DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR DETERMINATION OF RALTEGRAVIR POTASSIUM

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ABSTRACT

Raltegravir potassium is an antiretroviral drug. A simple and rapid stability indicating HPLC method of Raltegravir potassium was successfully developed. This method is based on HPLC separation followed by UV detection at 315 nm. HPLC method was developed on a symmetry HiQsil C_{18} (150 x 4.6mm id 5 µm) column with a mobile phase consisting of Methanol: Phosphate buffer: Acetonitrile 40:30:30 % v/v/v, pumped at 1.0 ml min-1 flow rate. The pH of buffer was adjusted to 3.0 with ortho phosphoric acid. The column was maintained at ambient temperature and 20µl of solutions were injected. The eluted compounds were detected by using PDA detector. Raltegravir potassium eluted at 3.3 ± 0.2 min. Stress degradation study shows that sample degraded with acid and base hydrolysis, under oxidation, thermal and photolytic stress conditions.

KEYWORDS

Raltegravir potassium, Stability indicating RP-HPLC, Forced degradation, Validation.

INTRODUCTION

Raltegravir potassium chemically, Potassium; 4-[(4-fluorophenyl) methylcarbamoyl]-1methyl-2-[2-[(5-methyl-1,3,4-oxadiazole-2-carbonyl)amino]propan2-yl]-6-oxopyrimidin-5-olate.It is a novel integrase stand transfer inhibitor active against human immunodeficiency virus [1-3].

Literature survey reveals that very few analytical methods have been reported for Raltegravir potassium viz. UV-Spectrophotometry.[4,5] RP-HPLC method for the determination of Raltegravir in pharmaceutical preparation.[6-8] RP-HPLC method for the determination of Raltegravir in human plasma.[9] RP-HPLC method

for determination validation and UPLC method for stability indicating method.[10] There are no reports for determination of Raltegravir potassium by stability indicating HPLC method, hence the work was undertaken.

METHOD

Instruments:

Quantitative HPLC was performed using isocratic high performance liquid chromatography (Jasco HPLC system) with a LC-PU 2080 Plus pump, manual injector with loop volume of 20µl (Rheodyne), programmable MD 2010 PDA detector and HiQsil C_{18} (150 x 4.6 mm i.d, 5 µm). The HPLC system was equipped with "Borwin- PDA software (version1.5). An electronic balance (Shimadzu AY-120), UV-Visible (Jasco model V-550) spectrophotometer, Elga Labwater (PURELAB UHQ-II) water purification system were used in this study.

Optimized chromatographic conditions:

The mobile phase consisting of Methanol: 10mM sodium dihydrogen phosphate buffer (PH 3.0): Acetonitrile in the ratio of (40:30:30) % v/v. It was then filtered through 0.45 µ membrane filter paper using vacuum filtration assembly and then sonicated on ultrasonic water bath for 15 min. The flow rate of mobile phase was maintained at 1ml/min and the column and the HPLC systems were kept in ambient temperature.

Preparation of solutions:

Preparation of standard stock solution:

Standard stock solution of Raltegravir potassium was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. Further dilution was made in methanol to get final concentration range of Raltegravir potassium 50,100,150,200,250 µg/ml.

Preparation of Buffer:

Preparetion of buffer procedure in IP Dissolve 90 mg of di sodium hydrogen phosphate buffer in 100 ml of HPLC grade water and adjust the pH 3.0 with 10% orthophosphoric acid.

Preparation of sample solution of blend:

For assay 1 gm Raltegravir potassium was mixed with 250 mg of starch and 250 mg of lactose 75 mg of this blend was weighed and diluted with 50 ml of methanol to get solution of concentration of 1000 µg/ml.

Selection of analytical wavelength:

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200–400 nm. The spectrum was obtained. It was observed that the drug showed considerable absorbance at 315 nm (Fig.2).

Selection of mobile phase:

The standard solution of Raltegravir potassium (100µg/ml) was injected into the HPLC system and run in different solvent systems. Different mobile phases like acetonitrile and water, methanol and water, acetonitrile and phosphate buffer, methanol and phosphate buffer in varying proportion of mobile phase components, varying conditions of pH were tried in order to obtain the desired system suitability parameters for the Raltegravir sodium. After several trials, methanol, 10 m disodium hydrogen phosphate PH 3.0 and acetonitrile in the ratio of 40: 30:30 % v/v was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Chromatogram and system suitability parameter of drug:

The column was equilibrated with the mobile phase (indicated by constant back pressure at desired flow rate). Working standard solution of drug (150µg/ml) was injected into the system. The retention time for the drug was found to be: 3.3± 0.2 min. System suitability parameters of Raltegravir potassium were summarized in Table 1.

Stress degradation studies of bulk drug: [11,12]

Stress degradation studies were carried under condition of acid, base, neutral hydrolysis, oxidation, dry heat and photolysis as per ICH Q1A R2 and Q1B. For each study, two samples were prepared: the blank subjected to stress in the same manner as the drug solution and working standard solution of Raltegravir potassium subjected to stress condition. Dry heat and photolytic degradation were carried out in solid state. Stress conditions were optimized to achieve 10-30% degradation.

Acid hydrolysis

Optimized condition:

Acid induced degradation was performed by adding 1 ml of 0.1N Hydrochloric acid (HCl) to volumetric flask containing 1ml of Raltegravir potassium standard solution (1500 µg/ml). The volume was made up to 10 ml with methanol & kept for 20 min. The solution was neutralized with 0.1ml of Sodium Hydroxide (NaOH) solution. Final solution (150 µg/ml) was injected.

After acid hydrolysis, 84.39% Raltegravir potassium was recovered with no peak of degradation

Alkaline hydrolysis

Optimized condition:

Base induced degradation was performed by adding 1 ml of 0.1N sodium hydroxide (NaOH) to volumetric flask containing 1ml of Raltegravir potassium standard solution (1500µg/ml). The volume was made up to 10 ml with methanol & kept for 18 hrs. The solution was neutralized with 0.1 ml of Hydrochloric acid (HCl) solution. Final solution (150µg/ml) was injected.

After acid hydrolysis, 70.99 % Raltegravir potassium was recovered with one peak of degradation at RT 2.9 min. (Fig. 4).

Neutral Hydrolysis

Optimized condition:

Neutral hydrolysis was performed by adding 1ml of Raltegravir potassium standard solution (1500µg/ml) was mixed with 1ml of water in 10 ml of volumetric flask and the volume was made upto the mark with methanol. Solution was kept for overnight dark place. Final solution (150µg/ml) was injected.

After neutral hydrolysis, 86.81% Raltegravir potassium was recovered with no peak of degradation.

Oxidative Hydrolysis

Optimized condition:

Oxidative degradation was performed by adding 1ml of Hydrogen peroxide (H_2O_2, 3% v/v) to volumetric flask containing 1ml of Raltegravir potassium standard solution (1500µg/ml). The volume was made up to 10 ml with methanol & kept for overnight in dark place.

After oxidative hydrolysis, 88.59% Raltegravir sodium was recovered with no peak of degradation.

Degradation under dry heat

Optimized condition:

Dry heat study was performed by exposing Raltegravir sodium in oven (80^0C) for a period of 6 hours. A sample was withdrawn after 6 hours, weighed and dissolved in methanol to get solution of 1000 µg/ml and further dilutions were made with methanol to get final concentration (150µg/ml)

After the dry heat degradation, 89.23% Raltegravir sodium was recovered with no peaks of degradation.

Photo-degradation studies

Optimized condition:

Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt hours /square meter and separately to fluorescence light illumination not less than 1.2 million lux hours. Sample was weighed, dissolved in methanol to get concentration of 1000 µg/ml. and further dilutions were made with methanol to get final concentration (40 µg/ ml)

After the photo degradation study under UV light 89.95% and Fluorescence light 90.79% Raltegravir potassium was recovered with no peak of degradation.

Stress degradation data summarized in table no. 2

Validation of Analytical Method:

The method was validated as per ICH Q2 (R1) guidelines [14].

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 990, indicating the non-interference of any other peak of degradation product or impurity as shown in table no.3

Linearity

Linearity was tested for the range of concentrations 50-250µg/ml. Each sample in Five replicates was analyzed and peak areas were recorded. The response factors were plotted against the corresponding concentrations of Raltegravir potassium to obtain the calibration curve. (Fig. 17)

Range

Raltegravir potassium: The range was found to be 50-250 µg/ml

Assay

Since marketed sample is not readily available Raltegravir potassium was spiked in excipient blend (prepared in the ratio expected in marketed product). Blend equivalent to 50 mg Raltegravir potassium was weighed and diluted with 50 ml of methanol to get solution of concentration of 1000µg/ml. This solution was filtered through whatmann filter paper from which 1 ml of solution was diluted to 10 ml with methanol to get concentration of 100µg/ml.

Peak area was extrapolated from linearity equation, assay data is summarized in Table 4

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to Blend (Excipients) at three different levels 80%, 100% and 120 %. Basic concentration of sample chosen was 100 µg/ml of standard. These solutions were injected into HPLC system in triplicate to obtain the chromatogram.

The drug concentrations were calculated by using linearity equation of Raltegravir potassium. The results obtained are shown in Table 5.

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Precision

The precision of the system was demonstrated by Intra-day and Inter-day variation studies. In the Inter-day studies, 3 replicates of three independent solutions of Raltegravir potassium were analyzed on three different days and percentage RSD were calculated. In the Intra-day studies 3 replicates of 3 concentrations were analyzed on the same day.

Precision data is summarized in table 6 and 7

Limit of Detection (LOD)

$$LOD = \frac{3.3\ \sigma}{S}$$

LOD is calculated from the formula: -

Where,

σ = standard deviation of peak area of drug for the lowest conc. in the range

S = slope of the calibration curve.

LOD of Raltegravir sodium= 1.85 µg/ ml

Limit of Quantification (LOQ)

The Quantitation limit is expressed as:

$$LOQ = \frac{10\,\sigma}{S}$$

LOQ of Raltegravir potassium= 5.61 µg/ ml.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which flow rate, Concentration (Strength) of buffer, mobile phase ratio were altered and the effects on the peak area were noted.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions:

The primary objective in developing this stability indicating HPLC method is to achieve the response of Raltegravir potassium free from interference by degradation products, if any. The chromatographic separation was achieved by using HiQsil C_{18} (150 x 4.6 mm i.d, 5 µm) column and mobile phase consisting of Methanol: 10mM sodium dihydrogen phosphate buffer (PH 3.0): Acetonitrile in the ratio of (40:30:30) % v/v. Detection was carried out by PDA detector. The retention time for Raltegravir potassium was found to be 3.3± 0.2 min.

Result of forced degradation studies:

Acid treatment

Raltegravir potassium on treatment with 0.1 N HCL and kept for 20 min at room temperature showed 15.61% degradation with no degradation product observed.

Alkali treatment

Raltegravir potassium on treatment with 0.1 N NaOH and kept for 18 hours at room temperatue showed 29.01% degradation with only one degradation product observed at RT 2.9 min.

Peroxide treatment

Raltegravir potassium on treatment with 3% $^{v}/_{v}$ H_2O_2 and kept for 24 hours at room temperature showed 11.41 % degradation with no degradation product observed.

Neutral treatment

Raltegravir potassium on treatment with H_2O for 24 hours at room temperature it showed 13.19% degradation with no degradation product observed.

Photo degradation Studies

Raltegravir potassium when exposed to ultraviolet light (200 Watt hours/Square meter) and when exposed to fluorescence light (1.2 million lux hours) it showed 10.05 % and 9.21% degradation respectively.

Dry heat degradation Studies

Degradation was observed for Raltegravir potassium when the bulk drug substance was exposed to dry heat at 80º C for 6 hours, it showed 10.77% degradation.

The only paper available in literature for stability indicating method by UPLC does not mention any product of degradation under any particular stress condition. In current study degradation product upon base catalyzed hydrolysis was detected. The degradation product upon base catalytic hydrolysis was characterized by LC-MS. In current work drug show sensitivity towards light, thermal stress condition which was not shown in sole SI-UPLC method reported.

CONCLUSION

The developed method is stability indicating, since the drug peak was found to be pure as confirmed by peak purity profiling study. This proves that there is no interference of degradation product in analytical peak. The method is specific, accurate, precise, and robust and can be used for routine quality control as well as assessing the stability of Raltegravir sodium.

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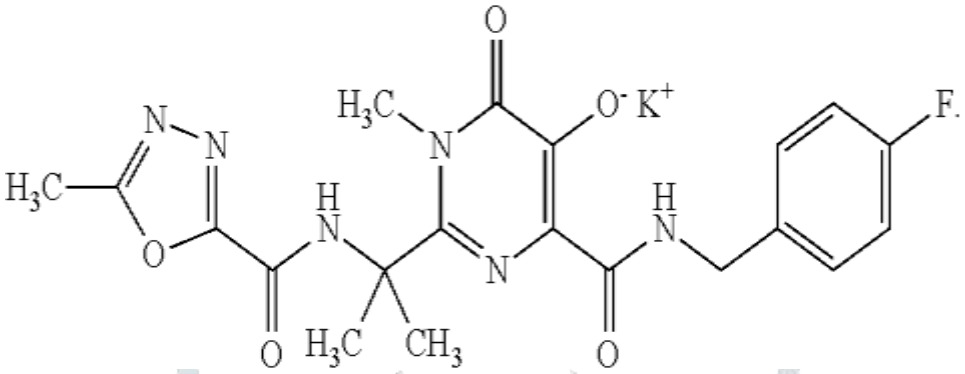

Fig. 1: Chemical structure of Raltegravir potassium

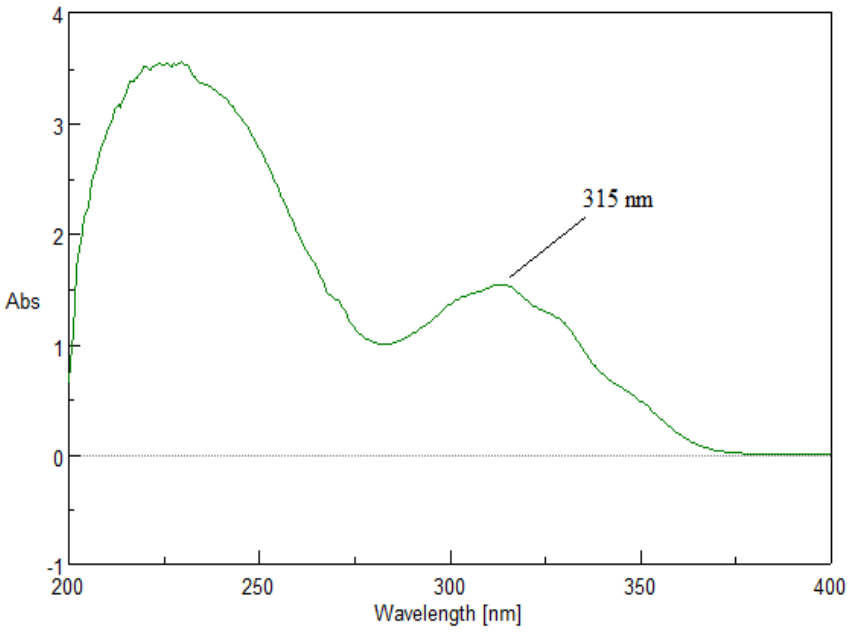

Fig. 2: UV Spectrum of Raltegravir potassium (100µg/ml)

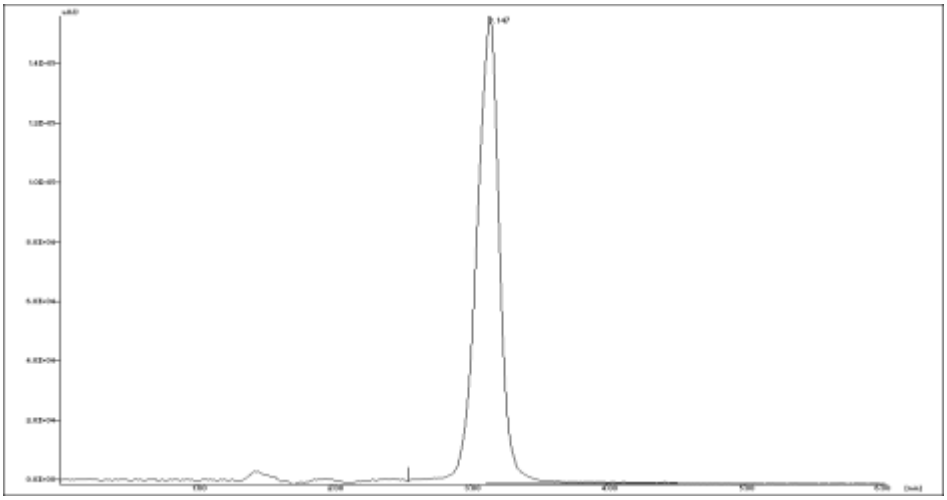

Fig. 3: Chromatogram of standard solution of Raltegravir potassium 100 mcg/ ml (, RT = 3.3 ± 0.2 min)

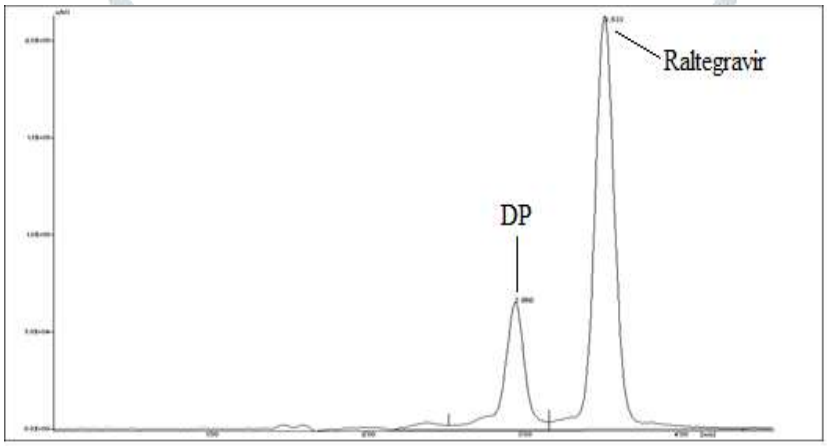

Fig. 4: Chromatogram of alkali treated Raltegravir potassium (150µg/ml)

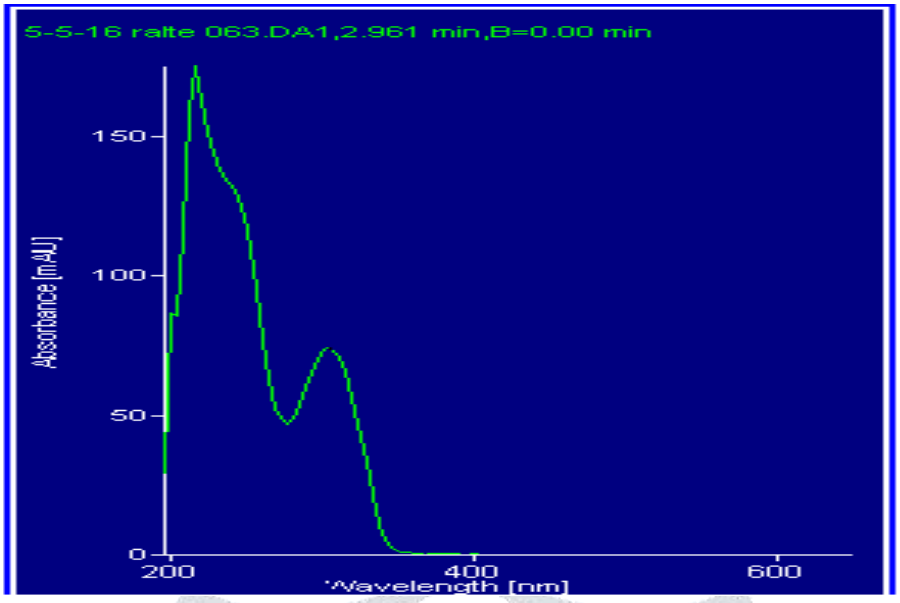

Fig. 5: UV Spectra of degradation product at RT 2.9 min

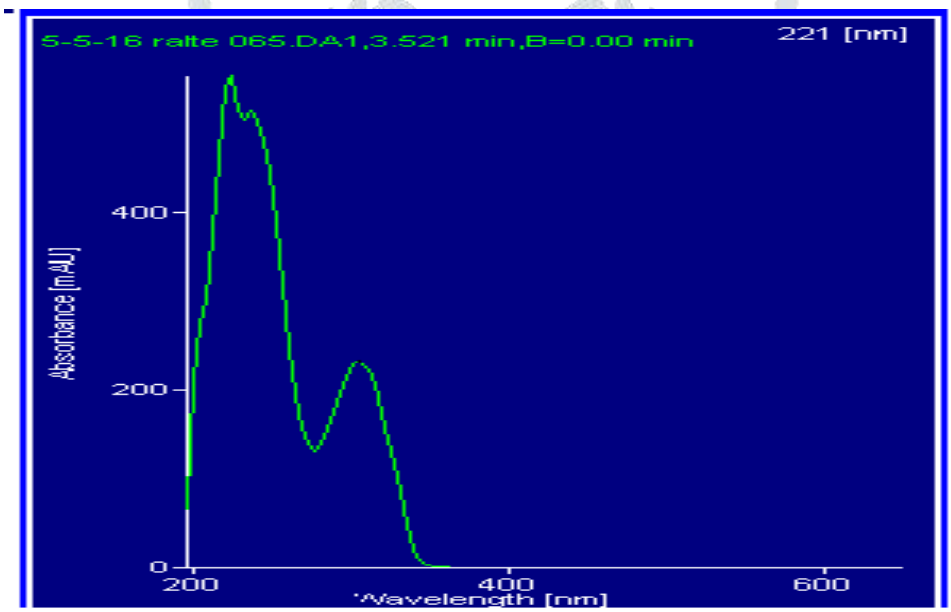

Fig. 6: UV Spectra of drug at RT 3.5 min

Fig. 16: Probable degradation reaction- Alkaline hydrolysis

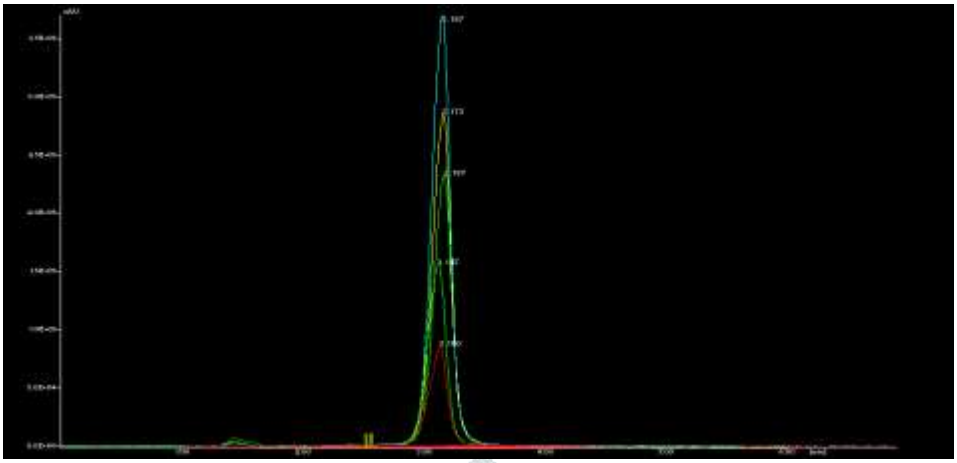

Fig. 17: Chromatogram of linearity of Raltegravir potassium (50-250µg/ml)

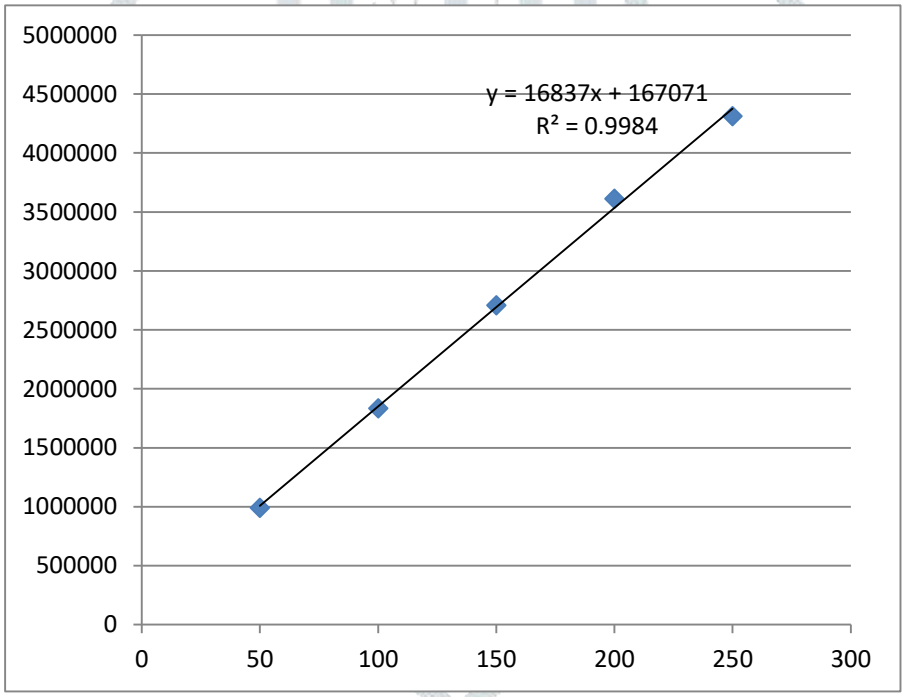

Fig. 18: Calibration curve for Raltegravir sodium

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Table 1: System suitability parameter

Name	RT (Min)	Conc. (µg/ml)	Area	Plates	Asymmetry
Raltegravir sodium	3.147	100	1818587.63	2246.2	0.91

Table 2: Summary of stress degradation study of Raltegravir potassium

Sr. No	Stress Degradation Conditions	%Recovery	Pear purity	
			Peak front	Peak tail
1	Acid hydrolysis (0.1N HCl for 20 min)	84.39	991.8	994.6
2	Base hydrolysis (0.1N NaOH for 18 hrs)	70.99	990.1	993.6
3	Oxidation (3 % H_2O_2 for overnight)	88.59	992.2	994.3
4	Neutral Hydrolysis (water for overnight)	86.81	990.5	992.8
5	Dry Heat (80^0C in oven for 6 hrs)	89.23	991.4	993.3
6	UV (200 watt hours./square meter)	89.95	992.1	993.7
	Florescent light, (1.2 million Lux. Hrs.)	90.79	990.5	992.8

Table 3: Specificity

Drug	Purity tail	Purity front	Asymmetry
Raltegravir sodium	990.8	993.6	0.91

Table 4: Assay of Spiked Blend

Sr. No.	Peak area of Raltegravir potassium blend	% Recovery
1	1856920.90	100.36
2	1877811.39	101.60
3	1857360.59	100.39
4	1858239.26	100.44
5	1859632.60	100.52
6	1875337.89	101.45
Mean	1864217.10	100.79
SD	9648.57	0.57
%RSD	0.517	0.56

Table 5: Recovery studies of Raltegravir potassium

Level (%)	Theoretical Conc. (µg/ml)	Amount Recovered (µg/ml)	% Recovery
80	80	80.28	101.21
100	100	100.58	99.08
120	120	122.25	101.07

Table 6: Intra-day precision study of Raltegravir potassium

Concentration (µg/band)	% amount recovered	Average	SD	% RSD
100	97.73	98.15	0.36	0.369
	98.29			
	98.41			
150	100.055	100.13	0.56	0.568
	99.60			
	100.73			
200	100.52	101.51	0.86	0.853
	101.86			
	102.14			

Table 7: Inter-day precision of Raltegravir potassium

Concentration (µg/band)	% amount recovered	Average	SD	% RSD
100	98.38	98.36	0.23	0.242
	98.11			
	98.59			
150	98.29	99.55	1.48	1.494
	99.18			
	101.19			
200	100.33	99.79	0.46	0.469
	99.51			
	99.52			

Table 8: Robustness study

Chromatographic changes				
Conditions	**Variation**	**Retention time (min)**	**Area**	**% RSD**
Mobile phase ratio % v/v/v)	39:30:31	3.28	1856920.90	0.57
	40:30:30	3.33	1857360.59	
	41:30:29	3.46	1858239.26	
Flow rate	0.9	3.21	1852174.80	0.70
	1.0	3.36	1853007.20	
	1.1	3.43	1855201.9	
pH	2.8	3.25	1844372.4	0.44
		3.33	1848212.6	
		3.28	1833821.7	
	3.2	3.42	1838221.9	0.55
		3.38	1858898.4	
		3.36	1848454.2	

Table 9: Summary of validation parameter

Sr. No.	Validation parameters	Raltegravir potassium
1.	Linearity Equation (r2) Range	y = 16837x + 167071 r^2 = 0.9984 50-250µg/ml
2.	Precision (% RSD) Interday Intraday	 0.59 0.73
3.	Accuracy	% Recovery
	80%	101.60
	100%	100.58
	120%	101.87
4.	Limit of Detection	1.85 µg/ml
5.	Limit of Quantitation	5.61 µg/ml
6.	Specificity	Specific
7.	Robustness	Robust

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