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Analysis of Dolutegravir, an HIV Integrase Inhibitor: A Comprehensive Pharmaceutical Assessment Profile

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Abstract: The present study evaluated the impact of HIV-Integrase inhibitors (IN) on HIV patients and the analytical methods used for their evaluation and measurement. The introduction of IN inhibitors has revolutionized antiretroviral therapy (ART), benefiting approximately 27.4 million individuals "since the launch" of the first antiretroviral drug "Azidothymidine". IN inhibitors have become the preferred first-line therapy due to their high resistance barrier. Elvitegravir, Raltegravir, Dolutegravir and Bictegravir are approved IN inhibitors by the US FDA. The literature suggests that liquid chromatography-tandem mass spectrometry and high-performance liquid chromatography are commonly used methods for drug estimation with a few reports mentioning spectrophotometric, spectro-fluorimetric and electro-chemical methods. The review also emphasizes the need for further research on determining impurity profiles and ensuring drug stability, which would improve the safety and quality of antiretroviral drugs in compliance with ICH guidelines. Overall, the advent of IN inhibitor has significantly impacted HIV treatment, and analytical techniques play a crucial role in their quantification and evaluation.

Keywords: Dolutegravir, Antiretroviral therapy, Integrase inhibitors, LC-MS, HPLC, HPTLC, QbD

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Introduction

Antiviral medication dolutegravir is combined with other antiretroviral medications to treat HIV-1 infections. Dolutegravir (Fig. 1) is sold under the brand names Juluca, Dovata, and Tivicay. Dolutegravir, an inhibitor of the HIV integrase, is

the third treatment in the collection. Only used in concert with other antiretroviral drugs to treat HIV infection, dolutegravir has a brief history of usage. Dolutegravir is not linked to cases of acute, clinically significant liver damage, but rather with

modest levels of medication-induced sera aminotransferase elevations. Dolutegravir is an integrase strand-transfer inhibitor (INSTI) that can be taken orally that prevents HIV-1 infection. Dolutegravir taken orally forms an attachment to the integrase's active site and helps transfer viral genetic information into human chromosomes (Back *et al.*, 2001). Integrase cannot connect to antiretroviral DNA as a result, blocking the strand transfer phase that is required for the HIV replication cycle and replication is thus halted. An HIV-1 antiviral drug is called dolutegravir. By attaching to the locations of activity and preventing the strand's exchange stage of retroviral Genome incorporation in the host cell, it prevents HIV integrase from completing its task. In the HIV replication cycle, strand transfer is a crucial stage that inhibits viral activity. In MT-4 cells and peripheral blood mononuclear cells (PBMCs), the median EC₅₀ for dolutegravir ranges from 0.50 nM (0.21 ng/ml) to 02.10 nM (0.85 ng/ml) (Hajimahdi and Zarghi, 2016). The pyridine carboxylic acids and derivatives class of organic compounds includes this chemical. These are chemicals with a derivative or pyridine ring joined to a carboxylic acid group. The IUPAC code for it is "3S,7R"-N-((2,4-di fluoro phenyl)methyl)"Di aza tri cyclo-11 hydroxy 7 methyl" 9,12 dioxo-4 "oxa-1,8 (8.4.0.0, 3,8)"tetra deca-10,13-di ene"- "13-carb oxa mide".

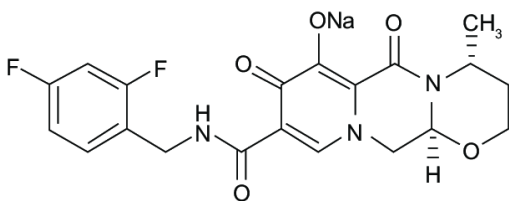


Fig. 1: Structure of Dolutegravir.

Pharmacokinetics:

Adults with HIV-1 infection received oral dolutegravir every day. Through three primary mechanisms, dolutegravir is extensively metabolised, however, it does not produce any persistent intermediates (Chaudhary *et al.*, 2021). The initial pathway is established by UGT1A1's glucuronidation, CYP3A4's carbon oxidation, and

the final step seems to be a "oxidative de-fluorination" and "glutathione conjugation" as the third process. The major metabolite in plasma is the glucuronide ether form M2, and because of its ineffectiveness in binding metal ions due to its chemical characteristics, it is inactive (Castellino *et al.*, 2013). 50 mg dose of dolutegravir causes a 17.4 L apparent volume of distribution (Song *et al.*, 2012). Dolutegravir is nearly completely recovered after a single oral dose when it is excreted in a ratio of 53% unchanged in faeces and 31% unchanged in urine. The renally eliminated recovered dose is composed of an unaltered drug (1%) an intermediate formed by benzylic carbon oxidation (3%) and a hydrolytic N-dealkylation product (3.6%) (Castellino *et al.*, 2013)

Analytical Methods:

Table 1 illustrates the analytical methods which are used for Dolutegravir analysis.

LC-MS Method:

Tert-butyl methyl ether was used for the liquid-liquid extraction of the sample with DTG-d5 serving as a standard for internal use (Bennetto-Hood *et al.*, 2014). DTG was extracted using a reverse phase C18 Waters XBridge (3.5 m: 2.150 mm) column and a MP containing formic acid 0.1% in deionized water or methanol. Over the calibration range of 10–4000 ng/ml the test's accuracy was confirmed.

DTG is obtained by utilising an ultrasound machine to separate it from human hair samples, and it follows by incubating for an entire night at 40°C in a mixture of 50:50 MeOH:ACN and formic acid 2% (Sykes *et al.*, 2018). Post the extraction process, samples are analysed using reverse-phase chromatography using a Waters Atlantis T3 (50 x 2.1 mm, 3-μm particle size) column on an Sciex AB quadrupole mass spectrometer. The outcomes are then determined using positive ion electrospray ionisation. As an internal reference, the assay uses stable, isotopically labelled ¹³C, d5-DTG. With a capacity to extract between 1 and 10 mg of hair/ml of extraction solvent, the calibration range is 5–10,000 pg DTG/ml.

Table 1: Analysis of Dolutegravir

S. No.	Drug	Method	Description	Reference
1.	Dolutegravir in bulk and tablet dosage form	UPLC –UV	Mobile phase : Ammonium acetate : Formic acid 50% (A) Acetonitrile 100 % (B) Column: EHB C8 column(2.1 x100mm Flow Rate : 0.3 ml / min Wavelength : 258 nm.	Xinzhu <i>et al.</i> (2016)
2.	Dolutegravir in bulk and pharmaceutical dosage form	HPLC and HPTLC	HPLC – Column: ODS C18 column(150 x 4.6 mm) Mobile phase: acetonitrile: water pH7.5 (80:20 %) Flow Rate : 1 ml / min U.V. detection range :260. nm. HPTLC – Column: G 60 F 254 column Mobile phase: Methanol: Chloroform: Formic acid (8:2:0.5%) U.V. detection range: 265 nm.	Girija <i>et al.</i> (2016)
3.	Dolutegravir in Human Plasma	HPLC	Column : ODS 2 C18 column (150 x 4.6) Mobile phase : Sodium Acetate (pH4.0) : Methanol (30:70) Flow Rate : 1.0 ml /min Retention time : 2.08 min Wavelength : 254 nm	Satyadev <i>et al.</i> (2015)
4.	Dolutegravir Pharmaceutical Dosage Form	RP- UPLC	Column : BEH C18 (50 cmX 3.0 mm) Mobile phase : Dipotassium HydrogenOrthoposphate : Methanol (30:70) Retention time : 2.857 min Wavelength : 260 nm	Murali Krishna <i>et al.</i> (2018)
5.	Dolutegravir in Rat Plasma	HPLC	Column : Phenomex C18 (150 x 4.6 mm, 5 m) Mobile phase : Ortho phosphoric acid: acetonitrile (60:40) Flow Rate : 1.0 ml/min Retention time : 4.35min Wavelength : 262 nm	Veeraswami and Naveen (2019)
6.	Dolutegravir Pharmaceutical Dosage Form	UV-HPLC	Column : Primesil C18 (150 x 4.6 mm, 5 m) Mobile phase : Methanol:water (pH 6) (70:30) Flow Rate : 0.8 ml/min Wavelength : 255 nm	Ujjwala <i>et al.</i> (2018)

Dolutegravir (DTG) in human plasma was quantified. Dolutegravir concentrations in plasma samples were determined using an LC-MS/MS device. In this LC-MS/MS method, the Multiple Reaction Monitoring (MRM) mode was used for the analysis. Solid Phase Extraction was used to separate Internal Standard Dolutegravir D6 and Dolutegravir from K3EDTA-based Human Plasma samples (Castellino *et al.*, 2013; Bennetto-Hood *et al.*, 2014). The prepared samples were then examined using an acetonitrile:water (80:20 v/v) MP with formic acid of 0.1 ml at 1 ml/min flowrate with 80% flow splitting on a Phenomenex Luna C18 (Hajimahdi and Zarghi, 2016), 50 mm 4.6 mm, 5 m column. For measurement, the "Peak area ratio" approach was applied. All of the models and the values used in the validation stage were created by the "Analyst Software".

Measuring the unbound Dolutegravir:

Measuring the free Dolutegravir (DTG) plasma level (Cu) may help with dolutegravir therapeutic medication monitoring, especially for patients who display virological failure or toxicity despite having adequate DTG total plasma concentrations. The two primary techniques for determining the concentration of the unattached DTG form are "Equilibrium dialysis (ED) and Ultrafiltration (UF)". The ultrafiltration settings were contrasted with ED (Hee *et al.*, 2014) in order to assess observations of concentrations in plasma made using this method. LC-MS/MS was used to determine DTG concentrations. Using a previously described liquid chromatography tandem mass spectrometry procedure, the amounts of unattached and total dolutegravir were quantified. This process requires mixing 50 ml of plasma, dialysate, or ultrafiltrate (ABSciex API-4500) with 200 ml of a precipitating reagent containing a deuterated internal standard.

In first-line antiretroviral therapy, dolutegravir is currently the drug of choice. For clinical pharmacology investigations in important populations, quantitative analytical techniques that can be applied in resource-constrained environments are recommended. The researchers

developed and evaluated a technique that combines liquid chromatography and ultraviolet light to find dolutegravir in dried blood spots (DBS). "Calibration standard" and "quality control samples" were generated by dropping 50 l of dolutegravir-laced whole blood onto each DBS card circle. Three sections were to be removed with methanol using two 6-mm punches in each. Pioglitazone was utilised as the internal standard and "acetonitrile/potassium phosphate monobasic buffer" (pH 5) was gradually eluted on a reverse-phase C18 column at 1 ml/min flowrate to accomplish chromatographic separation. UV detection was done at 260 nm. DBS was collected from individuals (n=10) by a finger prick over the period of 12 h at 8 different time points, with matched plasma at 1 and 12. The method was applied to quantify dolutegravir and ascertain its pharmacokinetic properties. The linearity and Bland-Altman plots were utilised to assess agreement between DBS and plasma concentrations.

Dolutegravir (DTG) clinical pharmacokinetics in plasma samples were investigated using a sensitive LC-MS/MS technique that was developed and validated. A simple acetonitrile protein precipitation, an internal standard that is a stably labelled isotope of DTG, and an assay method that only needs a 20-l aliquot of human plasma. A 2.1 mm x 50 mm X Bridge C18 reversed phase analytical column was used for chromatography, and MP 60:40 ACN:WATER and formic acid 0.1%. Utilising tandem mass spectrometry with ESI positive ionisation, the analyte and internal standard were located. The provided assay system gives a sensitive, exact, and accurate method for quantifying DTG. As part of a phase I/II paediatric clinical trial, it was successfully employed to examine clinical research samples.

HPLC and HPTLC Methods:

The drug was tested by HPLC using an ODS C18 column (150 mm x 4.6 mm, 5 m particle size) and an 80:20 v/v combination of acetonitrile:water (pH 7.5) as the mobile phase, with a flow rate of 1 ml/min at 260 nm. Silica gel G60 F254 was

analysed using the HPTLC procedure on aluminum-backed plates that had been pre-coated with formic acid, methanol, chloroform, and in the ratios of 0.5:8:2 as MP (Hemanth Kumar *et al.*, 2010; Gu *et al.*, 2020). An absorbance mode densitometric study of dolutegravir sodium was determined at 265 nm. Accuracy, specificity, LOQ, precision, and LOD were all validated. Both techniques are reproducible and specific for drug estimate, according to statistical analysis. The techniques can be applied to routine quality control evaluations of dolutegravir sodium.

A UV-Visible spectrophotometer (Ujjwala *et al.*, 2018) was used to assess the drug at a wavelength of 255 nm, and phosphate buffer was used as the solvent to compute the absorbance of dolutegravir sodium. Using a Primesil C18 column (250 x 4.6 mm, 5 µm particle size), an HPLC technique was used to analyse the drugs. The MP consists of methanol:water at pH 6, 70:30, 0.8 ml/min flowrate at 255 nm at 40 °C.

QbD:

The Quality by Design (QbD) methodology and ICH Q8(R2) criteria were used to create spectrophotometric techniques for dolutegravir quantification. By adjusting different parameters, the QbD technique was applied. Direct observation and principal component analysis were both employed to determine the critical parameters (Simiele *et al.*, 2017). The suggested method was validated using the ICH Q2 criteria for a number of factors, including system appropriateness, accuracy, precision, linearity, detection, and quantification limitations. Solvent: methanol, slit-width: 0.5, scan-speed, sample interval: 0.2 nm (R1), and wavelength 260 nm were estimated to be the key spectrophotometric technique parameters.

The USFDA, Canada, and European Regulatory Bodies have all given dolutegravir their approval as an antiretroviral medication. A thorough description of the findings of DLG forced degradation investigations conducted under the necessary stress conditions specified by the ICH.

DLG was shown to be stable under thermal and photolytic stress but unstable under acidity and oxidant conditions. While they were separated by preparative (Venkatnarayana and Siva, 2020) HPLC on a C18 column using the gradient elution method, one degradant from each solution that had been treated with acid and peroxide was identified by LC-MS as DP-1 and DP-2. The DP-1 and DP-2 peaks were then sent to HRMS for precise mass determination. For structural verification, an NMR spectroscopic investigation of DP-1 and DP-2 was also performed. An *in vitro* study employing HepG2 cells revealed that DLG and the byproducts of its forced breakdown are not harmful.

Conclusion

The introduction of HIV-Integrase inhibitors (IN) has revolutionized antiretroviral therapy and greatly benefited HIV patients worldwide. These inhibitors, such as Raltegravir, Elvitegravir, Dolutegravir, and Bictegravir, have become the preferred first-line therapy due to their high resistance barrier. The application of analytical methods for the measurement and characterization of IN inhibitors, including LC-MS/MS, HPLC, and other spectroscopic or electrochemical methods, is essential for monitoring drug levels, evaluating patient adherence, assessing drug interactions, and studying pharmacokinetics. While LC-MS/MS and HPLC are commonly employed methods, the literature also mentions spectrophotometric, spectrofluorimetric, and electrochemical techniques. Adherence to international guidelines, particularly the ICH guidelines, is crucial for developing and validating analytical methods to ensure their accuracy, reliability, and reproducibility. Further research is needed to explore impurity profiles and enhance drug stability, as this will contribute to the overall quality and safety of antiretroviral drugs.

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