ORIGINAL ARTICLE

Stability indicating reverse-phase high-performance liquid chromatography method for the determination of Raltegravir in bulk and pharmaceutical formulation

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Abstract

Introduction: A new sensitive and simple stability indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the determination of Raltegravir in tablet dosage forms. **Materials and Methods:** Chromatographic separation was achieved through C8 phenomenex column (250 mm × 4.6 mm i.d., 5 µm particle size) using ammonium formate:acetonitrile (20:80, v/v) mixture as the mobile phase. The Shimadzu Model CBM-20A/20 Alite HPLC system was monitored at detection wavelength 254 nm on isocratic mode with flow rate 1.2 ml/min and the method was validated. Raltegravir was exposed to different stress conditions, and the stability of Raltegravir was studied as per the ICH guidelines. **Results and Discussion:** Raltegravir follows Beer-Lambert's law over a concentration range 1–120 µg/ml with regression equation y = 15416x+10312 and correlation coefficient 0.9997. The limit of detection and limit of quantification are found to be 0.2885 µg/ml and 0.8743 µg/ml, respectively. Raltegravir was found to be more sensitive toward alkaline conditions. **Conclusions:** The proposed RP-HPLC method is accurate, precise, sensitive, and specific for the assay of Raltegravir in tablets.

Key words: ICH guidelines, Raltegravir, reverse-phase high-performance liquid chromatography, validation

INTRODUCTION

Raltegravir [Figure 1] is used to treat HIV infection.[1] It is an integrase inhibitor[2] with molecular formula $C_{20}H_{20}FKN_6O_5$, and the molecular weight is 482.51. Liquid chromatographic[3-5] methods, spectrophotometric techniques[6-8] were developed for the determination of Raltegravir in pharmaceutical formulations as well as biologicals. Khagga *et al.* reported a reverse-phase high-performance liquid chromatography (RP-HPLC) method and characterized the degradants using liquid chromatography-mass spectrometry/mass spectrometry.[9] In the present paper, the authors have proposed new stability indicating a liquid chromatographic method for the determination of Raltegravir and validated.[10] Stress degradation studies were also performed to study the stability of Raltegravir as per the ICH guidelines.[11]

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals are of AR grade (Merck), and all solvents are of HPLC grade (Merck). Raltegravir stock solution was prepared by dissolving 25 mg of Raltegravir in HPLC grade acetonitrile (1000 µg/ml) in a 25 ml volumetric flask and filtered. 0.1 M ammonium formate (molecular weight

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63.0559 g/mol) solution was prepared by dissolving 3.15 g ammonium formate in a 500 ml volumetric flask with HPLC grade water. The resulting solution was sonicated for half an hour and filtered.

Chromatographic Conditions

Shimadzu Model CBM-20A/20 Alite UFLC system (Shimadzu Co., Kyoto, Japan) equipped with SPD M20A prominence PDA detector. PhenomexC_8 column (250 mm × 4.6 mm i.d., 5 µm particle size) was employed for the entire chromatographic study. Chromatography work was performed on isocratic mode using a mixture of ammonium formate (0.1%):acetonitrile (20:80 v/v) as mobile phase with flow rate 1.2 ml/min and UV detection 254 nm and the study was observed at ambient temperature (25°C ± 2°C).

Method Validation

Linearity, precision, and accuracy

1–120 µg/ml Raltegravir solutions were prepared from the stock and 20 µL of each solution was injected into the HPLC system, and the peak area of the chromatogram was noted. A graph was plotted using concentration on the X-axis and the mean peak area on the Y-axis. Precision was studied at three different concentration levels (10, 20, and 50 µg/ml) of Raltegravir on the same day and 3 consecutive days, respectively. The accuracy of the method was proved by the standard addition method, and the recovery values were determined.

Assay of commercial formulations

Raltegravir is available as film-coated tablets with brand names Isentress, zepdon, etc., with label claim 400 mg. 20 film-coated tablets of Raltegravir were procured from the pharmacy store, powdered and powder equivalent to 25 mg Raltegravir was extracted with acetonitrile sonicated for half an hour and filtered through 0.45 mm membrane filter. Dilutions were made as per the requirement. 20 µL of solution from each brand was injected into the HPLC system, and the peak areas were noted from the respective chromatograms.

Stress degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Raltegravir was treated with different reagents to induce stress such as acidic hydrolysis, basic hydrolysis, and oxidation. All solutions for stress studies were prepared at an initial concentration of 50 mg/ml of Raltegravir and refluxed for 20 min at 80°C and then diluted with mobile phase.

Acidic degradation

Acidic degradation was performed by taking the drug solution mixture (50 mg/ml Raltegravir solution) and exposed to acidic degradation with 0.1 N hydrochloric acid for 20 min in a thermostat maintained at 80°C the stressed sample was cooled, neutralized with 0.1 N sodium hydroxide, and then diluted with mobile phase as per the requirement and 20 µL of the solution was injected in to the HPLC system.

Alkaline degradation

Alkaline degradation was performed by treating the drug solution mixture (containing each of 50 mg/ml Raltegravir) with 0.1 N sodium hydroxide for 20 min in a thermostat maintained at 80°C. The drug solution mixture was cooled, neutralized with 0.1 N hydrochloric acid and then diluted with mobile phase as per the requirement and 20 µL of the solution was injected into the HPLC system.

Oxidation

Oxidation was performed by treating the drug solution mixture (containing each of 50 mg/ml Raltegravir) with 30% H_2O_2 for 20 min in a thermostat maintained at 80°C. The drug solution mixture was cooled and then diluted with mobile phase as per the requirement, and 20 µL of the solution was injected into the HPLC system.

RESULTS AND DISCUSSION

A new stability is indicating reverse phase liquid chromatographic method has been developed for the determination of Raltegravir in active pharmaceutical ingredient and its tablet dosage forms using C8 Phenomenex column and a mixture of acetonitrile and ammonium formate as the mobile phase.

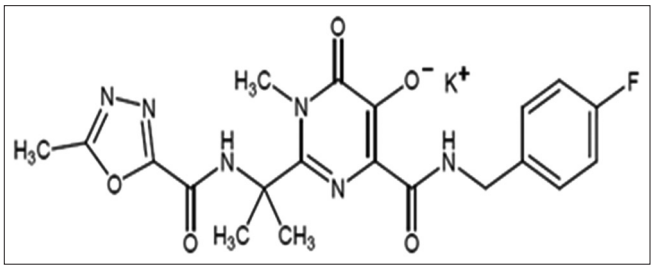

Figure 1: Chemical structure of Raltegravir

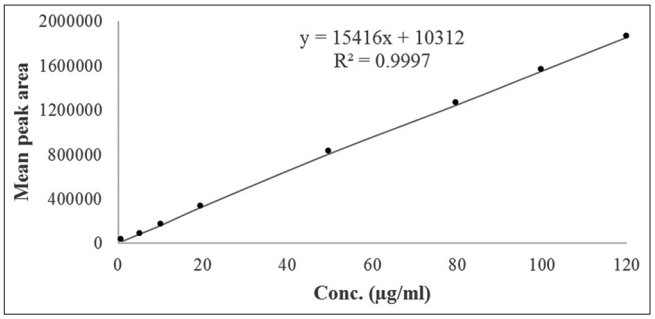

Figure 2: Calibration curve of Raltegravir

Method Validation

The proposed method was validated by linearity, precision, accuracy, and robustness as per the ICH guidelines. The calibration curve was drawn by taking a concentration of Raltegravir on X-axis and the corresponding mean peak area values on the Y-axis. Raltegravir follows Beer-Lambert's law over a concentration range 1–120 µg/ml [Table 1] with regression equation y = 15416x+10312 [Figure 2] and correlation coefficient 0.9997. The limit of detection and limit of quantification are found to be 0.2891 µg/ml and 0.8692 µg/ml, respectively. The typical chromatogram was shown in Figure 3. The assay of the marketed formulations was found to be 99.56–99.72. The % RSD in the precision study was found to be 0.46–1.02 indicating that the method is precise. The accuracy of the assay method was evaluated by standard addition method (80, 100, and 120%) and the percentage RSD was found to be 1.16–1.55 which is <2.0 indicating that the method is accurate [Table 2].

Table 1: Linearity of Raltegravir

Conc. (µg/ml)	*Mean peak area±SD	RSD (%)
1	16612.3±328.22	0.56
5	79443±116.65	0.83
10	158020±1614.5	1.02
20	328494.3±1647.9	0.5
50	805809±3772.9	0.46
80	1244662±1516.2	0.12
100	1548440±15872.9	1.02
120	1851550±19923.3	1.07

*Mean of three replicates. SD: Standard deviation, RSD: Relative standard deviation

Stress Degradation Studies

More than 20% of Raltegravir has undergone oxidation, acidic, and alkaline degradations indicating that the drug is very much sensitive. The degradants were well separated during the alkaline degradation. During the degradation study Raltegravir was well separated, and therefore the method is selective and specific. The system suitability parameters are within the acceptable criteria [Table 3] and the typical chromatograms obtained during the stress degradation studies were shown in Figure 4.

Table 2: Accuracy study of Raltegravir

Spiked conc. (µg/ml)	Total conc. (µg/ml)	*Mean peak area±SD (% RSD)	Drug found (µg/ml)	% Recovery
40 (80%)	90	1371722±16039.4 (1.16)	88.58	98.42
50 (100%)	100	1541068±207.65 (1.32)	99.52	99.52
60 (120%)	110	1667730±25884.1 (1.55)	107.48	97.70

*Mean of three replicates

Table 3: Stress degradation studies of Raltegravir

Stress conditions	*Mean peak area	Drug recovered (%)	Drug decomposed (%)	Theoretical plates	Tailing factor
Standard drug (untreated)	805809	-	-	5599.012	1.747
Acidic degradation	516628	35.89	64.11	3871.650	1.140
Oxidation	743099	7.79	92.21	5483.796	1.626
Alkaline degradation	210287	73.91	26.09	1782.24	1.170

*Mean of three replicates

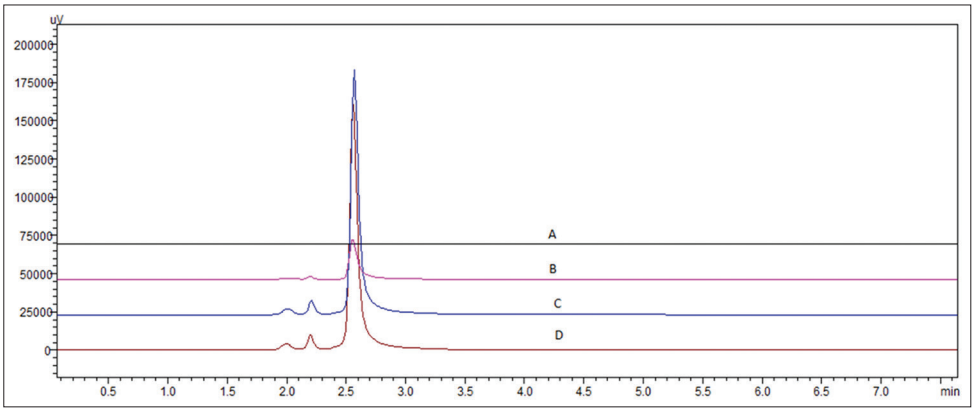

Figure 3: Typical chromatograms of (a) blank, (b) Raltegravir standard (10 µg/ml),(c) Raltegravir standard (50 µg/ml), (d) Raltegravir tablets (50 µg/ml) (Isentress)

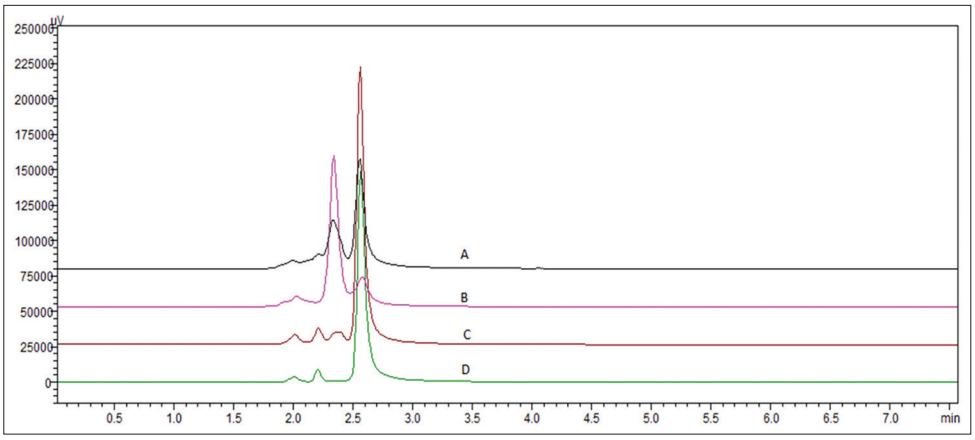

Figure 4: Typical chromatograms of Raltegravir (a) acidic degradation, (b) alkaline degradation, (c) oxidation, (d) standard drug (50 µg/ml) (untreated)

CONCLUSIONS

The validated stability indicating method developed for the determination of Raltegravir is specific and selective and more economical. Raltegravir is known to be more sensitive towards acidic, basic, and oxidation environments. This method can be excellently applied for the quantitative determination of Raltegravir tablets.

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