Novel Spectrophotometric Methods For The Determination of Raltegravir Potassium In Bulk And Pharmaceutical Formulations On The Basis of Coupled Red Ox Complexation Reactions

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Abstract: ***Four simple and sensitive visible spectrophotometric methods M_1,M_2,M_3 and M_4 are described for the assay of Raltegravir potassium in pure and solid dosage forms. These methods are based on the oxidation of Raltegravir potassium(RAL) by gallocyanine in the presence of chloramine T (M1) or FGFCF in the presence of $KMnO_4$ (M2) or Fe(III) in the presence of o-phenanthrolein (M3) metol-NBS (M4). The coloured complexes were measured at 515nm,630nm,510nm and 540nm for the methods 1,2,3, and4 resp.in all the methods the absorbance are found to increase linearly with increasing RAL concentrations. Beer's law obeyed over the concentration ranges 50µg/mL,20 µg/mL ,20 µg/mL and100 µg/mL .the calculated molar absorptivity values are 2.6×10^5,1.45×10^5,$0.66\times10^5$and 0.19×10^5 mol cm^{-1} resp.The proposed methods are applied to commercial available tablets and the results are statically compared with these obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the Raltegravir potassium in the presence of other ingredients that are usually present in dosage forms. These methods offer the advantages of rapidity, simplicity and sensitivity and normal cost can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.***

Keywords:. Raltegravir potassium, Assay, Beer's law, coupled red ox complexation, pharmaceutical formulation, Regression equation.

I. INTRODUCTION

Raltegravir with the chemical name [N-(4-Flurobenzyl)-5-hydoyl-1-methyl-2-(2-{[(5-methyl1,3, 4-oxadiazol-2-yl)carbonyl]amino}-2-propanyl)-6-oxo-1, 6-dihydro-4-pyramidinecarboxomide] is an antiretroviral drug produced by Merck & Co., used to treat HIV infection. It was approval by the U.S. Food and Drug Administration (FDA) on 12 October 2007, the first of a new class of HIV drugs, the integrase inhibitors, to receive such approval.

In December 2011, it was approved by FDA for pediatric use, taken in the form of pills orally twice a day with two other antiretroviral medications to form the cocktail (most anti-HIV drugs regimens for adults and children use these cocktails). Raltegravir is available in chewable form, but because the two tablet formulations are not interchangeable, the chewable pills are approved only for children, aged between 2 to 11. Older and adolescents are prescribed with the adult formulation.

Raltegravir 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 metabolized away via glucuronidation.

Raltegravir was initially approved only for use in individuals whose infection has proven resistant to other HAART drugs. However, in July 2009, the FDA granted expanded approval for Raltegravir for use in all patients. As with any HAART medication, Raltegravir is unlikely to show durability if used as monotherapy, due to the highly mutagenic nature of HIV.

In a study of the drug as part of combination therapy, Raltegravir exhibited potent and durable antiretroviral activity similar to that of efavirenz at 24 and 48 weeks but achieved HIV-1 RNA levels below detection at a more rapid rate. After 24 and 48 weeks of treatment, Raltegravir did not result in increased serum levels of total cholesterol, low-density lipoprotein cholesterol, or triglycerides.

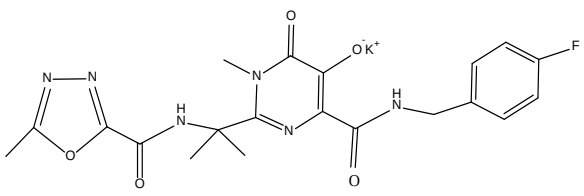


Fig:1 RALTGRAVIR POTASSIUM

II. MATERIALS AND METHODS

A Systronics UV-Visible double beam spectrophotometer 2203 with 1 cm matched quartz cells was used for all

spectral and absorbance measurements. A Systronics digital pH meter 361 was used for pH measurements.

2.1 Preparation of Solutions

1.Gallocyanine(GC) (0.05%): Prepared by dissolving 50 mg of GC in 500ml of distilled water.

2.ChloramineT (0.02%): Prepared by dissolving 20 mg of ChloramineT(CAT) in 100ml of distilled water and standardized iodometrically

3.HCl (Merck 5M): Prepared by diluting 217.5 ml of conc.HCl to 500ml distilled water and standardized.

4. $KMnO_4$ (0.032%): Prepared by dissolving 32 mg of potassium permanganate in 100ml of 2M H_2SO_4in distilled water.

5.Fast Green FCF Solution(FGFCF) (0.01%): Prepared by dissolving 100 mg of fast green FCF in 100 ml of 1.0M sulphuric acid. 10 ml of this solution was further diluted to 100 ml with the same strength of acid

6. H_2SO_4(Qualigens;2M): Prepared by diluting 112 ml of con.H_2SO_4 to 1000 ml with distilled water.

7. o-phenanthrolein solution (0.2%): Prepared by dissolving 200 mg of o-phenanthrolein in 100 ml of distilled water.

8. Ferric chloride solution (0.054%): Prepared by dissolving 54 mg of anhydrous ferric chloride in 100 ml of distilled water.

9. o-phosphoric acid solution: Prepared by mixing 1.27ml of o-phosphoric acid with 100 ml of distilled water.10ml of this stock solution was diluted to 100ml with distilled water.

10.N-Bomo succinamide(NBS) (0.01%): Prepared by dissolving 10 mg of N-bromosuccinimide in 100ml distilled water.

11. Acetic acid solution (Qualigens; 5%v/v): 5.0 ml of acetic acid was made up to 100ml of distilled water

12. Metol solution (0.3%): Prepared by dissolving 300mg of metol in 100ml of distilled water

13.Sulphonolic acid(SA) solution (0.2%): Prepared by dissolving 200mg of SA in 100ml of distilled water

2.2 Preparation of Standard Drug Solution

The drug was prepared by dissolving 100mg of distilled water. A portion of this stock solution was diluted stepwise with distilled water to obtain the working standard drug solution of concentrations of 50µg/mL (M_1), 20µg/mL (M_2), 120µg/mL (M_3) and 100µg/mL(M_4).The prepared stock solutions were placed in cool place which protected from light. From this stock solution a series of standard solutions were freshly prepared during the analysis.

2.3 Preparation of Sample Solution:

An accurately weighed portion of the powdered tablets equivalent to 100mg of drug was dissolved in20mL of distilled water shaken well and filtrate. The filtrate was diluted to 100mL with water to get 1mg/mL solution of drug in formulation. 2mL of this solution was further diluted to 50mL to get 40µg/mL solution. The absorbance of the solution was determined at λ_{max}250nm.The quantity of the drug was computed from Beer's law plot of the standard drug in water.

2.4 Methods

For Method M_1:

To each of 25 ml graduated tubes containing standard RAL solution (0.1-0.4 ml, 50 µg/ml), 1.25 ml of 5M HCl and 2.0 ml of 0.02% CAT were added and the solution was diluted to 20 ml with distilled water. After 10 min, 2 ml of GC solution was added, mixed thoroughly and the absorbance's were measured after 5 min at λ_{max} 515nm against reagent blank. The blank experiment was carried out in similar manner. The decrease in absorbance corresponding to consumed CAT, which in turn to the drug quantity was obtained by subtracting the absorbance of the blank solution from that of the test solution. The calibration graph was drawn by plotting the decrease in the absorbance of the dye (GC), against amount of drug. The amount of RAL in any sample was computed from its calibration graph(4.3.1)

For Method M_2:

Into a series of 25ml tubes containing aliquots of standard ECB solution (0.5-3.0mL, 20 µg/ml), 1.0ml of KMnO4 solution was added and the total volume in each tube was brought to 5ml with distilled water and kept aside for 10min at laboratory temperature. Then 2.0ml each of FG FCF solution and sodium sulfate solution were added successively and kept aside for 5min. The volume was made up to the mark with distilled water. The absorbance was measured at λmax 630 nm against distilled water. The decrease in absorbance corresponding to consumed permanganate and in turn the drug concentration was obtained by subtracting the decrease in absorbance of the test solution (dye – test) from that of the blank solution (dye – blank). The amount of RAL was deduced from its calibration curve(4.3.2)

For Method M_3:

Into a series of 25 ml calibrated tubes, aliquots of standard RAL solution (0.5 –2.0 ml, 500µg/ml) were transferred and then solutions of Fe (III) (1.5ml) and O-Phen 2.0ml was added successively. The total volume in each flask was brought to 10.0 ml with distilled water and heated for 30 min in a boiling water bath. After cooling to room temperature, 2.0 ml of o-phosphoric acid was added, the volume in each tube was made up to the mark with distilled water. The absorbance of the colored complex solution was measured after 5 min at λmax 510nm against a reagent blank prepared similarly. The content of the drug was calculated from its calibration graph(4.3.3)

For Method M_4:

Aliquots of the standard RAL solution (0.5-5.0ml,100 µg/ml), were transferred in to a series of 25 ml calibrated tubes containing 1.0 ml of AcOH and 1.0 ml of NBS solutions. The volume was brought to 10 ml with distilled water. The tubes were kept aside for 15 min at room temperature. Then 1.0 ml of metol solution and after 2 min 2.0 ml of SA solutions was added. The volume was made up to 25 ml with distilled water and the absorbance was measured after 10 min at λ_{max}540 nm against distilled water. A blank experiment was also performed. The decrease in absorbance corresponding to drug was obtained by subtracting the absorbance of test solution from that of the blank solution. The amount of RAL present was calculated from its calibration graph(4.3.4)

III. RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time volume and strength of CAT/GC,FGFCF/$KMnO_4$,Fe(III)/$K_3Fe(CN)_6$ and NBS-Metol/SA reagents, 5M HCl, 2M H_2SO_4 ,5%Acetic acid solutions and solvent for final dilution of the colored species were studied. CAT/GC,FGFCF/$KMnO_4$,Fe(III)/O-Phe and NBS-Metol/SA were preferred for this investigation as they yield high molar absorptive values among four dyes to different chemical classes. The optical characteristics such as Beer's law limit, sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing ¾ th of the amount of the upper Beer's law limits), regression characteristics like standard deviation of slope (s_b), standard deviation of intercept (s_a), standard error of estimation (s_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in Table-4.4 Commercial formulations containing RAL were successfully analyzed by the proposed methods. The values obtained by the proposed and referred methods for formulations were compared statistically by the t and F test and found not to differ significantly as additional demonstration accuracy; recovery experiments were performed by adding a fixed amount of the drug to the pure analyzed formulations at three different concentration levels. These results are summarized in table 4.5

IV. CHEMISTRY OF COLOUREDSPECIES

4.1 Methods M_1, M_2, & M_4:

RAL exhibits reducing property due to the presence of functional moieties vulnerable to oxidation selectively with oxidizing agents such as CAT in M_1, KMnO4in M_2 and NBS in M_4 under controlled experimental conditions. When treated with known excess of oxidant, RAL undergoes oxidation, giving products of oxidation besides unreacted oxidant. It is possible to estimate the drug content colourimetrically, which is equivalent to reduced form of the oxidant formed. The unreacted oxidant can be estimated colourimetrically either by decrease in the intensity of dye color due to disruption of chromophoric centers in the dye (CAT / GC in M_1, $KMnO_4$ /FGFCF in M_2) or color development due to in situ formation of charge-transfer complex (NBS / PMAP-SA in M_4).

step:I

$$\text{ralt grav ir pot ass ium} + KMnO_4 \text{ Oxidation} \longrightarrow \text{products} + \underset{\text{unreacted}}{KMnO_4} + Mn(II)$$

step:II

$$\underset{\text{unreacted}}{KMnO_4} + \text{FGFCF} \longrightarrow \underset{\text{coloured}}{\text{FGFCF}} + \underset{\text{colourless mixtures of unreacted compounds due to rutue of conjugatesystem (reproducible but not stoichiometric as several pathways possible)}}{\text{reacted FGFCF}}$$

M_1: CAT/GC

Step –I:

RTG + CAT ⟶ oxidation products + reduced CAT + unreacted CAT

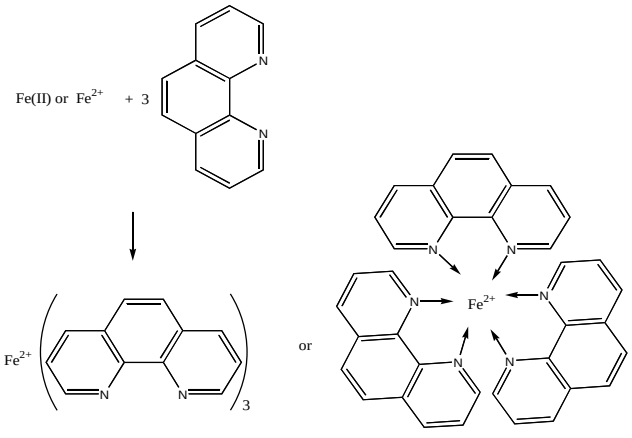
Step – 2:

CAT + GC ⟶ reduced form of CAT (Unreacted, colored)

M_2:KMNO4/FGFCF

4.2 FOR M_3:

When treated with known excess of Fe (III), RAL undergoes oxidation, giving products of oxidation inclusive of reduced form of oxidant, Fe (II) from Fe(III) besides unreacted oxidant. It is possible to estimate the drug content calorimetrically, which is equivalent to either reacted oxidant or reduced form of oxidant formed. The reduced form of Fe (II) has a tendency to give a colored complex on treatment with either o-Phen in [M_3].



Ferroin complex

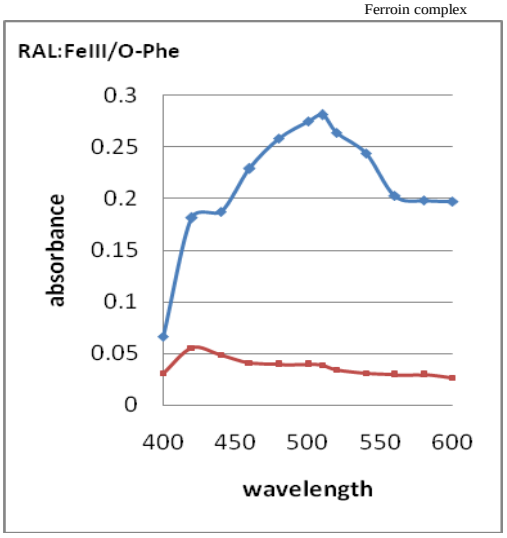
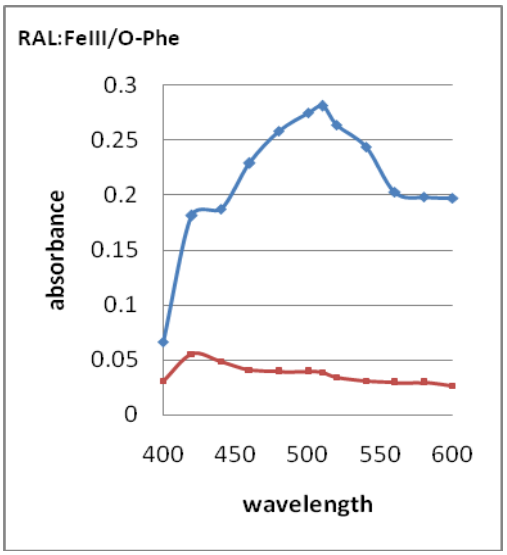
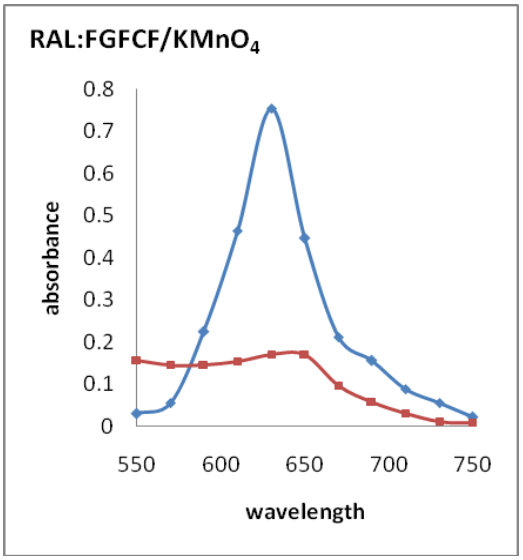
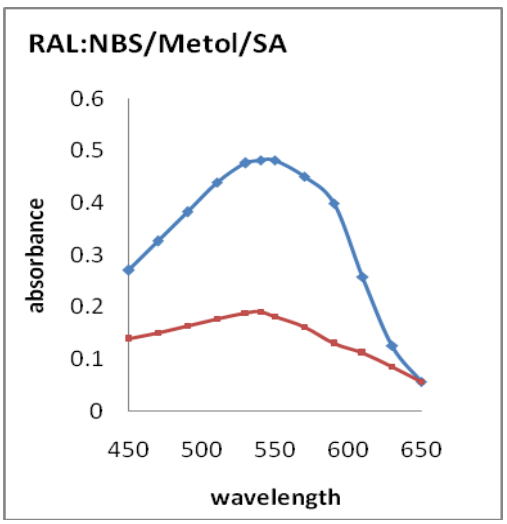


Fig 4.2.1, 4.2.2 Absorption spectra of RAL with CAT/GC and FGFCF/$KMnO_4$



Fig; 4.2.3, 4.2.4 Absorption spectra of RAL with Fe(III)/O-phe and NBS/Metol-SA





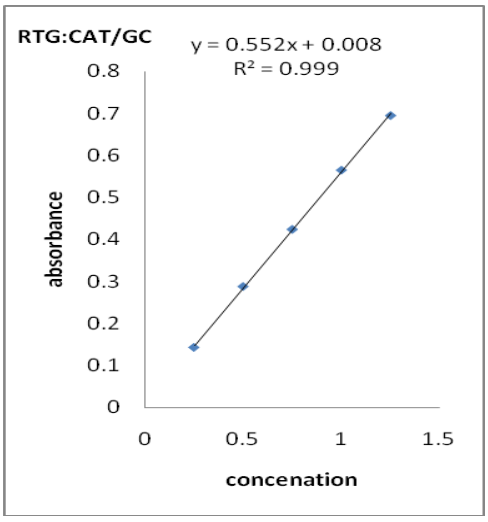
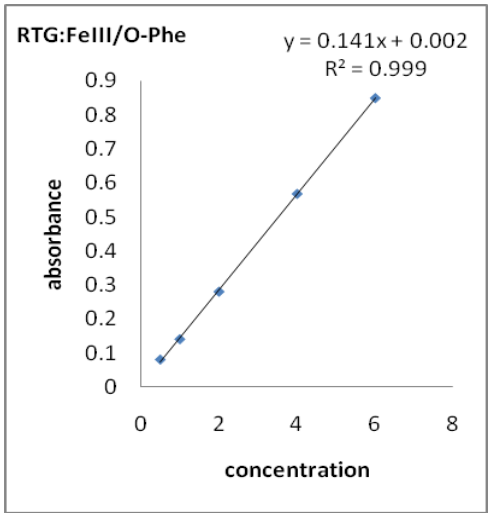
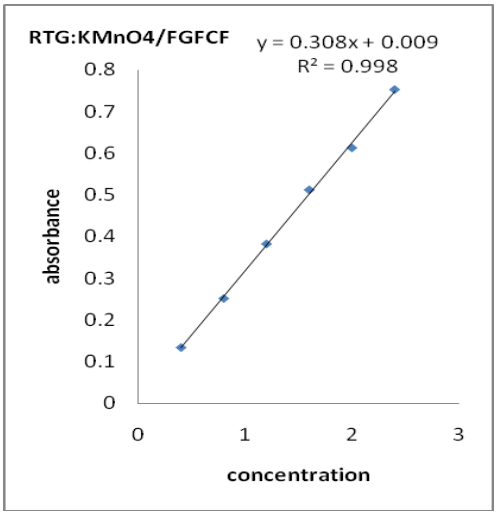
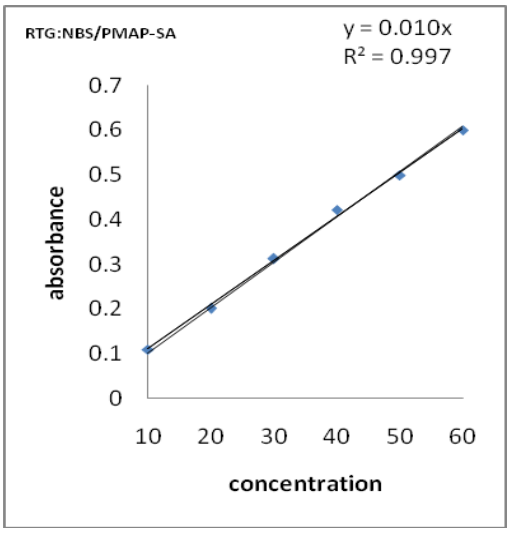


Fig: 4.3.1,4.3.2Beer's law plots of RAL with CAT/GC and FGFCF/$KMnO_4$



Fig;(4.3.3,4.3.4) Beer's law plots of RAL with Fe(III)/O-phe and NBS/Metol-SA





4.4 Table:1 Optical and egression characteristics, precision and accuracy of the proposed methods for RAL

Parameter	Method 1	Method 2	Method 3	Method 4
λ_{max} (nm)	515	630	510	540
Beer's law limits (µg ml^{-1})	0.1-0.4	0.5-3.0	0.25-5	10-60
Detection limits (µg ml^{-1})	2.19	2.72	0.79×10 1	1.14×10 1
Molar absorptivity (1 mole cm^{-1})	2.60×10 ^{5}	1.45×10 5	0.66×10 5	0.19007 x10^5
Sandell's sensitivity (µg cm^{-2} / 0.001)	0.0018	0.003314	0.0072	0.02538
Regression equation Slope (b)	Y=0.552x+0.008	Y=0.308x+0.009	Y=0.141x+0.002	Y=0.01 X+0.001
Standard deviation of slope (S_b)	0.4865	0.1795	0.1105	0.00242
Intercept(a	0.008	0.009	0.0072	0.001
S.D Intercept(S_a	0.4033	0.2794	0.3738	0.03815
Standard errorof	0.38466	0.3005	0.5044	0.4676

estimation (S_e)				
Correlation coefficient (r^2)	0.999	0.998	0.999	0.997
R.S.D %)*	0.5590	0.3630	1.344	0.5034
% Range oferror (Confidencelimits)* 0.05	0.586	0.3810	1.4106	0.5283
0.01 level	0.9201	0.5975	2.216	0.8286
% Error in bulk sam**	0.5306	0.3541	1.304	0.276

*: Average of six determinations considered
**: Average of three determinations

4.5 Assay of GLT In Pharmaceutical Formulations

formulation	Labeled amount in mg	Amount found by proposed methods Method 1 Method 2 Method 3 Method 4	%Recovery by proposed methods Method 1 Method 2 Method 3 Method 4
Tablet-I	200	(M1)199.61± 2.09 (M2) 203.49± 2.18 (M3)203.09 ±1.79 (M4) 198.78 ± 1.24	(M1)99.61± 1.04 (M2)101.74± 1.09 (M3)101.54 ± 0.898 (M4)99.39 ± 0.621
Tablet-II	400	(M1)401.08± 3.909 M2) 402.88± 2.72 (M3)405.0 ± 3.57 (M4)402.07 ± 3.58	(M)100.27± 0.977 (M2)100.72± 0.68 (M3)101.25 ± 0.892 (M4)100.51± 0.896

*: Average ± standard deviation of six determinations; the t- and F- values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit t=2.57, F=5.05.

**: After adding2different amounts of the pure labeled to the pharmaceutical formulations, each value is an average of 3 determinations

$: UV Reference method.

V. CONCLUSION

The proposed methods are found to be simple ,sensitive, accurate and economic for routine analysis of GLT in bulk and pharmaceutical formulation .Based on molar absorptive data and Beer's law range ,it may be concluded that among the proposed methods , it may be concluded that among the proposed methods ,method 1 more sensitive than methods 2,3 and 4 (1>2>3>4)

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