ORIGINAL ARTICLE

New Analytical Techniques for the Assay of Raltegravir (Anti-HIV Drug)

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Abstract

Introduction: Four first-order derivative, three difference and three zero-order spectrophotometric techniques were developed for the assay of Raltegravir in tablets. **Materials and Methods:** Spectrophotometric techniques such as zero order (D_0), first-order derivative (D_1) and difference spectroscopic methods have been developed in water and phosphate buffers (pH 3, pH 4, and pH 5) for the determination of Raltegravir. **Results**: Raltegravir has obeyed Beer-Lambert's law 1–150 in zero order (D_0) and 10–150 µg/mL in both first-order derivative (D_1) and difference spectroscopic methods. **Conclusion:** Raltegravir has shown a wide range of linearity in all the methods, and all the methods were validated as per the ICH guidelines. These simple methods can be successfully applied for the assay of Raltegravir in tablets.

Key words: Difference spectroscopy, first-order derivative (D_1), Raltegravir, validation, zero order (D_0)

INTRODUCTION

Raltegravir [Figure 1] is a new class of HIV drugs which is approved by the Food and Drug Administration, the integrase inhibitors.[1,2] Raltegravir is chemically N-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-(2-{[(5-methyl-1, 3, 4-oxadiazol-2-yl)carbonyl]amino}-2-propanyl)-6-oxo-1,6-dihydro-4-pyrimidinecarboxamide. The molecular formula is $C_{20}H_{20}FKN_6O_5$ with molecular weight is 482.51. Raltegravir can cause a condition that results in the breakdown of skeletal muscle tissue, leading to kidney failure.

Few spectrophotometric techniques[3-5] reverse-phase high-performance liquid chromatography (RP-HPLC) methods[6,7] and LC-mass spectrometry (MS), /MS method[8] have been reported for the determination of Raltegravir in pharmaceutical formulations in literature. A liquid chromatographic method has also been established for the estimation of Raltegravir in human blood plasma[9] using solid phase extraction technique. At present, the authors have proposed zero order, first-order derivative and difference spectrophotometric techniques for the assay of Raltegravir in phosphate buffers (pH 3, pH 4, and pH 5) and water and all the methods were validated as per the ICH guidelines.

MATERIALS AND METHODS

Ultraviolet (UV)-1800 Shimadzu double beam UV-VIS spectrophotometer is used for the present study, and all the solutions were scanned 200–400 nm I quartz cuvettes. Phosphate buffer solutions of pH 3, pH 4, and pH 5 were prepared as per the IP 1996 procedure. Stock solution of Raltegravir was prepared in methanol, and working solutions were prepared on dilution with phosphate buffer (pH 3, 4, and 5) and water as per the requirement for the established methods. Raltegravir is available as film-coated tablets with brand names Isentress, Zepdon, etc., with label claim 400 mg.

Method validation

Linearity and construction of calibration curve

Zero-order spectroscopy

Solutions 1–150 µg/mL were prepared from the stock solution on dilution with phosphate buffer pH 3.0 (Method A) and

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scanned 200–400 nm against reagent blank. The zero-order spectrum so obtained has shown λ_{max} at 303 nm. The absorbance of the solutions was noted at this λ_{max}, and a graph was drawn by taking the concentration on the X-axis and the corresponding absorbance on the Y-axis. Similarly, 1–150 µg mL were prepared on dilution from the stock solution with phosphate buffer pH 5.0 (Method B) and phosphate buffer pH 4.0 (Method C) buffer solutions and scanned 200–400 nm against reagent blank. The absorbance of these solutions was noted at λ_{max} 302 and 331 nm for Methods B and C, respectively, and calibration curves were drawn by taking the concentration on the X-axis and the corresponding absorbance on the Y-axis.

First-order derivative spectroscopy

The three zero-order spectra (D_0) obtained in three reagents such as phosphate buffer pH 3.0 (Method I), phosphate buffer pH 5.0 (Method II), phosphate buffer pH 4.0 (Method III), and water (Method IV) were transformed in to first-order derivative (D_1) spectra by the inbuilt software of the instrument. The derivative spectra so obtained have shown maxima, minima and therefore the amplitude has been chosen against the concentration to plot the calibration curves for Methods I-IV.

Difference spectroscopy

Three difference spectrophotometric methods have been developed for the determination of Raltegravir. A series of solutions 10–150 µg/mL were prepared in phosphate buffer pH 3.0, phosphate buffer pH 5.0, phosphate buffer pH 4.0, and water. The λ_{max} difference between phosphate buffer pH 4.0 (331 nm) and other reagents is more than 15 nm, and therefore the difference spectrophotometric technique was attempted. Difference spectroscopy was performed between phosphate buffer pH 4 and phosphate buffer pH 3 for Method A; between phosphate buffer pH 4 and phosphate buffer pH 5 for Method B and between phosphate buffer pH 4 and water for Method C, respectively. During the scanning process phosphate buffer pH 4.0 was taken in the reference cuvette, and phosphate buffer pH 3.0, phosphate buffer pH 5.0, and water were taken in sample cuvette for obtaining the zero line for Methods A–C, respectively, and the scanning of all drug solutions was done accordingly. The difference spectra so obtained for the Methods A-C have shown maxima as well as minima, and therefore the amplitude has been taken for the construction of calibration curve.

Precision and accuracy studies

The intraday and interday precision studies were performed at three different levels (10, 20, and 50 µg/mL) and accuracy studies were carried out by standard addition method (80%, 100%, and 120%) and the percentage recovery was calculated for all three techniques.

Assay of Raltegravir

A total of 20 Raltegravir tablets were procured from the local pharmacy store, weighed and crushed into powder. Powder equivalent to 25 mg of Raltegravir was extracted with methanol and dilutions were made with phosphate buffers (pH 3, 4, and 5) and water for the analytical techniques and assayed by following the above-detailed procedures.

RESULTS AND DISCUSSION

Three different analytical techniques zero order, first-order derivative spectroscopy and difference spectroscopy have been developed for the determination of Raltegravir in pharmaceutical dosage forms (tablets).

Zero-order spectroscopy

In zero-order spectrophotometric technique, the overlay absorption spectrum obtained in phosphate buffers - pH 3,

Table 1: Optical characteristics of Raltegravir zero-order spectroscopy

Parameters	Method		
	A	B	C
Reagent (phosphate buffer)	pH 3.0	pH 5.0	pH 4.0
Linearity range (µg/mL)	1–150	1–150	1–150
λ_{max} (nm)	303	302	331
Molar extinction coefficient (liter/mole/cm)	4.9775×10^3	4.3819×10^3	2.4665×10^3
Sandell's sensitivity (µg/cm²/0.001 absorbance unit)	0.0896	0.1014	0.1801
Slope	0.0117	0.0116	0.0113
Intercept	0.0221	0.0033	0.0275
Correlation coefficient	0.999	0.9993	0.9992
Precision (%RSD)	0.42–0.51	0.47–0.53	0.52–0.67
Accuracy (%RSD)	0.65–0.71	0.44–0.52	0.36–0.42
Assay (%)	99.48	99.54	99.71

RSD: Relative standard deviation

pH 4, and pH 5 was shown in Figure 2a and the optical characteristics observed were given in Table 1. The percentage relative standard deviation (RSD) in precision and accuracy studies was found to be <2 in all the techniques indicating that all the methods are precise and accurate. Linearity was observed over a wide concentration range in all the buffers, and all the methods are validated.[10] The linear regression equations are found to be y = 0.0117 x - 0.0221 (R^2 = 0.999) [Figure 2b], y = 0.0116 x - 0.0033 (R^2 = 0.9993) [Figure 2c], and y = 0.0113 x - 0.0275 (R^2 = 0.9992) [Figure 2d], respectively.

First-order derivative spectroscopy

In first-order derivative spectroscopic technique, the overlay spectra obtained in four reagents, i.e., phosphate buffer pH 3, phosphate buffer pH 5, phosphate buffer pH 4, and water (Methods I-IV), respectively, were shown in Figure 3 and the spectral characteristics observed were given in Table 2. Beer-Lambert's law has been followed over a wide concentration range (10–150 µg/ml) in all Methods I-IV [Figure 4] and the percentage RSD values in precision and accuracy studies were found to be <2 indicating that all the methods are precise and accurate [Table 3].

Difference spectroscopy

Indifference spectroscopic technique, the overlay difference spectra obtained in Methods A-C were shown in Figure 5a and the spectral characteristics observed were shown in Table 4. Beer-Lambert's law has been followed over a wide concentration range in all the buffers [Figure 5b-d] and in Methods A–C, respectively, and the percentage RSD values in precision and accuracy studies were <2 indicating that the methods are precise and accurate [Table 5].

CONCLUSION

These three validated spectrophotometric techniques zero order, first-order derivative and difference spectroscopic methods are very simple, precise, accurate, economical and are very useful for the quality control testing of Raltegravir in pharmaceutical formulations (tablets) successfully.

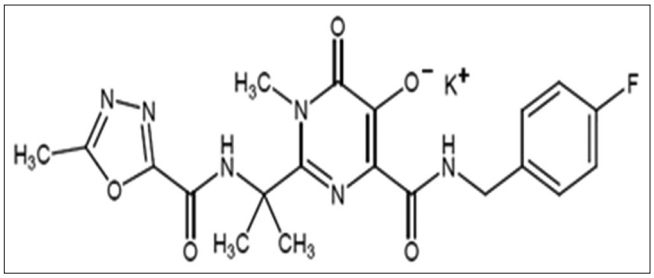

Figure 1: Chemical structure of Raltegravir

Table 2: Linearity of Raltegravir - first derivative spectroscopy

Concentration (µg/ml)	Method I phosphate buffer pH 3			Method II phosphate buffer pH 5			Method III phosphate buffer pH 4			Method IV water		
	Maxima	Minima	Amplitude	Maxima	Minima	Amplitude	Maxima	Minima	Amplitude	Maxima	Minima	Amplitude
10	0.002	0.010	0.012	0.002	0.004	0.006	0.003	0.006	0.009	0.001	0.007	0.008
20	0.005	0.019	0.024	0.004	0.008	0.012	0.005	0.014	0.019	0.005	0.020	0.025
40	0.010	0.037	0.047	0.009	0.018	0.027	0.009	0.028	0.037	0.004	0.032	0.036
50	0.013	0.049	0.062	0.011	0.022	0.033	0.012	0.036	0.048	0.012	0.048	0.06
100	0.023	0.090	0.113	0023	0.044	0.067	0.025	0.074	0.099	0.025	0.098	0.123
150	0.035	0.135	0.170	0.34	0.065	0.099	0.035	0.103	0.138	0.036	0.142	0.178

Table 3: Characteristics of Raltegravir - first derivative spectroscopy

Parameters	Method I	Method II	Method III	Method IV
Linearity range (μg/ml)	10–150	10–150	10–150	10–150
Accuracy % recovery (%RSD)	98.23–99.34 (0.23)	97.89–99.24 (0.32)	98.29–99.48 (0.28)	98.3–99.25 (0.34)
Precision intraday (% RSD)	0.23–0.43	0.19–0.56	0.26–0.45	0.23–0.47
Interday (% RSD)	0.73–0.92	0.54–0.91	0.53–0.89	0.53–0.97
Assay (%)	99.23	99.45	99.48	99.53l

RSD: Relative standard deviation

Table 4: Linearity of Raltegravir - difference spectroscopy

Concentration (μg/ml)	Method A (phosphate buffer pH 4 vs. phosphate buffer pH 3)			Method B (phosphate buffer pH 4 vs. Phosphate buffer pH 5)			Method C (phosphate buffer pH 4 vs. water)		
	Maxima	Minima	Amplitude	Maxima	Minima	Amplitude	Maxima	Minima	Amplitude
10	0.1207	0.0451	0.1658	0.0473	0.1007	0.0534	0.0818	0.0522	0.134
20	0.0893	0.2068	0.2961	0.0501	0.2064	0.1563	0.0829	0.1924	0.2753
40	0.2605	0.3374	0.5979	0.0576	0.4079	0.3503	0.2041	0.1532	0.3573
50	0.2760	0.4969	0.7729	0.2172	0.5275	0.3104	0.0008	0.4804	0.4812
100	0.5121	0.9364	1.4985	0.4197	1.0572	0.6375	0.5216	0.9192	1.4408
150	0.8186	1.4315	2.2501	0.8182	1.6465	0.8223	0.3409	1.4451	1.7860

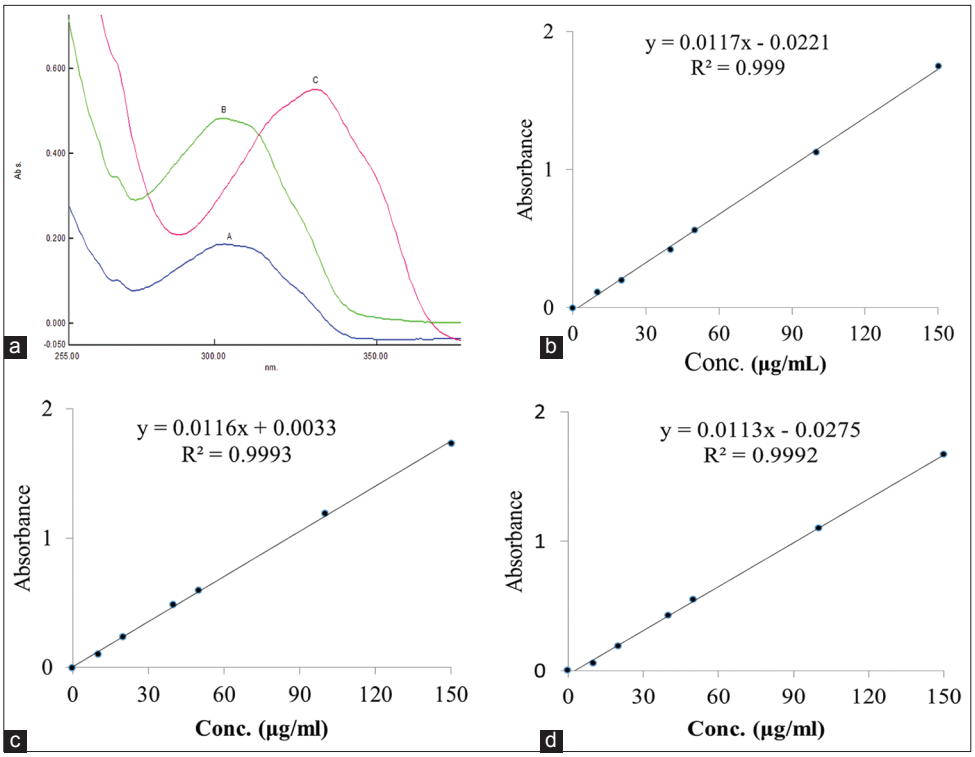

Figure 2: (a) Absorption spectrum and calibration curves (b-d) of Raltegravir in (a) phosphate buffer pH 3.0 (b) phosphate buffer pH 4.0 (c) phosphate buffer pH 5.0 (d) phosphate buffer pH 5.0

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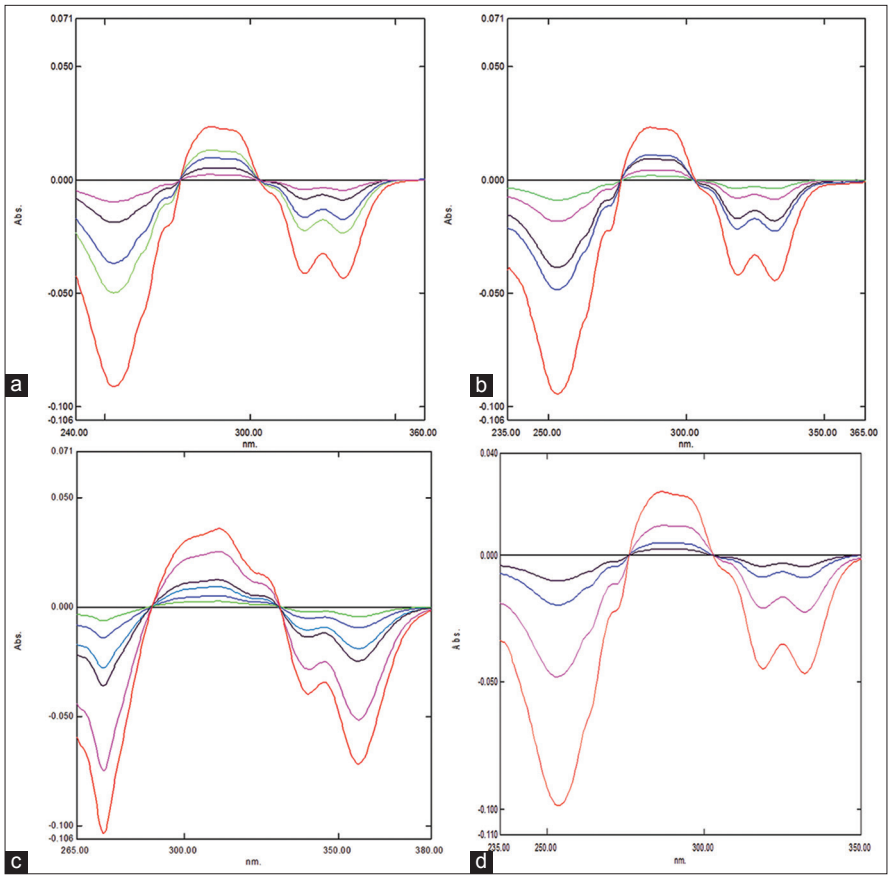

Figure 3: Overlay first-order derivative spectrum of Raltegravir in (a) phosphate buffer pH 3 (b) phosphate buffer pH 5 (c) phosphate buffer pH 4 (d) water

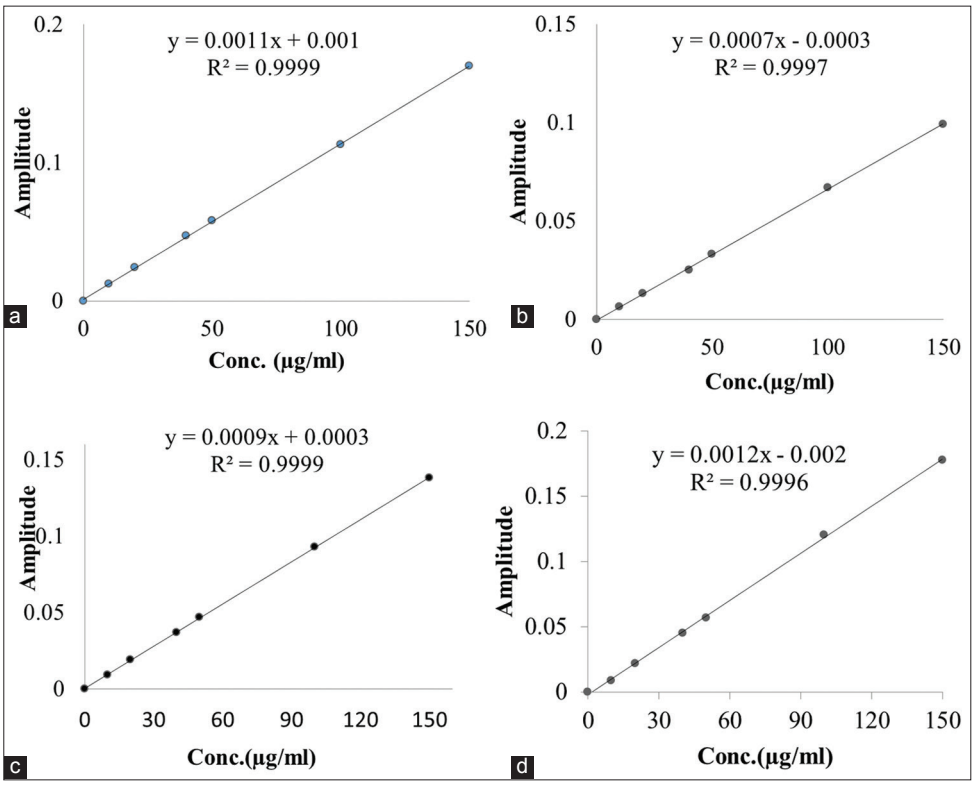

Figure 4: Calibration curve of Raltegravir in first derivative spectroscopy technique (D_1) (a) phosphate buffer pH 3 (b) phosphate buffer pH 5 (c) phosphate buffer pH 4 (d) water

Table 5: Characteristics of Raltegravir - difference spectroscopy

Parameters	Method A	Method B	Method C
Linearity range (μg/ml)	10–150	10–150	10–150
Accuracy (% recovery) (% RSD)	99.61–99.73 (0.23)	99.81–99.87 (0.41)	99.84–99.87 (0.41)
Precision			
Intraday (% RSD)	0.41–0.54	0.21–0.59	0.21–0.59
Interday (% RSD)	0.65–081	0.53–0.82	0.53–0.82
Assay (%)	99.64	99.91	99.91

RSD: Relative standard deviation

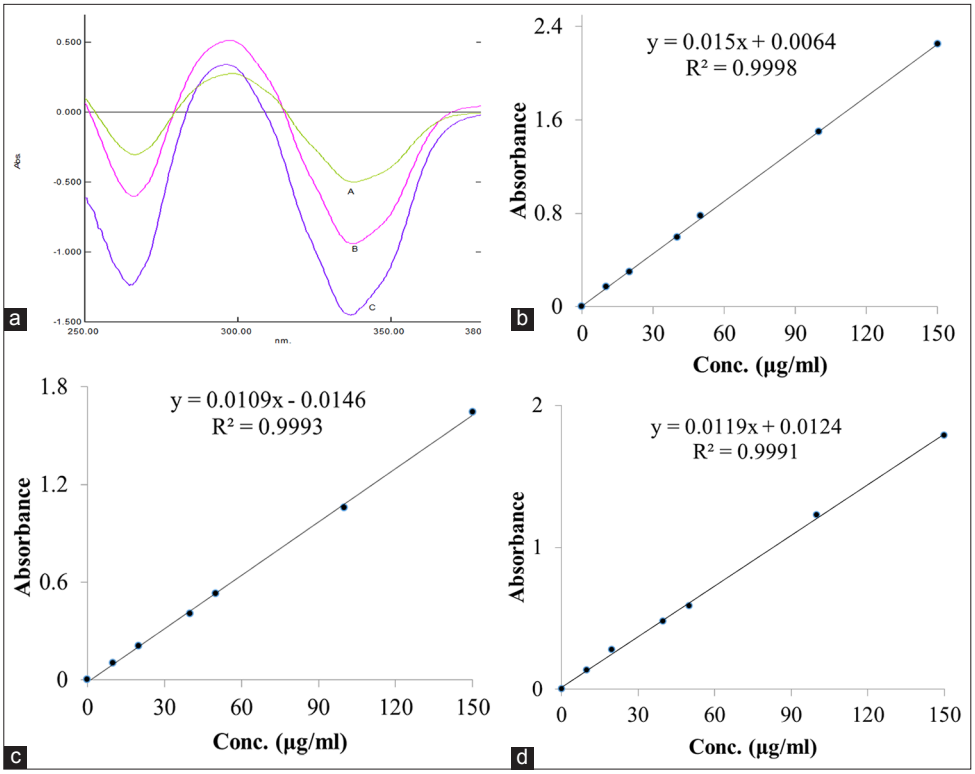

Figure 5: (a) Difference spectrum and calibration curves (b-d) of Raltegravir

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