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Simple Spectrophotometric Method for Estimation of Raltegravir Potassium in Bulk and Pharmaceutical Formulations

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ABSTRACT

A simple, rapid, precise, and economical spectrophotometric method has been developed for quantitative analysis of Raltegravir Potassium (RALP) in tablet formulations. The stock solution of RALP was prepared in water. The standard solution of RALP in water showed absorption maxima at 331.6 nm. The drug obeyed Beer–Lambert's law in the concentration range of 1–100 µg/mL with coefficient of correlation (R^2) was 0.9999. It showed coefficient of variation below 2 % in intra-run and inter-run precision. The recovery was obtained with values close to the 100 % of theoretical at three different concentrations. The results of analysis have been validated as per ICH guidelines. The method can be adopted in routine analysis of RALP in bulk and tablet dosage form and it involves water as a solvent and no complex extraction techniques.

INTRODUCTION

Raltegravir potassium (MK-0518) is an antiretroviral drug produced by Merck & Co., used to treat HIV infection (Savarino, 2006; US FDA, 2009). Raltegravir Potassium (RALP) chemically known as is N-[(4-Fluorophenyl)methyl]-1,6-dihydro-5-hydroxy- 1-methyl- 2- [1- [[(5-methyl - 1,3,4-oxadiazol-2-yl) carbonyl] amino] ethyl] -6-oxo-4-pyrimidinecarboxamide mono potassium salt (Savarino 2006; Serrao *et al.,* 2009; Cocohoba *et al.,* 2008; Humphrey *et al.,* 2011). It targets integrase, an HIV enzyme that integrates the viral genetic material into human chromosome. It is a critical step in the pathogenesis of HIV. The drug is metabolized away via glucuronidation (Summa *et al.,* 2008; Steigbigel *et al.,* 2008). It is recently approved by the US Food and Drug Administration (FDA) in October 2007, the first of a new class of HIV drugs, the integrase inhibitors, to receive such approval (US FDA, 2009). Raltegravir potassium is not official drug in any pharmacopeia till the date. In literature, the LC-MS/MS methods are reported for determination and quantification Raltegravir potassium alone and combination with other retroviral drugs (Poirier *et al.,* 2008; Long *et al.,* 2008; Merschman *et al.,* 2007; Quaranta *et al.,* 2009; Takahashi *et al.,* 2008; Vallano *et al.,* 2005; Fayet *et al.,* 2009; Jourdil *et al.,* 2009; Wang *et al., 2011*; Mosnier-Thoumas *et al.,2011*;Ter *et al., 2009*). Some high performance liquid chromatography (HPLC) methods are also reported in the literature for determination of Raltegravir potassium in human plasma (Goldwirt *et al.,* 2010; Talameh *et al.,* 2009; D'Avolio *et al.,* 2008; Rezk *et al.,* 2008) and pharmaceutical formulations (Rambabu *et al.,* 2011; Rami Reddy *et al.,* 2012; Satyanarayana *et al.,* 2011). However extensive survey revealed that currently there is no UV/Visible spectrophotometric method for quantitative determination of Raltegravir Potassium in Bulk API and or pharmaceutical formulations. Therefore it was felt necessary to develop an accurate, precise, specific, and rapid UV method for the determination of Raltegravir Potassium in pharmaceutical formulations. The main advantage of this developed method is that it involves water as a solvent and no other complex extraction techniques. Hence it can be adopted in routine analysis of RALP in bulk and tablet dosage form.

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MATERIALS AND METHODS

Materials

Raltegravir Potassium bulk drug was obtained from Hetero Labs Ltd, (Hyderabad, India), Whatman filter paper number 1 from Qualigens Fine Chemicals, India. The MilliQ water is used throughout the process. The commercially tablets of RALP are not available in Indian market; hence we have manufactured RALP immediate release tablet containing 400 mg of Raltegravir Potassium. The other ingredients used were lactose monohydrate, croscarmellose sodium, microcrystalline cellulose, PVP K-30 and magnesium stearate. Other chemicals used were analytical or HPLC-grade and glassware used were Class A grade.

Instruments

Shimadzu UV - 1700 UV/VISIBLE spectrophotometer with UV probe 2.10 software and 1 cm matched quartz cells were used for absorbance measurements. Analytical balance used for weighing standard and sample was Make-Mettler Toledo, Model-XP 105.

Preparation of standard stock solution

Accurately weighed 25 mg of RALP working standard was transferred into a 250 mL volumetric flask and dissolved in 150 mL of water. The solution was sonicate for 10 minutes. The volume was made up to 250 mL with water to give the solution containing 100 µg/mL of RALP.

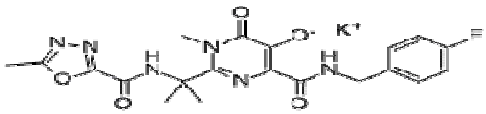

Fig. 1: Chemical structure of RALP.

Selection of λ_{max}

The standard stock solution was further diluted with water to get a 20 µg/mL of concentration. The solution was scanned between 200 and 400 nm using water as blank. The UV spectrum of RALP in water had shown λ_{max}, at 331.6 nm. Hence, it was selected for the analysis of RALP [Figure 2].

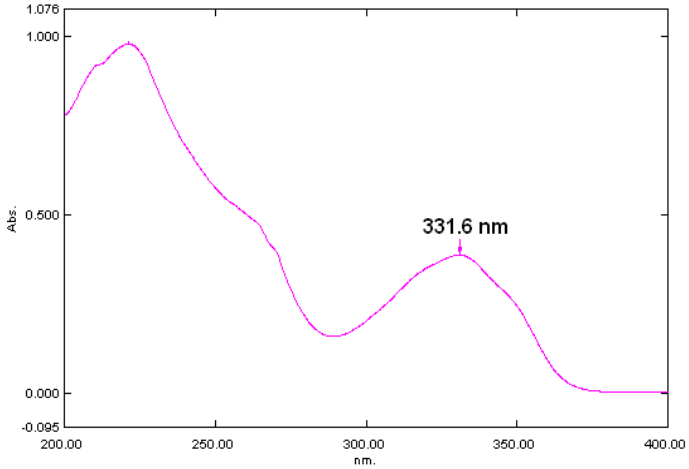

Fig. 2: UV spectrum of pure drug RALP in water.

Preparation of the calibration curve

Aliquots of standard stock solution were further diluted with water to get the solutions of concentration 1–100 µg/mL. The absorbance were measured at 331.6 nm against water as blank. All measurements were repeated three times for each concentration. The calibration curve was constructed by plotting mean of absorbance against corresponding concentration.

Preparation of the sample solution

The tablets of RALP are not available in Indian market; hence tablets manufactured in laboratory were assayed. These were labeled to contain 25 mg of RALP as an active substance per tablet.

Twenty tablets containing 400 mg of RALP were accurately weighed and powdered. The powder equivalent to 25 mg of RALP was weighed and transferred to a 250 mL volumetric flask; 150 mL water was added and sonicated for 10 min. The volume was adjusted to 250 mL with water. The solution was filtered through Whatman filter paper No. 01. From this filtrate, 10 mL was transferred to a 100 mL volumetric flask and diluted with water to 100 mL in order to obtain the final concentration of 10 µg/mL. The absorbance was measured at 331.6 nm using water as blank. This procedure was repeated for six times. The amount of RALP present in formulation was calculated by comparing it with standard absorbance. The results obtained are shown in Table 1.

Table. 1: Assay of tablet formulations.

Labeled claim (mg)	Amount Found (mg)	% Assay*	%RSD*
400	399.7	99.59	0.73

*Mean of six determinations.

Method validation

The developed method was validated as per ICH guidelines for following parameters.[13]

Linearity

The linearity was studied in the concentration range of 1–100 µg/mL at 331.6 nm. Linear regression data are shown in Table 2 and Figure 3.

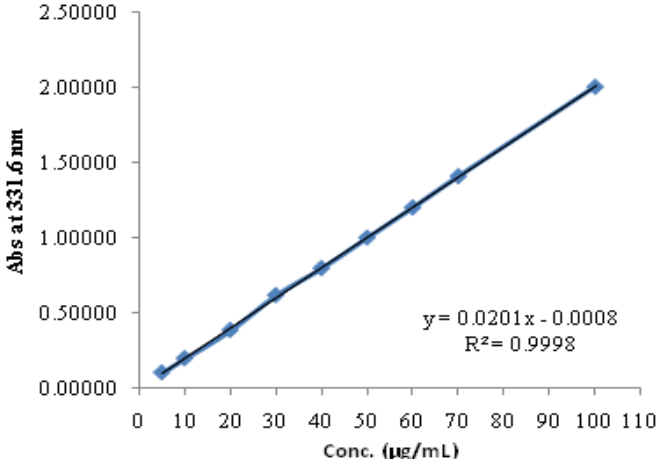

Fig. 3: Linearity plot of RALP at 331.6nm.

Table. 2: Linear regression data.

Sr. No.	Parameters	Results
1	λmax (nm)	331.6
2	Beer's law limit (µg/mL)	1–100
3	Correlation coefficient	0.9998
5	Regression equation ($y = mx + c$)	y = 0.0201x - 0.0008
6	Slope (m)	0.0201
7	Intercept (c)	-0.0008
8	Detection limit (µg/mL)	0.2
9	Quantification limit (µg/mL)	0.5

Specificity and selectivity

The spectra obtained from tablet solutions were identical with that obtained from standard solution containing an equivalent concentration of RALP. This showed that there was no any interference from excipients. Therefore, it could be said that developed method is highly selective.

Recovery studies

To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100%, and 120% level to preanalyzed samples and subsequent solutions were reanalyzed. At each level, three determinations were performed. The absorbances were measured at 331.6 nm using water as blank and the amount of drug recovered from the formulation were calculated, and the results obtained are shown in Table 3.

Table 3: Results of recovery studies.

Tablet amount (µg/mL)	Level of addition (%)	Amount of std drug added (µg/mL)	Amount recovered (µg/mL)*	% Recovery ±SD*	Mean % recovery
5	80	8	7.99	99.9 ± 0.50	
5	100	10	10.03	100.3 ± 0.84	100.1
5	120	12	12.01	100.1 ± 0.90	

*Mean of three determinations.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of drug. Graphs were recorded, and the area of each graph was measured. The results of this determination are reported in Table 4.

Table. 4: Result of repeatability, intraday, and Interday precision studies.

Method wavelength (nm)	Intraday		Interday		Repeatability	
	% Labeled claim ± SD*	% RSD	% Labeled claim ± SD*	% RSD	% Labeled claim ± SD*	% RSD
331.6	99.91 ± 0.50	0.51	99.47 ± 0.42	0.42	99.21 ± 0.25	0.25

*Mean of six determinations.

Intraday and inter-day precision

Intra-day precision was determined by analyzing the drugs at three different concentrations and each concentration for three times, on the same day.

Inter-day precision was determined similarly, but the analysis being carried out daily, for three consecutive days. The results are summarized in Table 4.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2 nm.

For changes of conditions, the sample was assayed in triplicate. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. Assay of RALP for all deliberate changes of conditions was within 98.0–102.0 %. The results are shown in Table 5.

Table. 5: Result of robustness studies.

Method wavelength (nm)	Condition (nm)	% Assay*	% RSD
331.6	329.6	99.17	0.63
	333.6	99.21	0.51

*Mean of three determinations.

Ruggedness

To determine ruggedness, two different analyst performed assay on manufactured tablets of the drug in similar operational and environmental conditions using developed method. The results are summarized in Table 6.

Table 6: Result of ruggedness studies.

Parameter	Analyst I	Analyst II
Label claim (mg)	400	400
% Assay*	99.93	99.53
% RSD	0.71	0.86

*Average of six determinations.

Solution stability

The stability of the standard solution was tested at intervals of 1, 5, 10, and 24 h. The stability of solutions was determined by comparing absorbance of RALP. The absorbance values were within 0.5% after 24 h.

These results indicate the solution was stable for 24 h at ambient temperature, because there was slight change in assay value. The %RSD of assay was 1.17 % after 24 h. The results are shown in Table 7.

Table. 7: Stability data.

Sr. No.	Ingredient	Time (h)	% Assay*	% RSD
1	RALP	1	100.3	0.35
		5	99.81	0.69
		10	99.83	0.75
		24	98.61	1.17

*Average of three determinations.

RESULTS AND DISCUSSION

The overlay UV spectra of standard and tablet solutions of RALP in water were found to be same. The UV spectrum of RALP in water has maximum absorption (λ_{max}), at 331.6 nm. The absorbance of excipients in tablet solution did not interfere with RALP at 331.6 nm. As a result, the wavelength was selected for quantitative analysis and validation. The developed method was found to be precise as the %RSD values for intraday and interday precision were found to be less than 2%. The method was also found to be accurate, indicated by % recoveries ranging from 99.1 to 100.1%.

CONCLUSION

The developed UV spectrophotometric method for the determination of RALP has the advantage of being fast, simple, inexpensive, and applicable over a wide concentration range with high precision and accuracy. The method was validated as per the guidelines laid by ICH. The results of the validation tests were found to be satisfactory and therefore this method can be applied successfully to analyze drug formulations.

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