



Reverse Phase-High Performance Liquid Chromatography and Ultra Violet Spectrophotometric Method for the Estimation of Raltegravir Potassium in Bulk and in Tablet Dosage form

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Abstracts - A High Performance Liquid Chromatographic (method A) and Ultra Violet spectrophotometric (method B) method were developed and validated for quantitative determination of Raltegravir potassium. The different analytical performance parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of Quantification (LOQ) were determined according to International Conference on Harmonization (ICH) Q2B guidelines. Reverse phase High Performance Liquid Chromatography (method A) and Ultra Violet (method B) were developed for the determination of Raltegravir potassium in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved on Symmetry C18 (4.6 x 150mm, 5 μ m XTerra) column using a mixture of phosphate buffer (pH 3.0): Methanol (45:55% v/v) as the mobile phase at a flow rate 0.6 mL min⁻¹. The Ultra Violet spectrophotometric determination was performed at 219 nm. The Linearity of the calibration curves for the analyte in the desired concentration range is good ($r^2 = 0.999$) by both High Performance Liquid Chromatography (method A) and Ultra Violet (method B) spectroscopic method. Both methods (A&B) were accurate and precise with recoveries in the range of 98-100 % and percentage relative standard deviation (RSD less than 2%). The proposed methods were highly sensitive, accurate, and precise and hence was successfully applied for the reliable quantification of Active Pharmaceutical Ingredient content in the commercial formulation.

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T.Sudha^α, T.Raghupathi^Ω

Abstract - A High Performance Liquid Chromatographic (method A) and Ultra Violet spectrophotometric (method B) method were developed and validated for quantitative determination of Raltegravir potassium. The different analytical performance parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of Quantification (LOQ) were determined according to International Conference on Harmonization (ICH) Q2B guidelines. Reverse phase High Performance Liquid Chromatography (method A) and Ultra Violet (method B) were developed for the determination of Raltegravir potassium in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved on Symmetry C18 (4.6 x 150mm, 5 µm XTerra) column using a mixture of phosphate buffer (pH 3.0): Methanol (45:55% v/v) as the mobile phase at a flow rate 0.6 mL min⁻¹. The Ultra Violet spectrophotometric determination was performed at 219 nm. The Linearity of the calibration curves for the analyte in the desired concentration range is good (r² = 0.999) by both High Performance Liquid Chromatography (method A) and Ultra Violet (method B) spectroscopic method. Both methods (A&B) were accurate and precise with recoveries in the range of 98-100 % and percentage relative standard deviation (RSD less than 2%). The proposed methods were highly sensitive, accurate, and precise and hence was successfully applied for the reliable quantification of Active Pharmaceutical Ingredient content in the commercial formulation.

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I. INTRODUCTION

Raltegravir potassium (Fig.1) is chemically N-[(4-fluorophenyl)methyl]-1, 6-dihydro-5-hydroxy-1-methyl-2-[1-methyl-1-[[5-methyl-1,3,4-oxadiazol-2-yl)ethyl]-6-oxo-4-pyrimidine carboxamide potassium salt [1]. Raltegravir potassium is an enzyme integrase inhibitor used for the treatment of Human Immunodeficiency Virus 1 (HIV-1) infection in treatment

experienced adult patients who have evidence of viral replication and HIV-1 strains resistant to multiple antiretroviral agents [2]. Raltegravir potassium targets integrase, an HIV enzyme that integrates the viral genetic material into human chromosomes, a critical step in the pathogenesis of HIV. The drug is metabolised away via glucouronidation .

Literature survey revealed that several analytical methods reported for the estimation of Raltegravir potassium in human plasma. Edward P.Acosta [3] was reported the sensitive HPLC-MS-MS method for the determination of Raltegravir potassium in human plasma. This work describes an assay system that has been developed to quantify Raltegravir concentration in human plasma using a liquid-liquid extraction technique paired with HPLC separation and MS-MS detection. Decosterd L.A [4] was reported the LC tandem MS assay for the simultaneous measurement of new antiretroviral agents: Raltegravir, Maraviroc, Darunavir, Etravirine. Single step extraction from plasma is performed by protein precipitation using 600 µl of acetonitrile. Ter Heine. R [5] was reported the quantification of the HIV integrase inhibitor raltegravir and detection of its metabolites in human plasma, dried blood spots and peripheral blood mononuclear cell lysate by means of HPLC tandem mass spectrometry. Naser L.Rezk [6] was reported an accurate and precise HPLC method for the quantification of the novel HIV integrase inhibitor raltegravir in human blood plasma after solid phase extraction. This is the first published method to use simple UV technology and reliable solid phase extraction methodology for the quantification of raltegravir in human plasma. Jean-Marie Poirier [7] was reported the quantification of the HIV integrase inhibitor raltegravir in human plasma by HPLC with fluorescence detection. Jasmine A. Talameh [8] was reported the quantification of raltegravir in female genital tract secretion using HPLC with UV detection. The method included sample preparation with perchloric acid followed by solid phase extraction. Separation with RP-

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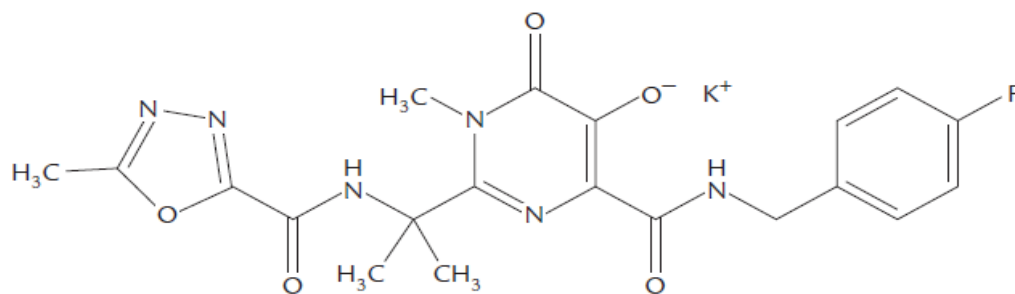


Fig. 1 : Chemical structure of Raltegravir potassium

HPLC and detection with an UV wavelength of 218 nm. Valerie Furlan [9] was reported the quantification of raltegravir in human plasma by HPLC with photodiode array detection. Notari.S [10] was reported the simultaneous determination of Maraviroc and Raltegravir in human plasma by HPLC-UV method. No methods have been reported for determination of Raltegravir potassium in bulk and tablet dosage form by RP-HPLC and UV spectrophotometric methods. The proposed research work describes the estimation of Raltegravir potassium in bulk and in tablet dosage form by RP-HPLC and UV spectrophotometric methods

II. MATERIALS AND METHODS

a) Chemicals

Raltegravir potassium pure drug was received from Divis pharmaceuticals, Hyderabad. The HPLC grade Methanol, acetonitrile, water was purchased from Qualigens fine chemicals, Mumbai, India. Analytical reagent grade potassium dihydrogen phosphate and ortho phosphoric acid from Merck were used. Tablets of Raltegravir potassium 400 mg(Isentress) were procured from Merck, U.S.

b) Instrumentation and analytical conditions

Chromatography was performed using a Water series HPLC with Empower software with UV detector. Symmetry X Terra C18 Column (100 × 4.6mm, 5μ) was used. The mobile phase consists of Buffer (pH 3.0): Methanol (45:55 % v/v). The flow rate was found to be 0.6ml/min and the wavelength was found to be 218 nm. The methods were conducted using an isocratic reverse phase technique. Temperature condition was ambient. Run time was adjusted to 5min. The UV method was performed on Lab India UV spectrometer with 1cm matched quartz cells. Photo Multiplier Tube was used as detector for all absorbance measurements. Electronic balance (AFCOSET ER-200A) was used for accurate measurements and pH meter (ADWA AD 1020) was used.

c) Preparation of standard stock solution

In method [A] 10 mg of Raltegravir potassium was dissolved in 7 ml of mobile phase and made upto

10 ml with mobile phase. In method [B] 10 mg of Raltegravir potassium was dissolved in 7 ml of mobile phase and made upto 10 ml with mobile phase. Both the solution contain (1 mg/ml)

d) Preparation of sample solution (Analysis of formulation)

In method [A] twenty tablets of Raltegravir potassium containing 400 mg of Raltegravir was weighed accurately. Tablet powder equivalent to 10 mg of raltegravir potassium was transferred to a 10 ml volumetric flask, dissolve in 7 ml of mobile phase and made upto volume with mobile phase. The resulting solutions were sonicate for 10 minutes and then filter through 0.45 μm filter. Transfer 0.3 ml of the filtrate into six separate 10 ml volumetric flask and made upto the volume with mobile phase (30 μg/ml). 20 μl of each solution was injected and the chromatograms were recorded. The peak area was determined and the procedure was repeated for six times. In method [B] powdered tablet equivalent to 10 mg of Raltegravir potassium was transferred to 10 ml volumetric flask dissolved and made upto the volume with solvent (1000 μg/ml). The solution was sonicate for 10 minutes and filter through 0.45 μm filter. Transfer 1ml of the filtrate to 10 ml volumetric flask and made upto the volume with mobile phase (100 μg/ml). From that, pipette out 0.3 ml solution and transferred to six volumetric flasks and made upto the volume with solvent (3 μg/ml). The absorbance was measured at 219 nm. The procedure was repeated for six times.

e) Calibration graph (or) Linearity

In method [A] aliquots of stock solution of Raltegravir potassium (0.1 to 0.5 ml of 1000 μg/ml) were transferred into 10 ml standard flask and made upto the volume with mobile phase. 20 μl of the solutions were injected and the chromatograms were recorded. The calibration was done by external standard calibration. Linearity was observed between (10-50 μg/ml). In method [B] aliquots of stock solution of raltegravir potassium (0.3 to 1.5 ml of 100 μg/ml) were transferred into 10 ml standard flask and made upto the volume with the solvent (methanol and phosphate buffer pH 3.0 in the ratio 55:45 %v/v). The absorbances of the

solutions of different concentration were measured at 219 nm against blank. Linearity was observed between (3-15 µg/ml).

f) Limit of detection and Limit of quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision, and variability. The LOD, LOQ were calculated as $LOD = 3.3 \sigma / S$ and $LOQ = 10 \sigma / S$ Where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve.

g) Accuracy

The accuracy of the method was checked by recovery studies of addition of standard drug solution to pre analysed sample solution at the different concentration levels (50%, 100%, and 150%) with in the range of linearity of the drug. The results of analysis of recovery studies obtained by the method [A&B] validated by statistical evaluation.

h) Precision

Precision is the degree of repeatability at an analytical method under normal operational condition. Repeatability and intermediate precision studies were done to the precision of the method [8]. Method [A] aliquots of standard stock solution of raltegravir potassium (0.3 ml of 1000 µg/ml) was transferred into 10 ml standard flask and made upto the mark with mobile phase (30 µg/ml). 20 µl of the solution were injected and the chromatograms were recorded. The procedure was repeated for five times. Method [B] aliquots of stock standard solution of Raltegravir potassium (0.9 ml of 100 µg/ml) was transferred into 10 ml standard flask and made upto the mark with solvent (9 µg/ml). The absorbance of the resulting solution was measured at 219 nm.

i) Repeatability

Repeatability is given by interday and intraday precision. The assay and recovery procedures were repeated for three times, on the same day and once for three successive days for both the methods.

j) Ruggedness

The degree of reproducibility of the test results obtained in method [A] and method [B] of raltegravir potassium was detected by analysing the drug sample under the variety of test conditions like different analyst and different instruments is ruggedness. The procedure was repeated under the above conditions.

k) Robustness

In method [A] as a part of robustness deliberate change in flow rate (0.5, 0.6, 0.7 ml/min), mobile phase composition (10% less, actual, 10% more) was made to evaluate the impact of the method.

l) Results and Discussion

Two simple, precise and accurate HPLC and UV methods were reported for the estimation of Raltegravir in bulk and tablet formulation. Method [A], A waters HPLC system with Xterra column C18 was used for analysis. The mobile phase was optimized with phosphate buffer (3.0) and methanol (45:55 %v/v) was used for the above mentioned composition of mobile phase. Sharp peak was obtained with the retention time 4.350 minutes. The UV detection was carried out 218 nm, as Raltegravir potassium showed very good absorbance at this wavelength. An optimized chromatogram of Raltegravir potassium was shown in figure 2. The system suitability parameters like tailing factor, asymmetric factor, number of theoretical plates and capacity factors were calculated and these values were compared with the standard limit as per USP [11]. It was found that the values were within the limits (Table 1). Method [B] is a simple UV method in which the drug, Raltegravir potassium shows a absorbance at 219 nm in the solvent (methanol and phosphate buffer pH 3.0 in the ratio 55:45 %v/v). The spectrum obtained for method [B] was shown in figure 3.

m) Calibration curve (Linearity)

Raltegravir potassium was found to be obeyed Beer's law in the concentration range of 10-50 µg/ml for method [A] and 3-15 µg/ml for method [B]. The correlation coefficient for method [A] and method [B] was found to be 0.999 and 0.999 respectively. This value indicates Raltegravir potassium has a good linearity in both the methods. In method [A] calibration curve was plotted by using peak area Vs concentration. In method [B] the curve was plotted by using absorbance Vs concentration. The optical characteristics such as Beer's law limit, correlation coefficient, molar absorptivity, sandell's sensitivity, slope and intercept values for method [B] were calculated and shown in Table 2.

n) Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) was calculated based on the signal to noise ratio. LOD and LOQ for Raltegravir potassium were found to be 0.027 µg/ml and 0.09 µg/ml respectively in HPLC. In UV LOD and LOQ was calculated with linearity studies. LOD and LOQ were found to be 0.0959 µg/ml and 0.290 µg/ml respectively. The results were shown in the Table 2.

o) Quantitation of formulation

The tablet formulation of Raltegravir potassium was selected for analysis and the percentage purity was found to be 100.62 % and 99.96 % for method [A] and method [B] respectively. The procedure was repeated for six times to validate the methods. The developed

methods were validated according to ICH guidelines [12]. The % RSD was found to be less than 2 %, which indicates the method [A] and method [B] had good precision. The results of analysis were shown in Table 3.

p) Accuracy

The accuracy of the method was confirmed by the recovery studies. To the pre analysed formulation a different concentration (50%, 100% and 150%) of the raw material was added and the amount of drug recovered was calculated. The percentage recovery was found to be 101.6 % \pm 0.2 for method [A] and 99.96 % \pm 1.0692 for method [B]. The procedure was repeated for three times. The % RSD value was calculated to be 0.19 and 1.0696 for method [A] and method [B] respectively. The low % RSD value indicates that there is no interference due to excipients used in the formulation. Thus both the developed methods are found to be accurate which is shown in Table 4

q) Precision and intermediate precision

The standard Raltegravir potassium solution of 30 μ g/ml was selected for analysis of method [A] and 9 μ g/ml for method [B]. The % RSD value was found to be 1.44 and 0.26 respectively for precision and intermediate precision in method [A] and % RSD value was found to be 1.13 and 0.31 respectively for precision and intermediate precision in method [B]. The low % RSD value reveals that the proposed methods were precise. The results were shown in table 5.

r) Repeatability

The precision of the method was confirmed by intraday and interday analysis. The assay and recovery procedures were repeated for three times on the same day and once for three successive days for both the methods. The assay % RSD value of intraday and interday analysis of Raltegravir potassium was found to be 0.56 and 1.72 respectively for method [A]. The % RSD value was found to be 0.82 and 1.51 respectively for method [B]. The recovery studies % RSD value of intraday and interday analysis was found to be 1.04 and 2.0 respectively for method [A]. The % RSD value of intraday and interday analysis was found to be 0.65 and 1.27 respectively for method [B]. The results were shown in table 6 and 7.

s) Ruggedness

Both the methods were validated for ruggedness. The results confirmed the ruggedness of the developed method. This is shown in table 8.

t) Robustness

Robustness was performed by changing the flow rate and by changing the organic composition of phase. It shows that there is no change in the values even after making deliberate change in the analytical procedure. The results were shown in the table 9.

III. CONCLUSION

The HPLC and UV methods developed for Raltegravir potassium shows good precision and accuracy. The low %RSD values in the recovery studies for both the methods shows that there is no interference due to excipients and for formulation. Hence it was concluded that the developed methods are simple, precise, accurate and rapid for the analysis of Raltegravir potassium in pure and in tablet dosage form. Thus the developed methods can be adopted for the routine analysis of Raltegravir potassium in bulk and tablet dosage form.

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REFERENCES RÉFÉRENCES REFERENCIAS

1. Savarino A. A historical sketch of the discovery and development of HIV 1 integrase inhibitor. Expert opin investing drugs 2006; 15(Suppl 12): 1507-22.
2. FDA approval of isentress (raltegravir). U.S Food & Drug Administration (FDA); June, 2009.
3. Edward P Acosta, Mary C.Long, Chantelle Bennetto-Hood. A sensitive HPLC-MS-MS method for the determination of Raltegravir in human plasma. J Chromatography B 2008; 867:165-171.
4. Decosterd LA, Fayet A, Beguin A, Zanolari B, Cruchon S, Guignard N et al.. A LC- tandem MS assay for the simultaneous measurement of new antiretroviral agents: Raltegravir, Maraviroc, Darunavir and Etravirine. J Chromatography B 2009; 877: 1057-1069.
5. Ter Heine.R, Hillebrand MJX, Rosing H, Van gorp ECM, Mulder JW, Beijnen JH. Quantification of the HIV-integrase inhibitor raltegravir and detection of its main metabolite in human plasma, dried blood spots and peripheral blood mononuclear cell lysate by means of HPLC tandem Mass spectrometry. J pharma Biomed analysis 2009; 49: 451- 458.
6. Nazer L Rezk, Nicole White, Angela DM Kashuba. An accurate and precise High performance liquid chromatography method for

the rapid quantification of the novel HIV integrase inhibitor raltegravir in human blood plasma after solid phase extraction. *Analytica Chimica Acta* 2008; 628: 204-213.

7. Jean Marie Poirier, Pascal Robidou, Patrice Jaillon. Quantification of the HIV-integrase inhibitor Raltegravir in human plasma by high performance liquid chromatography with fluorescence reduction. *J chromatography B* 2008; 867:277-281.
8. Jasmine A Talameh, Naser L Rezk, Angela DM Kashuba. Quantifying the HIV-1 integrase inhibitor raltegravir in female genital tract secretions using High Performance Liquid Chromatography with Ultraviolet detection. *J Chromatography B* 2010; 878: 92-96
9. Valerie Furlan, Anne-Marie Taburet, Maria Da Cruz, Aurelie Barrail-Tran, Lauriane Goldwirt. Quantification of Raltegravir in human plasma

by High Performance Liquid Chromatography with Photodiode array detection. *J Chromatography B* 2010; 878: 456-460.

10. Notari.S, Tommasi C, Nicastrì E, Bellagamba R, Tempestilli M, Percillo LP et al. Simultaneous determination of Maraviroc and Raltegravir in human plasma by HPLC-UV method. *Inter Union Biochem Mol Biology life* 2009; 61(Suppl 4): 470-475.
11. The United States pharmacopoeia. 23/NF, 18, United States of Pharmacopoeial convention, inc., Rock Ville, MD., 1995. p. 1063, 1961, 1988, 1990.
12. ICH Harmonized Tripartite Guidelines, Text on Validation of Analytical Procedures: Text and Methodology. Q2B. Geneva, 1996. p.8.

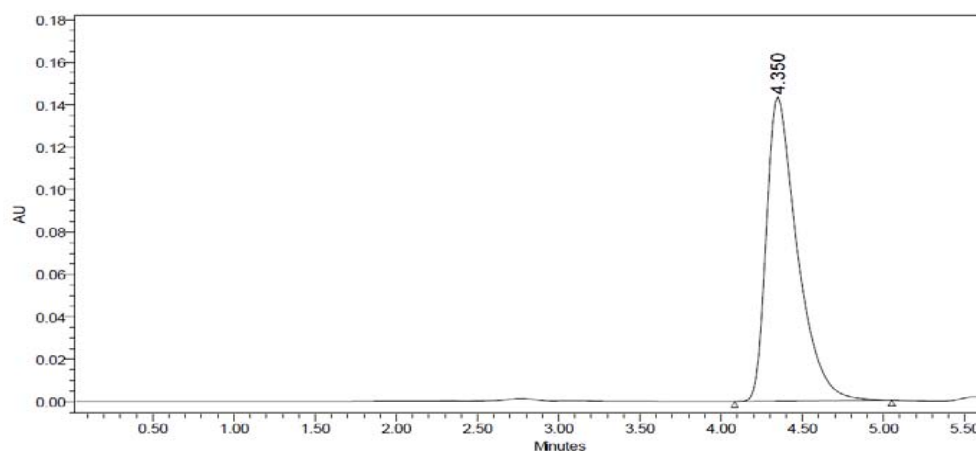


Fig. 2 : Optimized chromatogram of Raltegravir potassium

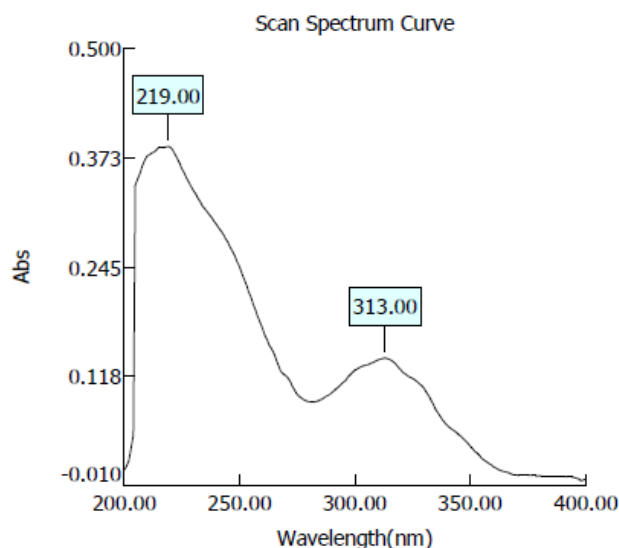


Fig. 3 : UV spectrum of Raltegravir potassium

Table 1: System Suitability Parameters

Parameter	Experimental value	Limit as per USP
Tailing factor	1.6	Less than 2
Asymmetric factor	1.0	Less than 2
No of theoretical plates	2524	More than 2000
Capacity factor	3.5	2-10
HETP	0.059	-
Theoretical plate per unit length	16.82	-

Table 2: Optical Characteristics of Raltegravir Potassium

Parameters	Methods	
	A (HPLC)	B (UV)
λ max`	218 nm	219 nm
Beer's law limit	10-50 μ g/ml	3-15 μ g/ml
Correlation coefficient (r ²)	0.999	0.999
Regression equation	$Y = 70827x + 8620$	$Y = 0.0433x - 0.0024$
Slope	70827	0.0433
Intercept	8620	-0.0024
LOD	0.027 μ g/ml	0.0959 μ g/ml
LOQ	0.09 μ g/ml	0.290 μ g/ml
Sandell's sensitivity	-	0.0228 μ g/cm ² /0.001
Molar absorptivity	-	20.6536 l/mol/cm

Table 3: Results of Analysis of Raltegravir Potassium in Tablet Formulation

No	Amount found (mg)		% label claim		SD		% RSD	
	A (HPLC)	B (UV)	A	B	A	B	A	B
1	399.190	398.642	99.79	99.6	1.091	0.4742	1.09	0.47
2	403.285	401.604	100.8	100.40				
3	394.190	400.114	98.5	100.02				
4	395.266	399.190	98.8	99.7				
5	392.000	397.164	98.0	99.2				
6	401.020	402.000	100.25	100.50				

Table 4: Accuracy Studies of Raltegravir Potassium

S.No.	Amount added (mg)		Amount found (mg)		% Recovery		Mean Recovery		SD		% RSD	
	A (HPLC)	B (UV)	A	B	A	B	A	B	A	B	A	B
1	5.36	5.1	5.46	5.11	101.8	100.2						
2	10.5	10.1	10.6	9.98	101.4	98.8	101.6	99.96	0.2	1.069	0.19	1.06
3	16.0	15.0	16.2	15.14	101.6	100.9						

Table 5: Precision and Intermediate Precision of Raltegravir Potassium

Parameters	Method [A] HPLC			Method [B] UV		
	Avg. Peak Area	SD	% RSD	Avg. Absorbance	SD	% RSD
Precision	1953328	28316.4	1.44	0.369	0.0041	1.13
Intermediate Precision	2127233	5601.7	0.26	0.367	0.0011	0.31

Table 6 : Intraday And Interday Analysis Formulation

Parameters	% Estimated		% Mean		SD		% RSD	
	A (HPLC)	B (UV)	A	B	A	B	A	B
Intraday	99.94 99.91 101.13 100.22	99.04 100.03 98.21 98.42	100.3	98.92	0.570	0.816	0.56	0.82
Interday	100.66 99.51 102.50 98.45	99.86 98.27 100.54 101.92	100.28	100.14	1.733	1.516	1.72	1.51

Table 7: Intraday and Interday Recovery Studies of Formulation

Parameters	% Estimated		% Mean		SD		% RSD	
	A (HPLC)	B (UV)	A	B	A	B	A	B
Average Intraday (50,100, 150 %)	100.84 100.81 99.01	98.72 100.02 99.41	100.22	99.38	1.047	0.650	1.04	0.65
Average Interday (50, 100, 150 %)	97.75 100.20 101.72	98.13 100.56 100.02	99.89	99.57	2.0	1.275	2.0	1.27

Table 8 : Ruggedness By Hplc And Uv Method

S.No	Type of analysis	% Estimated		SD		% RSD	
		A (HPLC)	B (UV)	A	B	A	B
1	Analyst-1	99.29	99.47				
2	Analyst-2	100.50	97.24				
3	Instrument-1	98.57	101.57	1.367	1.871	1.38	1.88
4	Instrument-2	97.23	98.20				

Table 9 : Robustness Studies For Raltegravir Potassium By Hplc

Parameters		System Suitability	
		USP plate count	USP Tailing
Flow rate (ml/min)	0.5	2451	1.7
	0.6	2669	1.6
	0.7	2773	1.5
Mobile phase composition (Organic)	10% less	2975	1.5
	Actual	2669	1.6
	10% more	2145	1.6



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