Asian Journal of Pharmaceutical Education and Research
Vol -7, Issue-4, October-December 2018
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 5.019

NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DOLUTEGRAVIR SODIUM IN SYNTHETIC MIXTURE BY USING RP-HPLC

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Received 21 August 2012; Revised 27 August 2018; Accepted 12 Sept. 2018, Available online 15 October 2018

ABSTRACT

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.

Keywords: RP-HPLC, Dolutegravi, Method development, Validation.

INTRODUCTION

Dolutegravir (DGV, S/GSK-1349572, Fig. 1) chemically known as (4R,12aS)-9-{[(2,4-difluorophenyl) methyl] carbamoyl}-4-methyl-6,8-dioxo-3,4,6,8,12,12ahexahydro-2H- pyrido [1`,2`:4,5] pyrazino [2,1-b][1,3]oxazol-7-olate. Its molecular formula is $C_{20}H_{18}F_2N_3O_5$ and molecular weight is 441.36. DGV is a newly developed human immunodeficiency virus (HIV) integrase inhibitor from ViiV Healthcare (Research Triangle Park, NC, USA). DGV is an integrase strand transfer inhibitor (INSTI) that does not require ritonavir for cytochrome P450 3A4 inhibition and preferentially blocks the strand transfer step of integration of the viral genome into the host cell's DNA[1], which is a two-step process mediated by the viral integrase enzyme. Like the other approved INSTIs raltegravir (RAL) and elvitegravir (EVG), DGV inhibits the binding of the integrase-viral DNA complex to host cell DNA by chelating Mg2+ions in the active site [2]. Once integration is blocked, HIV-1 cans no longer replicate, and the viral replication cycle is interrupted. In phase II trials, DGV has been shown to be highly effective at rapidly decreasing viral burden, with a concomitant increase in CD4+ cell count, in treatment-naïve patients receiving 10, 25 or

50 mg once-daily along with a nucleoside reverse transcriptase inhibitor (NRTI) background [3]. Moreover, when the DGV dosing groups were compared to the 600 mg efavirenz (EFV) dose group, the response was more rapid for all DTG groups. Phase III studies in treatment-naïve subjects demonstrate that DTG has sustained antiviral activity comparable to standard of care in combination with dual NRTIs [4,5]. DGV was also shown to be superior to RAL as part of a combination regimen in treatment-experienced, integrase inhibitor-naïve subjects [6]. In addition, DGV has been shown to retain in vitro activity against a large variety of viral phenol types no longer susceptible to RAL[7]. This translates into in clinical data demonstrating DGV's activity in subjects with resistance to RAL [8].DGV has been well tolerated in Phase III studies with a low incidence of discontinuation due to adverse events [4-6]. The most common adverse events of moderate to severe intensity in these trials were insomnia and headache. Additional studies to investigate the metabolism and disposition of DGV indicate the primary route of metabolism is glucuronidation via UDP-glucuronosyl transferase1A1 (UGT1A1), without induction or inhibition of cytochromeP450 enzymes [9, 10]. Since DGV will be administered as part of a multi-drug regimen, the lack of significant interactions with other antiretroviral agents is clinically advantageous. INSTIs are the newest class of anti retrovirals (ARVs) demonstrating potent anti-HIV activity. With DGV retaining activity in a variety of INSTI resistant phenotypes, having an excellent safety and tolerability profile and predictable pharmacokinetic (PK) profile with low to moderate inter-subject variability, it has the potential for treatment-experienced patients, but also holds promise to become a first-line antiretroviral agent, and will likely become a commonly used component of antiviral regimens.

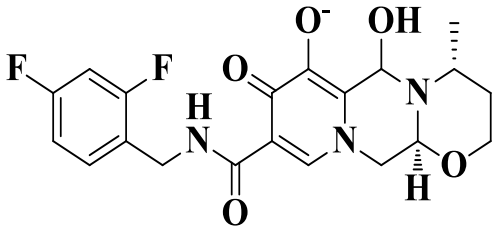


Figure 1 Chemical structure of Dolutegravir

In literature, various methods are available for determination of DGV in human plasma by HPLC [11], for combined formulated dosage form quantification HPLC methods [12-13], for pure dosage form by HPTLC and HPLC [14], Bhavar *et al.* published in 2015, UV Spectroscopic method for tablets forms [15]. In the present work, we are therefore focused on to achieve the optimum chromatographic conditions for the determination of DGV in a formulation. The developed method could be applied to quality control of the tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines, [16] which are mandatory also.

MATERIALS AND METHODS

Instrument

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

Reagents and chemicals

Dolutegravir were obtained as pure sample from Emcure Pharmaceutical Industries Ltd. Pune, as gift samples along with their analytical reports. HPLC grade methanol and acetonitrile was obtained from Merck (India) limited. All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house. Tablet Instgra 50mg Emcure Pvt. Ltd. Pune, India was purchased from local market.

Chromatographic conditions

The isocratic mobile phase consisted of methanol: acetonitrile in the ratio of (50:50 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 256 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Standard stock solution

Accurately weighed 10 mg of DGV was transferred into 10 ml volumetric flask, dissolved in 5ml of methanol and volume was made up to 10ml with methanol to get concentration of solution 1000 µg/ml (Stock-A), 5ml of stock-A was taken and diluted up to 50ml to get concentration of 100µg/ml (Stock-B).

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 5-25 µg/ml for DGV.

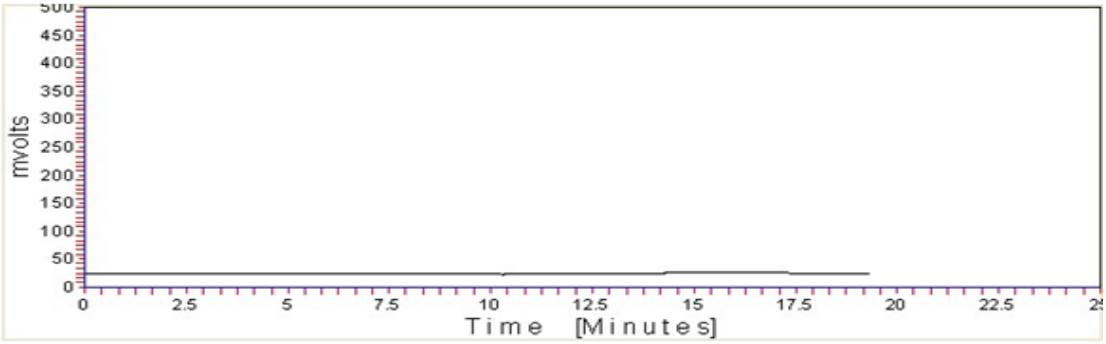
Sample preparation

Commercial formulations dolutegravir of Instgra was selected for analysis. Twenty tablets of instgra were weighed and powdered separately. Weight equivalent to 50 mg dolutegravir was dissolved in 50 ml diluents and then sonicated for 15min. and filtered through watmann paper no. 41. Then different concentration of solution were prepared by serial dilution technique, as per standard and each dilution was analyzed.

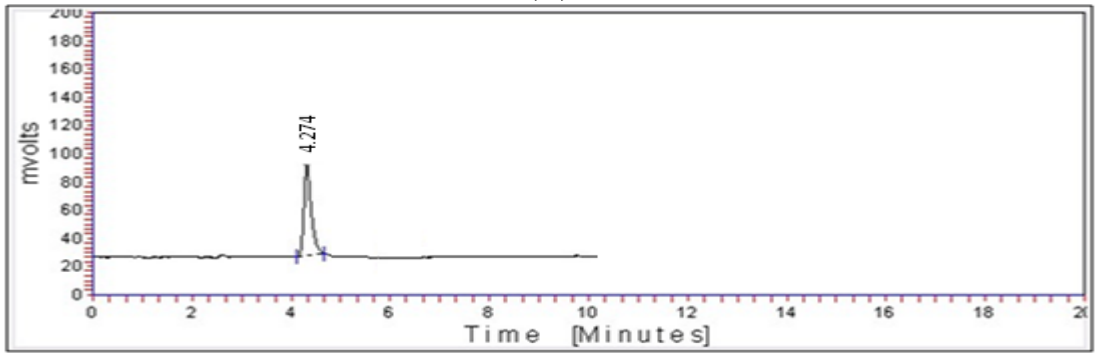
Results and discussion

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water, and buffer solutions in various proportions and at different pH values. A mobile phase consisting of acetonitrile/ methanol (50:50, v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 min were studied. A flow rate of 1 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using a reversed-phase C18 column, the retention times for DGV was observed to be 4.274± 0.03 min. Total time of analysis was less than 6 min. The maximum absorption of DGV was detected at 264 nm, and this wavelength was chosen for the analysis Fig. 2.



(A)



(B)

Figure 2 Chromatograms of (A) Blank mobile phase (B) DGV (15µg/ml) as reference substances

System suitability

System suitability parameters such as number of theoretical plates, HETP, and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for DGV was 2640.54.

Table 1 Results of system suitability parameters

Parameters	Dolutegravir
AUC*	465.582
No. of Theoretical Plates	2640.54 ± 12.04
Tailing Factor*	1.305
Retention time*	4.274
HETP*	0.199
Calibration range (µg/ml)	5-25

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 5-25 µg/ml for DGV. The linearity was represented by a linear regression equation as follows:

$$Y \text{ (TRZ)} = 42.73 \text{ conc.} + 14.01 (r2 = 0.999)$$

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve table 2.

Table 2 LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Dolutegravir	1.08	3.27

Accuracy

Method accuracy was performed by adding known amounts of DGV to the preanalysed synthetic mixture solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80%, 100%, and 120% of the nominal analytical concentration (10µg/ml for DGV). Each level was made in triplicate table 2. The mean percentage recoveries obtained for DGV was 99.78%, respectively, and RSD was less than 3.

Table 3: Results of recovery study

Statistical data	Dolutegravir		
	80%	**100%**	**120%**
% Mean*	98.77	99.71	98.90
SD*	0.29	0.091	0.174
%R.S.D*.	0.29	0.092	0.176

*Mean of nine determinations (three replicates at three concentration level)

Precision

Repeatability

Five dilutions in three replicates were analyzed in the same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in table 3.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variations, and results were found within acceptable limits (RSD < 2) as shown in table 4.

Table4 Statistical data for precision

Statistical parameter	Dolutegravir		
	Mean*	S.D*	R.S.D*
Repeatability	99.65	0.076	0.076
Intermediate Precision	99.86	0.150	0.150
(I) (A day to day)			
(II) Analyst to Analyst	100.62	0.99	0.99
Robustness	99.67	0.106	0.106

*Mean of 15 determinations (three replicates at five concentration level)

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was chain from, Methanol: ACN (50:50% V/V), to (55: 45% V/V) and method is found robust as RSD is again found < 2.0 table 4.

Specificity and selectivity

Commonly used excipients were spiked in to a preweighed quantity of drugs. The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPLC method is illustrated in Fig. 3. Where complete separation of DGV in presence of tablet excipients.

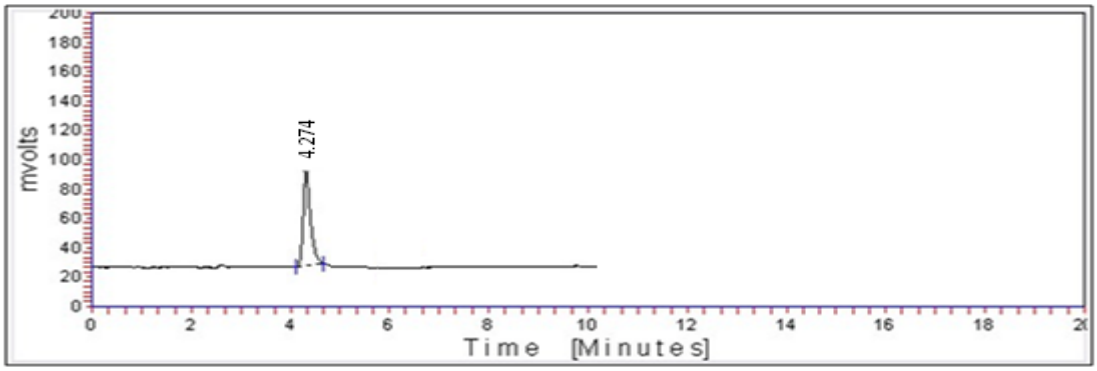


Figure 3 Chromatograms of DGV (15µg/ml) in a tablet formulation

Analysis of Tablets

The concentration of DGV in the tablet formulation was found to be 99.95%. The low values of % RSD indicate that the method is precise and accurate in table 5.

Table 5 Results of tablet analysis

S.NO.	Parameter	Sample
		Dolutegravir
1	% Found	99.95
2	S.D.	0.123
3	% R.S.D.	0.256
4	SEσ*	0.230

* Mean of nine determinations

Conclusion

The proposed HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of Dolutegravir by HPLC in pharmaceutical dosage form. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of trazodone hydrochloride with no interference from other formulation excipients. The proposed method was highly reproducible, reliable, rapid, robust and specific. Therefore, a high percentage of recovery and the run time of less than six minutes allow its application for the routine determination of DGV in the tablet dosage form.

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