Here's an overview of the main components:

1. Solvent Reservoir

- **Function**: Holds the mobile phase (solvent or mixture of solvents) that will flow through the column.
- **Details**: The mobile phase can be a single solvent or a mixture, and the solvent reservoir maintains the consistency of the mobile phase.

2. Pump

- **Function**: Delivers the mobile phase to the column at a constant flow rate and pressure.
- **Details**: The pump must be capable of providing precise, reproducible flow rates. Common types include:
  - **Isocratic pump**: Uses a single mobile phase composition.
  - **Gradient pump**: Uses a changing mobile phase composition over time for more complex separations.

3. Injector

- **Function**: Introduces the sample mixture into the mobile phase stream.
- **Details**: The sample is typically injected through an automatic or manual injector. The most common types include:
  - **Manual injector**: Requires the operator to load the sample.

- **Auto sampler**: Automatically injects samples from a sample tray.

4. Column

- **Function**: The column is where the separation of the sample components takes place.
- **Details**: The column is packed with a stationary phase (e.g., silica or polymer beads) that interacts differently with the components of the sample, causing them to separate based on their affinity for the stationary phase. Columns come in different sizes and packing materials, depending on the type of analysis.

5. Detector

- **Function**: Detects the components as they elute from the column.
- **Details**: There are various types of detectors used in HPLC, and the choice depends on the nature of the sample and the required sensitivity. Some common types include:
  - **UV-Vis Detector**: Measures the absorbance of UV or visible light by the sample components.
  - **Refractive Index (RI) Detector**: Measures changes in the refractive index as sample components pass through.
  - **Fluorescence Detector**: Measures the fluorescence emitted by components after excitation.
  - **Evaporative Light Scattering Detector (ELSD)**: Measures light scattering from non-volatile components.
  - **Mass Spectrometer (MS)**: Can be used for highly sensitive and specific detection, providing molecular identification of analytes.

6. Data System

- **Function**: Collects and processes data from the detector.
- **Details**: The data system typically includes software that records the detector's signal as a chromatogram and helps to analyze and interpret the data. It also controls and monitors other instruments in the system.

7. Waste Container

- **Function**: Collects the mobile phase and eluted sample components after they pass through the detector.
- **Details**: The waste container stores the used solvents and sample residues safely.

Method Development

The development of an accurate HPLC method involves careful optimization of mobile phases, columns, and detection systems. Various studies have explored different mobile phase compositions, such as mixtures of water and methanol or acetonitrile with phosphate buffers, which are essential for achieving reliable separation of raltegravir [5]. The column selection is often a C18 reversed-phase column, known for its high separation efficiency and stability in the analysis of pharmaceuticals like raltegravir. [6]

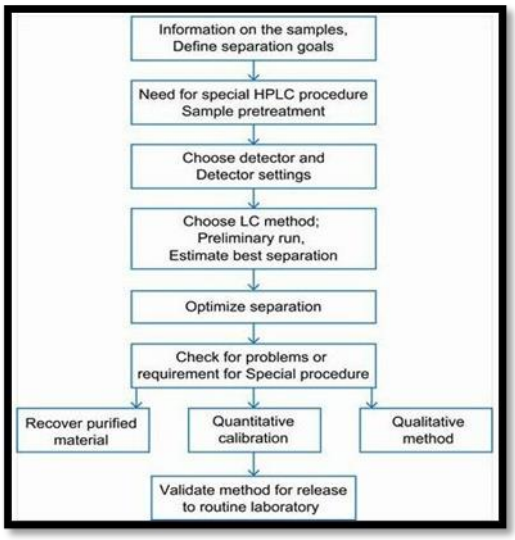

Fig-5: Steps involved in HPLC Method Development

a. Mobile Phase Optimization

For reversed-phase HPLC, the mobile phase commonly used is a mixture of water and organic solvents like methanol or acetonitrile**.** The pH is adjusted (between 3.0 and 4.0**)** to ensure proper solubility and stability of raltegravir during analysis. This range helps prevent degradation or interaction between raltegravir and other components [7] [8].

b. Column Selection

Reversed-phase C18 columns are the most commonly used for the analysis of raltegravir. The choice of column depends on factors such as particle size, column dimensions, and surface chemistry. C18 columns, known for their hydrophobicity, ensure the efficient separation of raltegravir from other potential contaminants in biological matrices.

c. Detection Method

The most widely employed detection technique for raltegravir estimation is UV detection, typically set at 246 nm, a wavelength at which raltegravir absorbs strongly. This UV wavelength is optimal for obtaining sharp, well-defined peaks for the analyte. Fluorescence detection has also been used, but it requires derivatization, which can complicate the method.

Equation for detector response:

$$A = \epsilon \cdot c \cdot l$$

Where:

- A is the absorbance,
- ϵ is the molar absorptivity,
- c is the concentration of raltegravir,
- l is the path length of the cuvette (usually 1 cm).

Method Validation Parameters

The validation of an HPLC method for raltegravir estimation includes testing for specificity**,** linearity**,** precision**,** accuracy**,** and sensitivity. A variety of studies have established the minimum standards for method validation in terms of limits of detection (LOD)**,** limits of quantification (LOQ)**,** and relative standard deviation (RSD) of repeated measurements [9].

Table-1: Parameters of method validation

VALIDATION PARAMETER	REQUIREMENT
Specificity	The ability to measure raltegravir in the presence of interfering substances.
Linearity and Range	Linearity is tested with known concentrations; range defines the accurate quantification limits.
Precision (RSD)	Measures the variability in repeated experiments (intraday and interday).
Accuracy	The closeness of the measured concentration to the true value.
Sensitivity (LOD and LOQ)	Determines the smallest amount of raltegravir that can be detected or quantified.
Robustness	Tests how small changes in conditions affect the results.

Specificity

Specificity is crucial for ensuring that only raltegravir is detected without interference from endogenous substances or potential impurities. Studies have demonstrated that under optimal conditions, HPLC can distinguish raltegravir from matrix components like plasma proteins and excipients in tablet formulations [10].

Linearity and Range

The linearity of the HPLC method is crucial for establishing a reliable concentration-response relationship. The calibration curve must be plotted over a wide concentration range for accuracy. As noted by Sharma and Tyagi [11], the HPLC method for raltegravir is linear between concentrations of 50 mg/mL to 5000 mg/mL**.**

Equation for linearity:

$$y=mx+b$$

Where:

- y is the peak area (or height),
- x is the concentration of raltegravir,
- m is the slope,
- b is the intercept [12].

Precision

Precision is typically reported as Relative Standard Deviation (RSD)**,** which measures the variability between repeated injections of the same sample. Intraday precision (within-day) and interday precision (between-day) should be ≤ 2% for a method to be deemed reliable. In a study by Arooj et al. [13], the RSD for raltegravir was found to be within the acceptable range for both intra-day and inter-day analyses.

Equation for Precision (RSD):

$$RSD= (\mu/\sigma) \times 100$$

Where:

- σ is the standard deviation,
- μ is the mean of the measured values [14].

Accuracy

Accuracy is determined by comparing the measured concentration of raltegravir to a known reference or standard. As per Patel et al. [15], the percentage error in the quantification of raltegravir is usually below 2%, indicating the high accuracy of the method.

Equation for Accuracy (Percentage Error):

$$Percentage\ Error = \{(Cmeasured - Ctrue)/ Ctrue\} \times 100$$

Where:

- Cmeasured is the concentration measured,
- Ctrue is the true or known concentration [16].

Sensitivity (LOD and LOQ)

Limit of Detection (LOD) is the smallest amount that can be detected, while Limit of Quantification (LOQ) is the smallest amount that can be quantified with acceptable accuracy. Kim et al. [17] reported an LOD of 10 mg/mL and an LOQ of 30 mg/mL for raltegravir in plasma.

Equation for LOD and LOQ:

$$LOD = \{(3 \times \sigma)/S\},\ LOQ = \{(10 \times \sigma)/S\}$$

Where:

- σ is the standard deviation of the blank,
- S is the slope of the calibration curve [18]

Table-2: Literature review of Raltegravir

S.NO	COLUMN	MOBILE PHASE	RESULT	REFERENCE
1	Symmetry C8	Methanol: phosphate(40:60v/v)	Flow rate: 0.6mL/min, wavelength: 247nm Injection volume: 20µL Retention Time: 2.881min Linearity: 5-25µg/Ml	[19]
2	Symmetry Develosil ODS C18	Methanol: Phosphate buffer (Ratio - 30:70 v/v)	Flow rate:1.0mL/min Run time: 5.0min Injection volume: 10µL wavelength: 246nm Linearity: Raltegravir 20-70µg/mL	[20]
3	RP Shim packC18	ACN: ammonium acetate buffer: Methanol (50:50v/v)	Flow rate:0.8mL/min Retention Time: 4.31min wavelength: 271nm Linearity: Raltegravir 10-50µg/mL	[21]

Conclusion

HPLC remains a cornerstone analytical technique for the quantification of raltegravir in pharmaceutical formulations and biological matrices. The method's development and validation ensure accurate**,** precise, and sensitive estimation, making it indispensable in clinical applications such as therapeutic drug monitoring**,** quality control**,** and bioequivalence studies**.** Through continuous advancements in column technology, mobile phase optimization, and detection methods, HPLC continues to offer robust solutions for raltegravir analysis.

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