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A REVIEW ON METHOD DEVELOPMENT AND VALIDATION OF DOLUTEGRAVIR BY DIFFERENT ANALYTICAL TECHNIQUES

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ABSTRACT

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, micro organisms, minerals and various synthetic products. As a consequence, analytical method development has become the basic activity of analysis. Recent development in analytical methods has been resulted from the advancement of analytical instruments. The improvement of the analytical method development and analytical instruments like UV, HPLC, LC-MS are required for reduced the time of analysis, increased precision and accuracy and reduced costs of analysis. Dolutegravir (DTG) is an antiretroviral medication used, together with other medication, to treat HIV/AIDS. It may also be used, as part

of post exposure prophylaxis, to prevent HIV infection following potential exposure. Dolutegravir is an HIV-1 integrase inhibitor that blocks the strand transfer step of the integration of the viral genome into the host cell (INSTI). The effect of this drug has no homology in human host cells, which gives it excellent tolerability and minimal toxicity.

KEYWORDS: Analytical method development, validation, Dolutegravir, UV, HPLC, LC-MS.

INTRODUCTION

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results. The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte(s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report. The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many *standard analytical methods* have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards organizations and statutory authorities, instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own *in-house methods* or adapt existing ones for specific purposes.

Method development forms a significant part of the work of most analytical laboratories, and *method validation and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

The purpose of the analysis, the required time scale and any cost constraints;
The level of Analyte(s) expected and the detection limit required;

The nature of the sample, the amount available and the necessary sample preparation procedure;
The accuracy required for a quantitative analysis;
The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;
Possible interference with the detection or quantitative measurement of the analyte(s) and the possible need for sample clean-up to avoid matrix interference;
The degree of selectivity available − methods may be selective for a small number of analytes or specific for only one.
Quality control and safety factors.

Table No. 1: Analytical techniques and principal applications.

Technique	**Property measured**	**Principal areas of application**
Gravimetry	Weight of pure analyte or compound of known as stoichiometry	Quantitative for major or minor components
Titrimetry	Volume of standard reagent solution reacting with the analyte	Quantitative for major or minor Component
Atomic molecular spectrometry	Wavelength and intensity of electromagnetic radiation emitted/ absorbed by the analyte	Qualitative, quantitative or structural or for major down to trace level components
Mass spectrometry	Mass of analyte or fragments of it	Qualitative or structural for major down to trace level components isotope ratios
Chromatography and electrophoresis	Various physicochemical properties of separated analytes	Qualitative and quantitative separations of mixtures at major to trace levels
Thermal analysis	Chemical/physical changes in the analyte when heated or cooled	Characterization of single or mixed major/minor compounds
Electrochemical analysis	Electrical properties of the analyte in solution	Qualitative and quantitative for major to trace level components
Radiochemical analysis	Characteristic ionizing nuclear radiation emitted by the analyte	Qualitative and quantitative at major to trace levels

Table No. 2: Spectrometric Techniques and Principal Applications.

Technique	Basis	Principal applications
Plasma emission spectrometry	Atomic emission after excitation in high temperature gas plasma	Determination of metals and some non-metals mainly at trace levels
Flame emission spectrometry	Atomic emission after flame excitation	Determination of alkali and alkaline earth metals
Atomic absorption spectrometry	Atomic absorption after atomization by flame or electro thermal means	Determination of trace metals and some non-metals
Atomic fluorescence spectrometry	Atomic fluorescence emission after flame excitation	Determination of mercury and hydrides of non-metals at trace levels
X-ray emission spectrometry	Atomic or atomic fluorescence emission after excitation by electrons or radiation	Determination of major and minor elemental components of metallurgical and geological samples
©-spectrometry	©-ray emission after nuclear excitation	Monitoring of radioactive elements in environmental samples
Ultraviolet/visible spectrometry	Electronic molecular absorption in solution	Quantitative determination of unsaturated organic
Infrared spectrometry	Vibrational molecular absorption	Identification of organic compounds
Nuclear magnetic resonance spectrometry	Nuclear absorption (change of spin states)	Identification and structural analysis of organic compounds
Mass spectrometry	Ionization and fragmentation of molecules	Identification and structural analysis of organic compounds

Table No. 3: Separation techniques and principal applications.

Technique	Basis	Principal applications
Thin-layer chromatography	Differential rates of migration of analytes through a stationary phase by movement of a liquid or gaseous mobile phase	Qualitative analysis of mixtures
Gas chromatography	-Do-	Quantitative and qualitative determination of volatile compounds
High-performance liquid chromatography	-Do-	Quantitative and qualitative determination of non-volatile compounds
Electrophoresis	Differential rates of migration of analytes through a buffered medium	Quantitative and qualitative determination of ionic compounds

Analytical method development

It is a process whose main purpose is to prove if any analytical method in the pharmaceutical industry is suitable for use in the measurement of drug substance or drug product.

Analytical chemistry deals with methods for identification, separation, and quantification of the chemical components of natural and artificial materials. The choice of analytical methodology is based on many considerations, such as: chemical properties of the analyte and its concentration sample matrix, the speed and cost of the analysis, type of measurements i.e., quantitative or qualitative and the number of samples. A qualitative method yields information of the chemical identity of the species in the sample. A quantitative method provides numerical information regarding the relative amounts of one or more of the analytes in the sample.

The steps of method development and method validation the steps of method development and method validation.

Method development plan definition

Background information gathering

Laboratory method development, it includes various stages namely sample preparation, specific analytical method, detection and data processing

Generation of test procedure

A well-developed method should be easy to validate. A method should be developed with the goal to rapidly test preclinical samples, formulation prototypes, and commercial samples. There are five common types of analytical methods, each with its own set of validation requirements

Identification tests

Potency assays

Quantitative tests for impurities

Limit test for the control of impurities

Specific tests

The first four tests are universal tests, but the specific tests such as particle-size analysis and X ray diffraction are used to control specific properties of the active pharmaceutical ingredient (API) or the drug product.

The most widely used methods for quantitative determination of drugs and metabolites in biological matrices such as blood, serum, plasma, or urine includes Gas chromatography, (GC) High-performance liquid chromatography, (HPLC) Thin layer chromatography, (TLC) combined GC and LC mass spectrometric (MS) procedures such as LC-MS-MS, GC-MS, and GC-MSMS, techniques like NMR is used for structure identification.

Chromatography in different forms is the leading analytical method for separation of components in a mixture. The chromatographic procedure for the separation of substances is based on differences in rates of migration through the column arising from different partition of the compounds between a stationary phase (column packing) and a mobile phase transported through the system. Chromatographic methods can be classified according to the physical state of the mobile phase into the following basic categories: gas chromatography, (GC) supercritical fluid chromatography (SFC) and liquid chromatography (LC). The technique was originally developed by the Russian botanist M.S. Tswett in 1903.

Analytical method validation

It establishes documented evidence that the procedure adapted for a test is fit for the intended purpose in terms of quality, reliability and consistency of results.

Parameters to be checked for method validation

Selectivity/Specificity

Precision

Accuracy

Linearity

Range

Stability

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Selectivity/specificity

Specificity is ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The terms selectivity and specificity are often used interchangeably. According to ICH the term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said

to be selective. Since there are very few methods that respond to only one analyte, the term selectivity is usually more appropriate.

Precision

ICH defines the precision of an analytical procedure as 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 is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility. Ruggedness as defined in USP XXII <1225>, 1990 incorporates the concepts described under the terms *"intermediate precision"*, *"reproducibility*" and *"repeatability*" of this guide.

Accuracy

The accuracy of an analytical method is the degree of agreement of test results generated by the method to the true value.

Accuracy is measured by spiking the sample matrix of interest with a known concentration of analyte standard and analyzing the sample using the "method being validated." The procedure and calculation for Accuracy (as% recovery) will be varied from matrix to matrix and it will be given in respective study plan or amendment to the study plan.

Linearity

The linearity of an analytical method is its capability to elicit check consequences which might be at once, or with the aid of well described mathematical adjustments, proportional to the concentration of analytes in within a given range.

Linearity is determined by injecting a series of standards of stock solution/diluted stock solution using the solvent/mobile phase, at a minimum of five different concentrations in the range of 50–150% of the expected working range. The linearity graph will be plotted manually/using Microsoft Excel or software of the computer (Concentration vs. Peak Area Response) and which will be attached to respective study files.

Range

The range of an analytical method is the interval between the upper and lower levels that have been demonstrated to be determined with precision, accuracy and linearity using the set method. This range will be the concentration range in which the Linearity test is done.

Stability

Many analytes readily decompose prior to chromatography investigations, for example during the preparation of the sample solutions, during extraction, clean-up, phase transfer, and during storage of prepared vials. Under these circumstances, method development should investigate the stability of the analyte. Accuracy test takes care of stability. It is required to mention in the method how long a sample after extraction can be stored before final analysis, based on the duration taken for accuracy test.

Limit of detection and limit of quantitation

The limits of detection (LOD) and quantification (LOQ) are defined as the lowest concentration of the analyte that can be reliably detected and quantified, respectively. Usually the LOD and LOQ refer to the limits associated with 95% probability of obtaining a correct result Limit of detection.

High Performance Liquid Chromatography (HPLC)

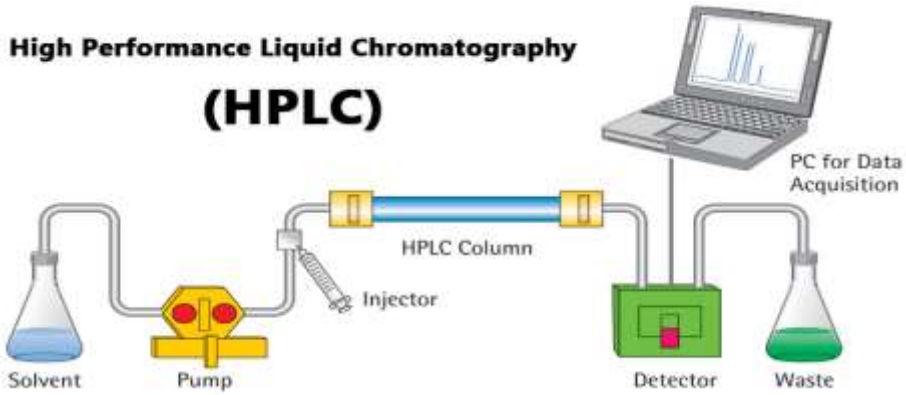
High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify specific components in mixtures. The mixtures can originate from food, chemicals, pharmaceuticals, biological, environmental and agriculture, etc, which have been dissolved into liquid solutions.

It relies on high pressure pumps, which deliver mixtures of various solvents, called the mobile phase, which flows through the system, collecting the sample mixture on the way, delivering it into a cylinder, called the column, filled with solid particles, made of adsorbent material, called the stationary phase.

Each component in the sample interacts differently with the adsorbent material, causing different migration rates for each component. These different rates lead to separation as the species flow out of the column into a specific detector such as UV detectors. The output of the detector is a graph, called a chromatogram. Chromatograms are graphical representations

of the signal intensity versus time or volume, showing peaks, which represent components of the sample. Each sample appears in its respective time, called its retention time, having area proportional to its amount.

HPLC is widely 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. Chromatography can be described as mass process involving adsorption and/or partition. As mentioned, HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with adsorbent, leading to the separation of the sample components. The active component of the column, the adsorbent, is typically a granular material made of solid particles (*e.g.*, silica, polymers, etc.), 1.5–50 μm in size, on which various reagents can be bonded. The components of the sample mixture are separated from each other due to their different degrees of interaction with the adsorbent particles. The pressurized liquid is typically a mixture of solvents (*e.g.*, water, buffers, acetonitrile and/or methanol) and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and adsorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination.



Liquid chromatography–Mass spectrometry (LC–MS)

Liquid chromatography–mass spectrometry (LC–MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography - MS systems are popular in chemical analysis because the individual capabilities of each technique

are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity. This randem technique can be used to analyze biochemical, organic, and inorganic compounds commonly found in complex samples of environmental and biological origin. Therefore, LC-MS may be applied in a wide range of sectors including biotechnology, environment monitoring, food processing, and pharmaceutical, agrochemical, and cosmetic industries.

Ultra violet (UV) Spectroscopy

Ultraviolet (UV) spectroscopy or ultraviolet–visible (UV–VIS) spectrophotometry refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum. Being relatively inexpensive and easily implemented, this methodology is widely used in diverse applied and fundamental applications. The only requirement is that the sample absorb in the UV-Vis region, i.e. be a chromophore. Absorption spectroscopy is complementary to fluorescence spectroscopy. Parameters of interest, besides the wavelength of measurement, are absorbance (A) or transmittance (%T) or reflectance (%R), and its change with time.

A UV-vis spectrophotometer is an analytical instrument that measures the amount of ultraviolet (UV) and visible light that is absorbed by a sample. It is a widely used technique in chemistry, biochemistry, and other fields, to identify and quantify compounds in a variety of samples.

UV-vis spectrophotometers work by passing a beam of light through the sample and measuring the amount of light that is absorbed at each wavelength. The amount of light absorbed is proportional to the concentration of the absorbing compound in the sample.

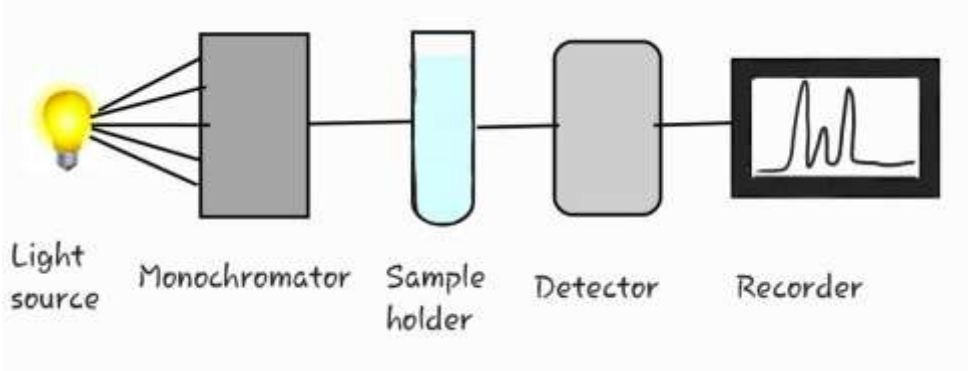


Fig. UV Instrumentation.

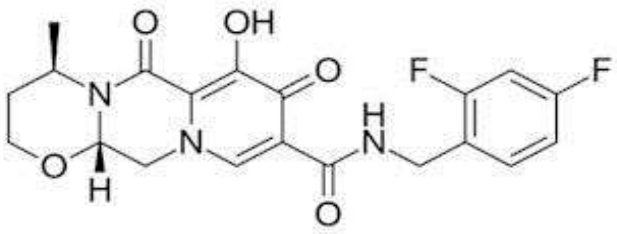
Ultra Performance Liquid Chromatography (UPLC)

Ultra Performance Liquid Chromatography (UPLC) is the newest technology in liquid chromatography based analysis. UPLC is the upgrade high performance liquid chromatography with high pressures, outstanding in both peak resolution and sensitivity. As well as higher sample throughput obtain more available sample information and the decrease separation time consumption. UPLC can provide us with impactful results within their organization. Ultra Performance Liquid Chromatography (UPLC) Based Analysis Services at Creative Proteomics offers you a state-of-the-art liquid separations platform that includes standard UPLC with frequently used separation mechanisms.

DRUG PROFILE

Name: Dolutegravir

Description: Dolutegravir, a second-generation integrase inhibitor for the treatment of HIV-1 infection.



IUPAC Name: 4R,12As)-N-(2,4-diflurobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido(1,2,4,5)pyrazino(2,1-b)(1,3)oxazine-9-carboxamide.

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

Molecular mass: 419.38g/mol

Category: Dolutegravir is a class of medications called HIV integrase inhibitors.

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.

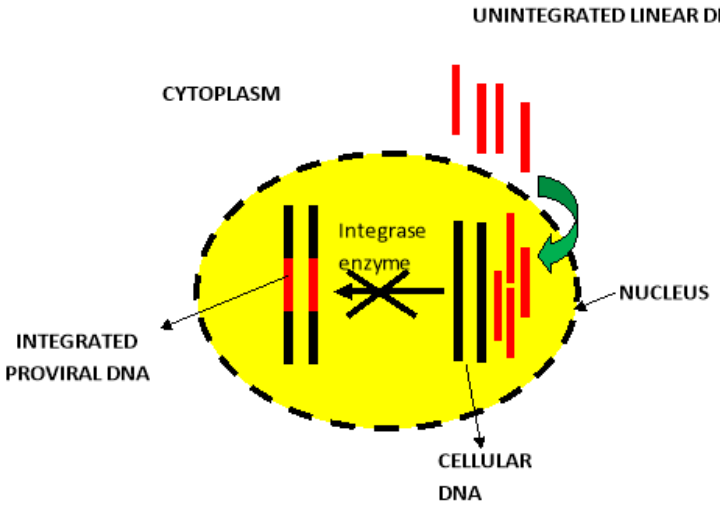


Fig. Mechanism of action of Dolutegravir.

Pharmacodynamics: 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.

Absorption: rapid oral absorption.

Volume of distribution: 17.4L

Protein binding: Human plasma proteins reaching a percentage 98.9%.

Metabolism: UDP-glucuronosyltransferase (UGT)1A1 & cytochrome P450(CYP)3A4

Route of elimination: Fecal excretion is the primary route of elimination and reflects both elimination and reflects both elimination of unabsorbed material and biliary secretion of dolutegravir or its metabolic products.

Half-life: 13 to 14 hours.

Toxicity: Minimal toxicity.

Brand names: Dolulivir, instgra, doluvir, naivex, tivicay, myltega, tegrad-50, xapavir.

Table 4: Solubility studies of Dolutegravir.

SOLVENTS	SOLUBILITY
Water	Soluble
Sodium Tri citrate	Soluble
Orthophosphoric acid	Soluble
Methanol	Slightly Soluble
Sodium benzoate	Soluble
Acetonitrile	Soluble

REVIEW OF LITERATURE

1. **Dongshengli, et al., (2023):** Dolutegravir (DTG) has been the first-line drug in many human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) guidelines for the treatment of naïve and experienced HIV-infected individuals, which calls for cost-effective and convenient methods for quantitative detection of DTG in human plasma for pharmacokinetic studies and patient adherence evaluation. Here, an HPLC–ultraviolet method in combination with liquid–liquid extraction with isocratic elution was developed for the first time. The analysis was performed on a CLC-ODS column (6 mm internal diameter × 15 cm, 5 μm) using a mixture of acetonitrile and phosphate buffer (40:60, *v/v*) as the mobile phase at the flow rate of 1 mL/min. Using triamcinolone as the internal standard, 100 μL of plasma sample was extracted by methyl tert-butyl ether, followed by evaporating under nitrogen stream, re-dissolving with 100 μL mobile phase, and injection of 20–40 μL of supernatant into the chromatographic system. The linearity of DTG was good in the range of 0.05–10 μg/mL ($r = 0.9995$), and the inter- and intra-day variabilities were 0.4%–4.3% ($n = 10$) and 1.2%–6.2% ($n = 10$) for the lower limit of quantification, low-, medium-, and high-concentration quality control samples (0.05, 0.1, 0.8, and 8 μg/mL), respectively, while the methodological and extraction recoveries were 98.0%–103.0% ($n = 20$) and 65.2%–75.7% ($n = 3$), respectively. This method was successfully applied to analyze DTG plasma concentration in 84 Chinese patients with HIV.
2. **Naveen pathakala, et al., (2022):** High Performance Liquid Chromatography (HPLC) is one of the effective separation analytical tools to determine and quantitate the impurities. By using HPLC, we can separate a mixture of compounds to identify and quantify into individual components. In literature survey, various quantification methods are available for the determination of dolutegravir in combined dosage form. The present research paper describes a simple, a precise reversed-phase high performance liquid chromatography (RP-HPLC) method for quantification of dolutegravir in bulk and pharmaceutical dosage form (tablets). Detection was carried out at 256 nm. The mobile phase used as pH-3.6 phosphate buffer : acetonitrile in ratio of (40:60) v/v with flow rate of 1 ml/min. All the parameters of validation were found in the acceptance range of ICH guidelines. The method was found to be linear and correlation coefficient obtained was 0.9996. The system suitability parameters were found to be within the limits.
3. **Manikandan velu swamy, et al., (2022):** A simple, rapid, and robust reverse phase HPLC method was developed and validated for the determination of related substances

for Dolutegravir dispersible tablets 10mg. The primary goal of this research is to develop and validate a new RP-HPLC method for validating the amount of Impurity B (degradation impurity) as a related substance following USP guidelines. Dolutegravir and its impurities were separated using chromatographic conditions on a Phenyl-Hexyl (250 × 4.6 mm), 5µ column with a 45% buffer (sodium dihydrogen phosphate dihydrate and EDTA): 49% methanol: 6% acetonitrile mixture and a pH of 2.5 ± 0.05 adjusted with orthophosphoric acid. The flow rate in isocratic elution mode was 1.2 mL/min. The column temperature was kept constant at 35°C, and the eluted compounds were measured at a wavelength of 258 nm using the PDA detector. The system suitability and other validation parameters were found to be within the limits. The method was sensitive because the LOD and LOQ demonstrate its sensitivity. The linearity curves for Dolutegravir and Impurity B were found to be linear, with a correlation coefficient of at least 0.997. The average percentage of impurities recovered ranged between 80% to 120%.

4. **Vaishnavi dulange, et al., (2021):** UV spectroscopic method was developed for the estimation of Dolutegravir in bulk and Formulation. The UV spectrum of Dolutegravir in methanol and water mixture showed ? max at 254nm. Beer's law is valid in the concentration range of 10-50µg/ml. This method was validated for linearity, accuracy, precision, LOD and LOQ. The method has demonstrated excellent linearity over the range of 10-50µg/ml with regression equation y = 0.030x + 0.008 and regression correlation coefficient r2= 0.998. Moreover, the method was found to be highly sensitive with LOD (2.056µg/ml) and LOQ (6.230µg/ml). Depending on results the given method can be successfully applied for assay of Dolutegravir in formulation.
5. **A Poonam, et al; (2021):** To develop an environment-safe aqueous solubility enhancement method of poorly water-soluble drugs is the need of the pharmaceutical field. Because when organic solvents were used for solubility enhancement, there are many disadvantages like carcinogenicity, environment pollutant, flammable, toxicity, and cost. The hydrotropic method has been used to enhance the aqueous solubility of poorly water-soluble drugs. Dolutegravir is slightly soluble in water. To enhance aqueous solubility various hydrotropes were used and optimized the concentration of each hydrotrope. From these hydrotropes, a mixture of 10% sodium tri citrate and 10% sodium benzoate was selected because Dolutegravir was completely soluble in a mixed hydrotropic solution. The maximum wavelength of Dolutegravir in the hydrotropic mixture was found a 268nm, and the linearity curve in the range of 5-25µg/ml. A

regression coefficient was found to be 0.999. Percent label claim, accuracy (% RSD) were found 99.58%, 0.13(80%), 0.11(100%), 0.13(120%), respectively. The LOD for Dolutegravir was determined to be 0.037µg/ mL, and LOQ was found to be 0.11µg/mL.

6. **M. venkatanarayana, 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 eluMtion mode and the wave length for detection is 240 nm (UV detector). 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.
7. **Girja B. bhavar, et al., (2015):** Simple, sensitive, precise, and specific high-performance liquid chromatographic (HPLC) and high-performance thin-layer chromatographic (HPTLC) methods for the determination of dolutegravir sodium in bulk drug and pharmaceutical dosage form were developed and validated. In the HPLC method, analysis of the drug was carried out on the ODS C18 column (150 × 4.6 mm, 5 µm particle size) using a mixture of acetonitrile: water (pH 7.5) in the ratio of 80:20 v/v as the mobile phase at the flow rate 1 mL/min at 260 nm. This method was found to be linear in the concentration range of 5–35 µg/mL. The peak for dolutegravir sodium was observed at 3.0 ± 0.1 minutes. In the HPTLC method, analysis was performed on aluminum-backed plates pre-coated with silica gel G60 F254 using methanol: chloroform: formic acid in the proportion of 8:2:0.5 v/v/v as the mobile phase. This solvent system was found to give compact spots for dolutegravir sodium with the *Rf* value 0.77 ± 0.01. Densitometric analysis of dolutegravir sodium was carried out in the absorbance mode at 265 nm.

CONCLUSION

As we have done the literature review on the dolutegravir, majority of the ones are with HPLC. UV, GC analytical methods is used lesser times. Despite the fact that only a few strategies of estimation of on top of medicine square measure offered, several of them suffer from one disadvantage or the opposite. Hence its planned to enhance the present strategies

and to develop new strategies for the assay & stability studies of dolutegravir in pharmaceutical dose forms adapting totally different offered analytical techniques like Ultraviolet illumination spectrophotometry, UPLC and LC-MS.

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CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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