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ANALYTICAL METHODS FOR THE DETERMINATION OF DOLUTEGRAVIR IN PHARMACEUTICALS: A MINI REVIEW

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Abstract:

Ganciclovir is a purine analogue used in the treatment of cytomegalovirus diseases. The literature survey on dolutegravir revealed a number of analytical methods for its quantification. Present review is focused on method conditions, linearity offered, sensitivity, accuracy, precision and assay results of various analytical methods.

Keywords: *Ganciclovir, Cytomegalo virus, Linearity, Spectroscopy, HPLC, HPTLC.*

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1. INTRODUCTION:

Dolutegravir is used as second-generation HIV-1 integrase strand transfer inhibitor[1]. Chemically it is known as 4*R*,(12a*S*)-*N*-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5] pyrazino[2,1-*b*][1,3]oxazine-9-carboxamide[2]. The molecular weight of dolutegravir is 441.36 corresponding to the molecular formula $C_{20}H_{18}F_2N_3NaO_5$. The structure of dolutegravir was shown in Figure 1. There were a number of literatures on analytical methods of dolutegravir are available. Present article describes various Visible, UV spectrophotometric, spectrofluorimetric and reverse phase high performance liquid chromatographic (RP-HPLC) methods reported since 2010 for the quantification of dolutegravir in bulk and its formulations.

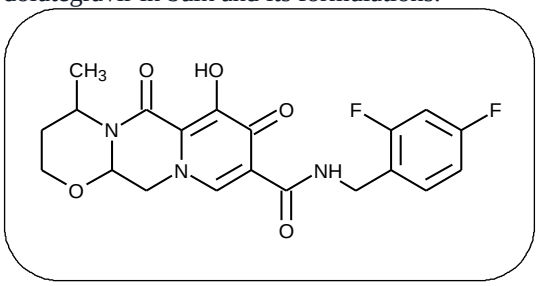

Figure 1: Structure of Dolutegravir

2. Analytical Methods for dolutegravir

2.1 Visible spectroscopic methods

There are several methods for analyzing the same, however, they are time-consuming and costly. Hence, created a new spectrophotometric method for determining Dolutegravir (DLT) in tablet dosage forms that is simple, accurate, and precise. In methanol, the initial stock solution of Dolutegravir was prepared. The method is based on the formation of a blue color chromogen complex from Dolutegravir oxidation-reduction with Ferric chloride in the presence of potassium ferricyanide. Result: The color complex was measured at 710nm. Beers law was observedin the concentration range of 3.5-6.5µg/ml witha coefficient of correlation (R2) was 0.998. The system suitability criteria were found to be within the limits. The LOD and LOQ were found to be 0.91 and 2.47, indicating that the method is sensitive. Conclusion: The relative standard deviation (RSD) and percent recovery values were found to be satisfactory, indicating that the proposed method is suitable, accurate, and precise and that it can be used in routine analysis of Dolutegravir in tablet dosage forms, with relatively low-cost solvents[2].

A simple and sensitive spectrophotometric method was developed for the quantitative measurement of dolutegravir in pure form and pharmaceutical formulation. The present method was based on redox reaction between dolutegravir and ferric chloride, which upon complexation with 1,10-phenanthroline formed an orange-coloured complex that showed absorption maximum at 520.0 nm. The developed method obeyed linearity in the concentration range of 40.00 – 140.00 µg/mL. The method was also validated as per International Council for Harmonization guidelines and the results were within acceptance values. The validated method was employed for the determination of dolutegravir in pharmaceutical dosage form and the percentage assay value was found to be 102.5, which agrees with its label claimed. The developed redox-based colorimetric method could be used in the routine quality control analysis of dolutegravir present in various pharmaceutical dosage forms[3].

In the present research work, developed and validated a simple, accurate and precise colorimetric method for analysis of dolutegravir tablet dosage form as per International Conference of Harmonization guidelines The initial stock solution of Dolutegravir was prepared in methanol. Based on the method the formation of orange colour by oxidation-reduction of dolutegravir with Ferric chloride in the presence of 1,10-phenanthroline. The wavelength was measured at 505nm. Beers law was observed in the conc. range of 3-21µg/ml with coefficient of correlation (R2) was 0.999. The system suitability criteria found to be within the limits. LOQ and LOD was found to be 0.711, 2.134 respectively and it demonstrate that the method is sensitive[4].

2.2 UV-spectrophotometric methods

A simple, rapid, precise and accurate spectrophotometric method has been developed for quantitative analysis of Dolutegravir sodium in tablet formulations The initial stock solution of Dolutegravir sodium was prepared in methanol solvent and subsequent dilution was done in water. The standard solution of Dolutegravir sodium in water showed maximum absorption at wavelength 259.80 nm. The drug obeyed Beer–Lambert's law in the concentration range of 5–40 µg/mL with coefficient of correlation (R2) was 0.9992. The method was validated as per the ICH guidelines. The developed method can be adopted in routine analysis of Dolutegravir sodium in bulk or tablet dosage form and it involves relatively low cost solvents and no complex extraction techniques[5].

A simple, rapid, precise and accurate spectrophotometric method has been developed for quantitative analysis of Dolutegravir sodium in tablet formulations. The initial stock solution of Dolutegravir sodium was prepared in methanol solvent and subsequent dilution was done in water. The standard solution of Dolutegravir sodium in water showed maximum absorption at wavelength 259.80 nm. The drug obeyed Beer–Lambert's law in the concentration range of 5–40 µg/mL with coefficient of correlation (R2) was 0.9992. The method was validated as per the ICH guidelines. The developed method can be adopted in routine analysis of Dolutegravir sodium in bulk or tablet dosage form and it involves relatively low-cost solvents and no complex extraction techniques[6].

The term hydrotropy has been used to designate the increase in solubility in water of various substances due to the presence of large amount of additives. Concentrated aqueous hydrotropic solutions of sodium benzoate, urea, nicotinamide, sodium salicylate, sodium ascorbate and sodium glycinate have been observed to enhance the aqueous solubility of many poorly water-soluble drugs. In the present investigation hydrotropic solubilization technique has been employed to solubilize the poorly water-soluble anti - retroviral drug, Dolutegravir. Determination of solubilities of the drug in 8 M urea hydrotropic solution and distilled water was carried out at room temperature. There was more than 50 fold enhancement in aqueous solubility of Dolutegravir with 8 M urea (as compared to aqueous solubility). Therefore, it was thought worthwhile to solubilize the poorly water soluble Dolutegravir from fine powder of its laboratory mixture to carryout spectrophotometric analysis at 258 nm in method A, 248-268 nm in method B and 256nm in method C. urea does not show any absorbance above 250 nm. Beer ś law was obeyed in the concentration range of 52.5 -20µg/ml in method A, B and C, with correlation coefficients (R) of 0.996, 0.995 and 0.996 respectively. Laboratory mixture containing piroxicam have been analyzed successfully. Recovery studies and statistical data proved the accuracy, reproducibility and the precision of the proposed method. Based on the same principle a large number of drugs having λ_{max} above 250nm can be estimated by 8 M urea (inexpensive hydrotropic agent). Thus, hydrotropic solutions can be used in place of organic solvents (which are pollutants, toxic and give error due to volatility)[7].

Spectrophotometric methods were developed according to Quality by Design (QbD) approach as per ICH Q8(R2) guidelines for estimation of dolutegravir. QbD approach was carried out by varying various parameters, and these variable parameters were designed into Ishikawa diagram. The present work deals with the development of sensitive, simple, accurate, precise, and cost-effective UV-spectrophotometric method for the determination of dolutegravir, an anti-retroviral drug, in bulk and pharmaceutical dosage form by UV spectrophotometric method as per International Conference on Harmonization (ICH) guidelines. The critical parameters were determined by using principle component analysis as well as by observation. Estimated critical parameters in the spectrophotometric method were solvent methanol, wavelength: 260nm, slit width: 0.5, scan speed fast, sampling interval: 0.2nm and proposed method was validated for various parameters like system suitability, linearity, precision, accuracy, detection limits and quantification limits as per the International Conference on Harmonization guidelines ICH Q2(R1). The method's linearity was found to be excellent over the concentration range 5 to 25µg/ml with high correlation coefficient value of 0.999. Limits of detection and quantification were found to be 0.20µg/ml and 0.60µg/ml, respectively. The mean recovery was found to be 100.35% with low percentage relative standard deviation (% RSD) value[8].

2.3. High performance liquid chromatographic methods

Dolutegravir (DTG) is an integrase strand transfer inhibitor, which is a newly approved antiretroviral drug used for the treatment of HIV-infected naive and experienced individuals. Many aspects of DTG pharmacology remain to be studied. Our aim was to develop and fully validate a robust analytical method for the quantification of DTG in plasma using liquid chromatography coupled with UV detection. A simple and rapid protein precipitation method was used for analyte extraction from 100 µL plasma. The separation was achieved on a C8 reverse-phase analytical column using a gradient elution with 50 mmol/L formic acid and 50 mmol/L ammonium acetate in water (mobile phase A), and 100% acetonitrile (mobile phase B) and at a flow rate of 0.3 mL/min and a total run time of 10 minutes. The detector wavelength was set at 258 nm. The linearity of the calibration curve ($r > 0.9999$, $n = 6$) was validated over a concentration range of 0.25–10 mcg/mL. Intra-assay variability ranged from 3.3% to 6.1% and inter-assay variability ranged from 4.5% to 5.7%. The overall accuracy ranged from 90.7% to 97.7% for the 3 different concentrations of quality control samples. Recovery efficiency of extraction ranged from 94.3%–100%. This method is highly

selective with no interferences from commonly concomitant antiretroviral drugs or endogenous metabolites. The described method is simple, robust, selective, accurate, precise, and cost-effective. Thus, this assay can be readily transferred and implemented in clinical settings and used for pharmacokinetic studies and therapeutic drug monitoring programs[9].

Simple, sensitive, precise, and specific high-performance liquid chromategraphic (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. Linear regression analysis showed good linearity with respect to peak area in the concentration range of 200-900 ng/spot. The methods were validated for precision, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and specificity. Statistical analysis showed that both of the methods are repeatable and specific for the estimation of the said drug. The methods can be used for routine quality control analysis of dolutegravir sodium[10].

There is rare discussion available on quantitative separation of optical isomers of Dolutegravir, Dolutegravir is most recent FDA approved antiretroviral drug. The author has described simple, sensitive, precise, and specific RP-HPLC method for the separation of (R,R)-diastereomer, (S,S)-diastereomer and (S,R) enantiomer of Dolutegravir from its Process related and degradant impurities using Chiralpak IF-3, HPLC column. The method is validated as per ICH Q2(R1) guideline and can be used in routine analysis[11].

A rapid, specific and accurate high performance liquid chromatographic method for the determination of dolutegravir in human plasma using hydrochlorothiazide as internal standard was developed and validated by UV detection. The extraction process involved a liquid-liquid extraction using methyl-t-butyl ether. Both dolutegravir and the internal standard were eluted under isocratic mode using a 150 X 4.6 mm i.d, 5 µm Phenomenex ODS 2 C18 column. The mobile phase composed a mixture of 30:70 % v/v 20 mM Sodium acetate buffer (pH 4.0) and Methanol at a flow rate of 1.0 mL/minute. The wavelength of detection is 254 nm. The injection volume is 20 µL. The runtime of the method is 6 minutes with retention times 2.08 minutes and 4.16 for Hydrochlorothiazide and Dolutegravir respectively. The method showed good linearity in the range of 101.90 to 7004.49 ng/mL. The recovery of Dolutegravir is 59.21 % with a co-efficient of variation 3.72 % and recovery of internal standard was 60.61 % with a co-efficient of variation 3.33 %[12]. A chiral HPLC method was developed for the quantification of dolutegravir enantiomer and dolutegravir diastereomer in Dolutegravir sodium drug substance. Both of these isomers are resolved on Lux cellulose-4, 250 mm x 4.6 mm, 5µ column using a mobile phase consisting of the mixture of acetonitrile, water, and orthophosphoric acid in the ratio of 980:40:2 v/v/v. The mobile phase was pumped through the column at the flow rate of 1.5 mL min-1 . The resolution between Dolutegravir enantiomer and Dolutegravir was found to be more than 3.0. The developed method was validated and proved to be specific, accurate, and precise according to ICH. The experimentally established limit of detection and quantification for dolutegravir enantiomer is 0.006 and 0.018% w/w respectively and for dolutegravir diastereomer are 0.007 and 0.021%w/w. The average percentage recoveries of enantiomer were ranged between 102.8% and 103.2 % and diastereomers was ranged between 97.5% and 96.2%. The linearity curve was found to be linear and correlation coefficient obtained was 0.9997 for enantiomer and 0.9993 for diastereomers[13].

A novel isocratic reversed phase high performance liquid chromatographic method was developed and validated for the determination of human immune deficiency virus drug Dolutegravir (DGV) present in formulation known as Instgra which consists 50 mg of DGV. Chromatographic separation achieved isocratically on thermo C18 column (5µm, 150mm x 4.60mm) and acetonitrile: methanol in the ratio of 50:50 (v/v) as the mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 264 nm. The retention times for DGV was found to be 4.274 ± 0.3 min. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. The method was linear in the concentration range of 5-25

µg/ml with correlation coefficient of 0.999. The mean recoveries obtained for DGV 99.71% and RSD was less than 2. The correlation coefficients for all components are close to 1. Developed method was found to be novel, accurate, precise, selective and rapid for estimation of DGV[14].

A sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) assay was developed and validated to facilitate the assessment of clinical pharmacokinetics of dolutegravir (DTG) in plasma samples. This work describes an assay system requiring only a 20µL aliquot of human plasma that is subjected to a simple acetonitrile protein precipitation containing a stably labeled isotope of DTG used as an internal standard. Chromatography was performed on an XBridge C18, 2.1mm×50mm, reversed phase analytical column, using a 60:40 acetonitrile/water mobile phase containing 0.1% formic acid. Detection of the analyte and internal standard was achieved by ESI positive ionization tandem mass spectrometry. The precursor/product transitions (m/z) monitored were 420.1/136.0 and 428.1/283.1 for DTG and DTG-IS, respectively. The dynamic range of this assay extends from 5 to 10,000ng/mL, with a mean coefficient of determination (r, mean±SD) of 0.9996±0.0003. The mean precision values for calibration standards ranged from 0.7 to 4.1%, while accuracy values were 98.3 to 102.0%. Validation results demonstrated high accuracy (≤6.5% deviation) and high precision (≤9.1% CV) for the quality control samples. This assay system provides an accurate, precise, and sensitive method for DTG quantitation and was successfully applied to clinical research samples as part of a phase I/II pediatric clinical trial[15].

3. CONCLUSIONS:

A number of analaytical methods for the quantification of dolutegravir were consulted and details were provided in this article. UV-Visible spectroscopic methods developed based on reaction of dolutegravir with various chromogenic reagents affords colored products, which showed absorption maximum at a particular wavelength. The HPLC methods, in addition to their high accuracy and precision, they offer greater flexibility in the selection of stationary and mobile phases. By implementation of various extraction procedures, this feature will be useful in the quantification of dolutegravir in biological fluids also.

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