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Research Paper

Spectrophotometric Determination of Raltgravir Potassium and Galantamine Hydrobromide Pharmaceuticals using Folin-Ciocalteu reagent and Iron (II) - Orthophenanthraline as Chromogenic agents

***S. Hemambika[1], K. Raghu Babu[2], N. Annapurna[2]**
[1]Department of Organic and Analytical chemistry,Dr.lankapalli Bullayya PG college, Visakhapatnam, (AP), INDIA
[2]Department ofEngineering Chemistry, Andhra university, AUCE(A), Visakhapatnam, (AP), INDIA

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Abstract: *Two simple, sensitive and selective methods are proposed for the determination of Raltgravir potassium(RAL) and Galantamine Hydrobromide(GLT) in pure drug and tablets. The first method is based on the reduction of Folin-Ciocalteu reagent (F-C) by RAL and GLT in Sodium carbonate to form a blue coloured complex ,which was measured at 810 nm and 800nm resp. (Molybdenum –Tungsten blue method).In the second method (Prussian blue method),Iron (III) was reduced to Iron(II) by RAL and GLT in HCl medium, in which Iron (II) is complexed with Ferricyanide and the resulting Prussian blue was measured at 510nm ,630nm respectively. The absorbance measured in each case was related to the RAL and GLT concentrations. The experimental conditions were carefully studied and optimised. Beer's law was obeyed in concentration ranges 0.1-0.5 µg/ml(RAL and GLT for method I) and 0.4-2.4µg/ml(RAL and GLT for method (II) corresponding molar absorptivity values 0.458×10^4 (1 mole cm^{-1}) RAL and 2.75×10^5 (1 mole cm^{-1}) for method I, 6.65×10^4 mole cm^{-1} 1.166×10^5 mole cm^{-1} (RAL and GLT for method II. The LOD is 1.37(µg ml^{-1}) and 1.35(µg ml^{-1}) for method I, 7.95 µg ml^{-1} and 2.716 µg ml^{-1}(RAL and GLT for method II.*

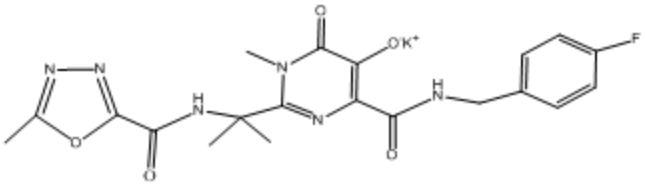
Keywords: Folin-Ciocalteu reagent , Iron (III), Galantamine hydrobromide spectrophotometry, Raltgravir potassium, Pharmaceuticals Ferricyanide.

Introduction

Raltegravir potassium (RAL, chemically known as N-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-(2-{[(5-methyl-1,3,4-xadiazol-2-l)carbonyl]amino}-2-propanyl)-6-oxo-1,6-dihydro-4-pyrimidine carboxamide is an antiretroviral drug produced by Merck & Co., used to treat HIV infection[1]. It received 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[2,3]. 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[4]. Literature survey revealed that several analytical methods reported for the estimation of Raltegravir potassium in human plasma. Edward P. Acosta was reported the sensitive HPLC-MS-MS method for the determination of Raltegravir potassium in human plasma[5]. 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 was reported the LC tandem MS assay for the simultaneous measurement of new antiretroviral agents: Raltegravir, Maraviroc, Darunavir, Etravirine[6]. Singlest epextraction from plasma is performed by protein precipitation using 600µl acetonitrile. Ter Heine R. 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 [7].

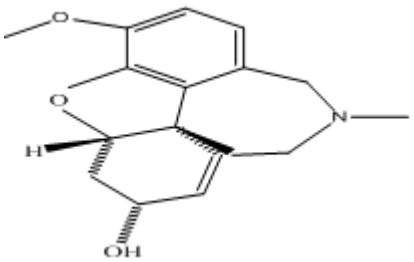
Naser L.Rezk was reported an accurate and precise HPLC method for the quantification of the novel HIV integrase inhibitor raltegravir in human blood plasma after solid phase extraction[8]. This is the first published method to use simple UV technology and reliable solid phase extraction

methodology for the quantification of raltegravir in human plasma. Jean-Marie Poirier was reported the quantification of the HIV integrase inhibitor raltegravir in human plasma by HPLC with fluorescence detection[9]. Jasmine A. Talameh was reported the quantification of raltegravir in female genital tract[10]. HPLC and detection with an UV wavelength of 218 nm. Valerie Furlan was reported the quantification of raltegravir in human plasma by HPLC with photodiode array detection[11]. Notari S. was reported the simultaneous determination of Maraviroc and Raltegravir in human plasma by HPLC-UV method[12]. 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 UV spectrophotometric methods.



Chemical structure of Raltegravir potassium

Galantamine hydrobromide : (Nivalin, Razadyne, Razadyne ER, Reminyl, Lycoremine) is used for the treatment of mild to moderate Alzheimer's disease and various other memory impairments, in particular those of vascular origin. It is an alkaloid that is obtained synthetically or from the bulbs and flowers of *Galanthus caucasicus* (*Caucasian snowdrop*), *Galanthus woronowii* (Voronov's snowdrop), and some other members of the family Amaryllidaceae such as Narcissus (daffodil), *Leucojum aestivum* (snowflake), and Lycoris including Lycoris radiata (red spider lily)[1]. Studies of usage in modern medicine began in the Soviet Union in the 1950s. The active ingredient was extracted, identified, and studied, in particular in relation to its acetyl cholinesterase (AChE)-inhibiting properties, Galantamine is a potent allosteric potentiating ligand of human nicotinic acetylcholine receptors (nAChRs) $\alpha_4\beta_2$, α_7/5-HT_3, $\alpha_3\beta_4$, and $\alpha_6\beta_4$ in certain areas of the brain, as well as a weak competitive and reversible cholinesterase inhibitor in all areas of the body[15].



Chemical structure of Galantamine hydrobromide

It increases the concentration and thereby action of acetylcholine in certain parts of the brain. It has shown activity in modulating the nicotinic cholinergic receptors on cholinergic neurons to increase acetylcholine release[16]. It is hypothesized that this action might relieve some of the symptoms of Alzheimer's.

Material and Methods

Apparatus: 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.

A pure Raltgravir potassium (RAL) and Galantamine hydrobromide (GLT) sample certified to be 99.86% pure, was procured from Aurabindo Laboratories, Hyd, India, as a gift and was used as received.

RAL containing tablets i.e., isentress Merck & co., Inc (100mg, 400mg) dutrebis Merck & co.,Inc 150mg were purchased from local commercial sources.

(i) FC reagent (2N): Prepared by dissolving 10 ml of 2N Folin Ciocalteu reagent (Merck, Mumbai, India) in 10ml distilled water.

(ii)Sodium carbonate merck 10%) : Prepared by dissolving 10g of sodium carbonate (S.D.Fine hem..Ltd, Mumbai, India) in 100ml distilled water.

(iii)Ferric chloride solution (0.054%): Prepared by dissolving 54 mg of anhydrous ferric chloride (Merck, Mumbai,india) in 100 mL of distilled water.

(iv)K_3 [Fe(CN)$_6$] solution (0.1%): Prepared by dissolving 100 mg of potassium ferricyanide (Merck, Mumbai,india) in 100 mL of distilled water.

The stock solution (1mg/ml) of Raltgravir potassium (RAL, GLT) was prepared by dissolving 100 mg of it in 100 ml of water. A portion of this stock solution was diluted stepwise with the water to obtain the working standard (RAL,GLT) solution of concentrations 150 µg/ml (for method M_1 molybdenum blue method), 50 µg/ml (for method M_2 Prussian blue method).

General procedures

Preparation of calibration curves

Molybdenum-tungsten blue method (MTB method)

Different aliquots (0.1-1.0ml,150 µg/ml) standard solution were accurately transferred into a series of 25ml calibrated flasks. Subsequently 2.0 ml of FC solution was added. After 5 min 7.0 ml of Na_2CO_3 was added and kept aside for 30 min. The volume was made up to the mark with distilled water. Blue coloured complex is formed .The absorbance was measured at λmax810 nm (RAL), 800nm (GLT) against a similar blank reagent. The amount of RAL was deduced from its calibration curve.

Prussian blue method (PB method)

To a series of 25 ml calibrated tubes, aliquots of standard solution (0.5– 5.0 ml, 50µg/ml) was transferred and 1 ml of 3.32 x 10^{-3}M $FeCl_3$ solution was added. The tubes were stoppered immediately and shaken well for 5 min. Then 0.5 ml of potassium ferricyanide solution was added into each tube and was closed with lids immediately. After 5 min 1 ml of IN HCl was added and final volume was made up to 25 ml with distilled water. The absorbance of the solution in each tube was measured immediately at λ_{max} 510nm (RTG), 630nm (GLT) against the similar reagent blank. In each case ,standard graph was prepared by plotting the measured absorbance versus the concentration of RTG/GLT, and the concentration of the unknown was computed using the regression equation derived from Beer's law data.

Procedure for tablets

Twenty tablets were accurately weighed and ground into a fine powder. a portion of the powder equivalent to 100 mg of the RAL/GLT ,was dissolved in 20 ml of methanol, shaken for 20min.the volume was diluted to the mark with methanol and mix well. And the insoluble residue was filtered through whatman 42 filter paper. The filtrate was further diluted to 100 ml with methanol to get 1 mg/ml level for the MTB method assay and PB method assay respectively. One ml of this solution was further diluted to 25 ml to get 40 µg/ml solutions. The absorbance of the solution was determined at 250nm (RTG), 265nm (GLT). The quantity of the drug was computed from the Beer's law plot of the standard drug in methanol.

Procedure for method validation

All assay validation procedures were carried out according to current ICH guidelines[17],which include linearity, limit of detection(LOD),and quantification (LOQ) ,intra-day and inter –day precision and accuracy, robustness and ruggedness, selectivity and recovery.

Linearity, LOD and LOQ

Linearity was assessed by analysing a set of six calibration standards .calibration curves were prepared as described above in the "preparation of calibration curves" section 2.3.1.the linearity of the methods was determined by plotting the absorbance(Y) against the theoretical concentration(X) of RAL/GLT. calibration measurements were subjected to least square regression analysis to obtain information relate to the slope, the Y-intercept, the correlation coefficient (r) .he coefficient of correlation should be 0.9800 or greater. The LOD and LOQ values were calculated using the following formulae[18]:

LOD=3.3S/m and LOQ=10S/m

Where S is the standard deviation of replicate (n=6) blank absorbances, and m is the slope of the calibration curve.

Precision and accuracy

To evaluate the precision and accuracy of the methods, a standard drug solution at three conc levels was subjected to same day (intra-day) analysis in six replicate ,as well as on five consecutive days(inter-day) analysis by preparing all solutions a fresh each day. The mean and standard deviation (SD) were obtained; the accuracy and precision were evaluated in terms of relative error (RE) and relative standard deviation (RSD) resp.

Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation in method variables, including the amounts of analytical reagents and the reaction time, on the performance of the proposed methods.

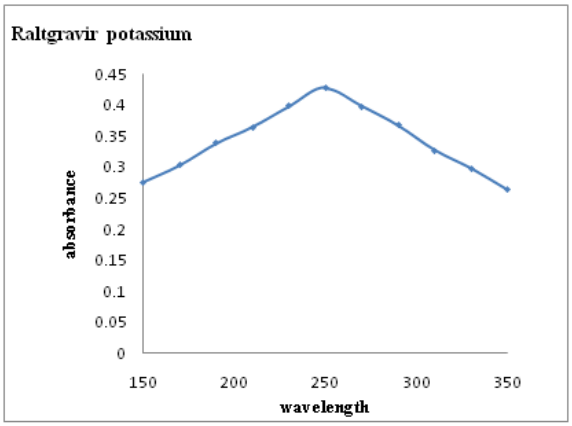


Figure 1: Absorption spectra of RTG in methanol (UV reference method

In these experiments the parameters was changed while the other kept unchanged, and the %RSD was calculated each time .ruggedness was also tested by applying the proposed methods to determine RAL/GLT using the same experimental conditions by three analysts using the same instrument (inter-personal) and also by the single analyst using three different instruments (inter-instrument).these assays were performed at three concentration drug levels.

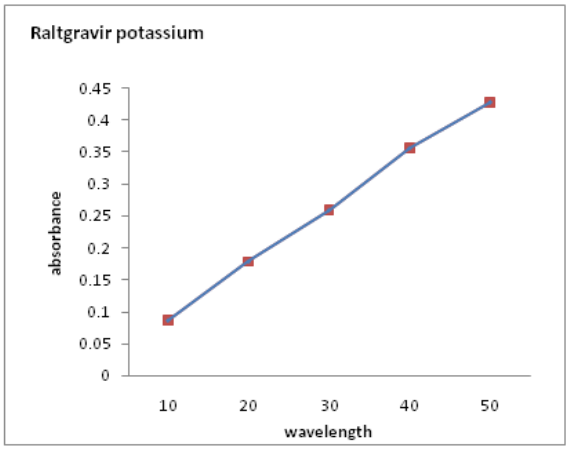


Figure 2: Beer's law plot of RAL in methanol (UV reference method)

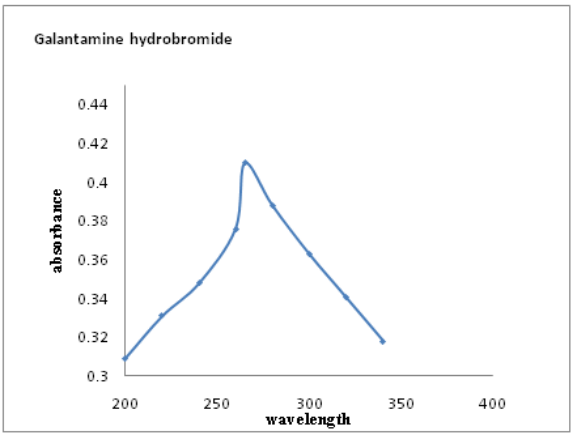


Figure 3: Absorption spectra of GLT in methanol (UV reference method

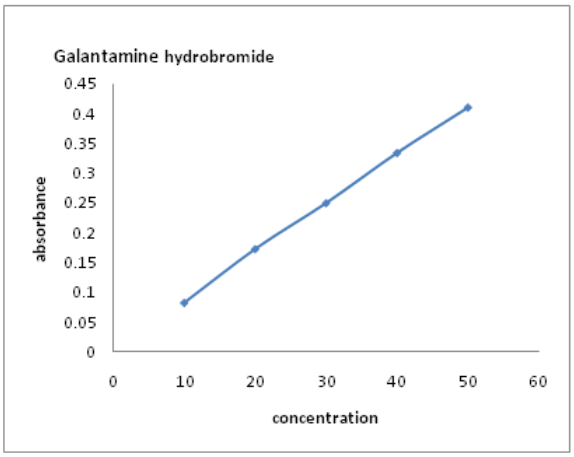


Figure 4 : Beer's law plot of GLT in methanol (UV reference method)

Results and Discussion

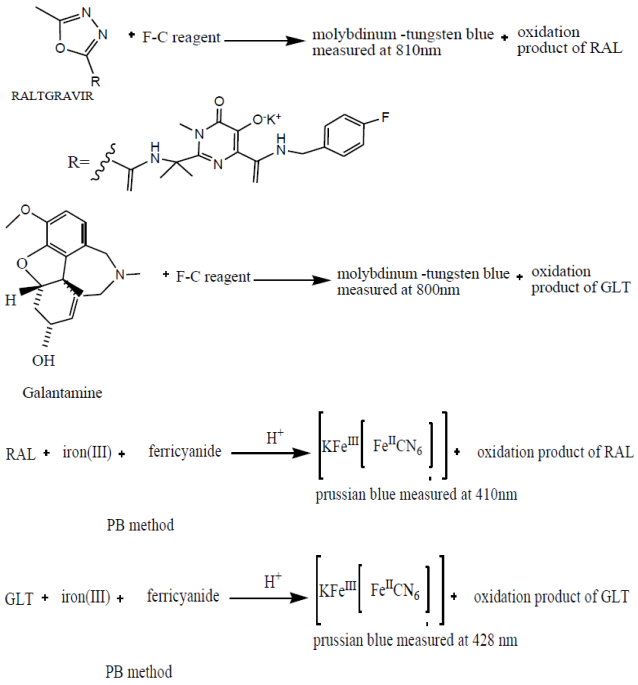
Absorption spectra

The quantitative determination of many phenolic and amino compounds of pharmaceutical importance using F-C reagent is well known[19-26]. and involves the reduction of the yellow molybdotungsto phosphoric acid heteroployanion reagent by the phenols or amines and the measurement of the absorbance of the resultant molybdotungstrate blue[27,28]. In the present MTB method F-C reagent was reduced by RAL/GLT in sodium carbonate medium to produce an intense, blue coloured product that peaks at 800nm,810nm for RAL and GLR resp[29,30].

In the PB method due to the reducing nature of RAL/GLT Iron(III) was reduced to Iron (II) in acid medium. Subsequently the Iron(II) formed in situ reacted with Ferricyanide to form soluble Prussian blue [$KFe^{III}[Fe^{III}(CN)_6]$] similar to previous reports[31-36].The absorption maxima at 510nm and 630nm for RAL And GLTresp.

Method development

In the drug ,the experimental variables,i.e.,the contact time, the amount of F-C reagent and sodium carbonate and addition order of the reactants, were carefully studied and optimised,maxium absorbance was reached 10min after mixing the reactants, and the colour remained stable for as long as 24h equal volumes (2.0ml)of F-C reagent (2N) and 7.0ml sodium carbonate (10%) solution were found to be optimal at a total volume 10ml with respect to addition order of rectants,the order indicated in the recommended procedure yields the coloured species with higher sensitivity and stability.



Blue colour formation in the pb method was found to be somewhat slow, and maximum colour intensity as achieved after 30min, remaining stable for an addition 60min under optimum conditions 1ml of Iron(III) (0.054%) and 0.5ml of Ferricyanide(0.1%) solutions, as well as 1N HCl, added sequentially, resulted in maximal sample colour intensity and minimal blank absorbance .

Method Validation

Linearity and sensitivity

A linear correlation was found between the absorbance at λ_{max} and the RAL /GLT concentration in the ranges given in the table 1. The graphs (figure 5-12) showed a negligible intercept ,as described by the regression equation:

Y=b+mX where Y is the measured absorbance is the concentration in µg/ml ,m and b are the slope and the intercept ,respectively.

Regression analysis of beer's law data using the method of linear least squares was used to calculate the slope (m), the intercept(b) and the correlation coefficient(r) of each system. These values are presented in table 1. Beer's law ranges, molar absorptivity values and Sandell's sensitivity values are shown in table 1. The calculated values of limit of detection also included, high lighting the high sensitivity of these methods.

Table 1: Optical and Regression characteristics, precision and accuracy of the proposed methods for RAL/GLT

Parameter	Raltgravit potassium		Galantamine hydrobromide	
	M_1	M_2	M_1	M_2
λ_{max} (nm)	810	510	800	630
Beer's law limits (µg ml^{-1})	0.1-0.5	0.5-6.0	0.1-0.5	0.4-2.4
Detection limits (µg ml^{-1})	1.37	7.95	1.35	2.7165
Molar absorptivity (1 mole cm^{-1})	0.458×10^4	6.65×10^4	2.75×10^5	1.166×10^5
Sandell's sensitivity (µg cm^{-2} / 0.001 absorbance unit)	0.0204	0.00711	0.04335	0.00107
Regression equation (Y = a + bC) Slope (b)	1.445	0.010	0.697	0.308
Standard deviation of slope (S_b)	1.99	0.1105	0.9521	0.1791
Intercept (a)	0.011	0.006	0.002	0.008
Standard deviation of intercept (S_a)	0.66	0.3738	0.3157	0.2789
Standard error of estimation (S_e)	0.6319	0.5044	0.3011	0.2998
Correlation coefficient (r^2)	0.999	0.998	0.998	0.999
Relative standard deviation (%)*	0.5486	1.334	0.7672	0.4113
% Range of error (Confidence limits)* 0.05 level	0.5759	1.1406	0.8053	0.4317
0.01 level	0.9030	2.216	1.2628	0.677
% Error in bulk samples **	0.4547	0.277	1.156	0.3579

*: 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 adding 2 different amounts of the pure labeled to the pharmaceutical formulations, each value is an average of 3 determinations.

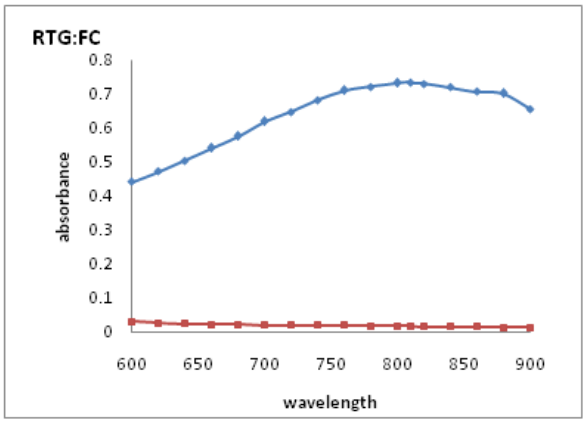


Figure 5: Absorption spectra of RAL:FC

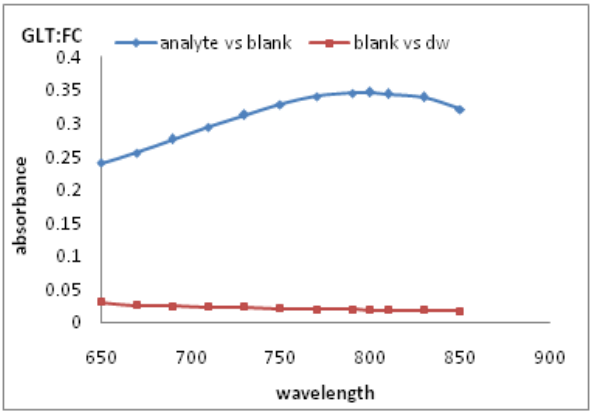


Figure 7: Absorption spectra of GLT:FC

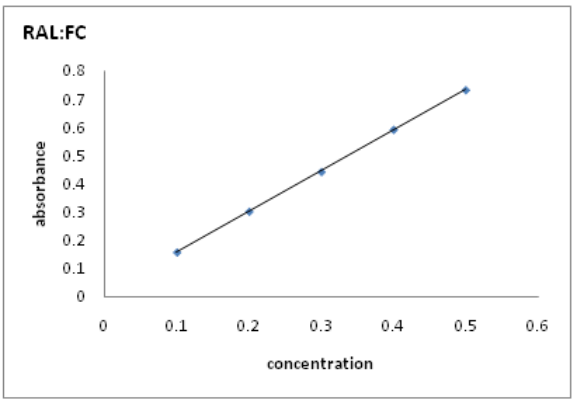


Figure 6: Beer's law plot of RAL: FC

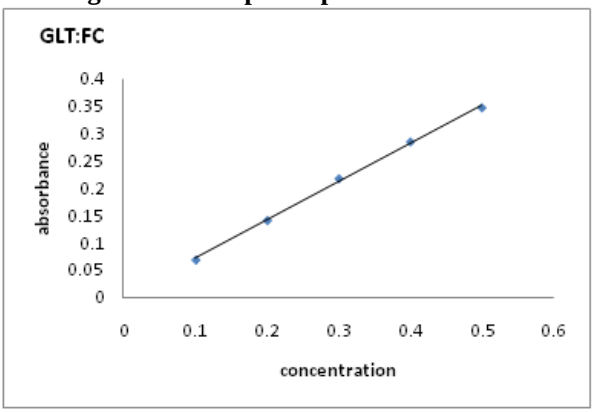


Figure 8: Beer's law plot of GLT: FC

Applications to tablets

Two brands of 200mg and 500mg strength tablets of RAL and 8mg and 24 mg strength tablets of GLT were analysed by the proposed methods, and the results are presented in the table2.the same tablets were also analysed by the

reference method for comparision.the results revealed that there is a close agreement between the results obtained by proposed methods and those of reference method.

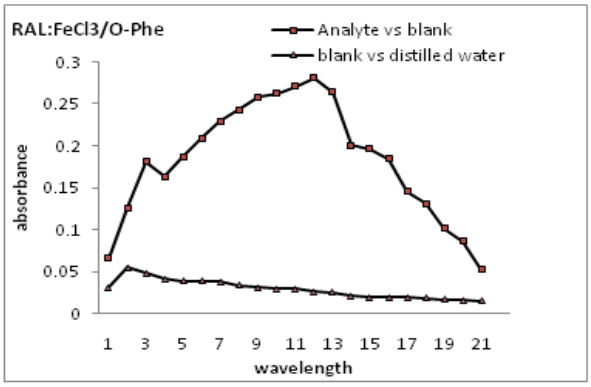


Figure 9: Absorption spectra of RAL:FeCl3/o-phe

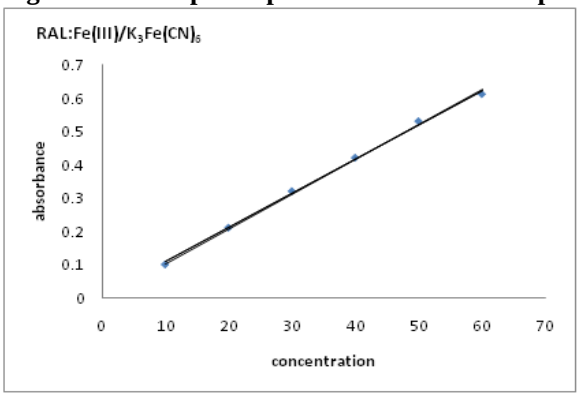


Figure 10: Beer's law plot of RAL:FeCl3/o-phe

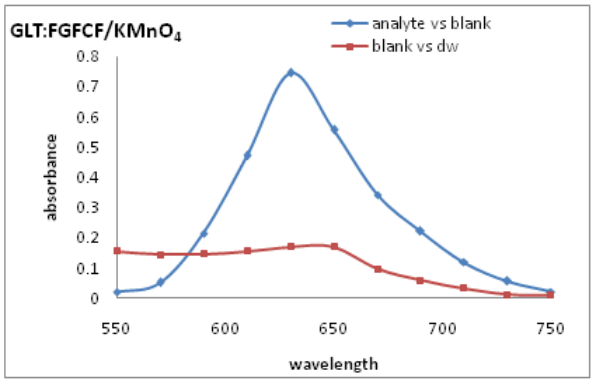


Figure 11: Absorption spectra of GLT: FeCl3/o-phe

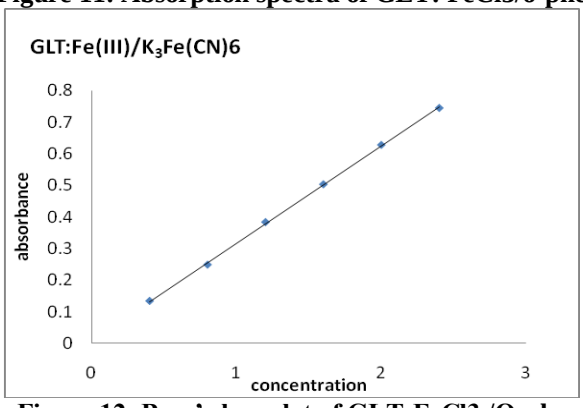


Figure 12: Beer's law plot of GLT:FeCl3 /O-phe

Table 2: Assay of Ral in Pharmaceutical Formulations

Sample	Amount taken (mg)	Amount found by proposed methods*		Reference methods	Percentage recovery by proposed methods**	
		M_{13} (MTB)	M_9 (PB)		M_{13}	M_9
Tab –I	200mg	199.3± 0.736 F=2.76 t =1.276	196.49 ±2.1 F=4.36 t = 1.265	198.21± 1.043	98.05±0.86	97.66±1.09
Tab -2	500mg	496.07± 3.3 F=2.56 t =0.876	492.88± 2.72 F=1.710 t = 0.170	498.21± 1.043	97.26±0.83	95.32± 0.68

Table 3: Assay of GLT in Pharmaceutical Formulations

Sample	Amount taken (mg)	Amount found by proposed methods*		Reference methods	Percentage recovery by proposed methods**	
		M_{13} (MTB)	M_9 (PB)		M_{13}	M_9
Tab –I	8mg	7.94 ±0.15 F=2.506 T=0.978	7.92± 0.063 F=2.32 T=0.913	7.988±0.09	99.27± 1.91	99.095 ±0.796
Tab -2	24mg	23.60 ±0.42 F=2.19 T=0.849	23.75± 0.41 F=2.07 T=1.279	23.95±0.28	98.35 ±1.787	98.99± 1.73

Conclusion

The spectrophotometric methods described in this study were found to be simple and did not involve any critical experimental variables compared to most reported methods. The methods were demonstrated to be both accurate and precise well ass robust and rugged. Both systems have wide linear dynamic ranges of applicability. molybdenum–tungsten blue method the absorbance is 2.75×10^5 (GLT) furthermore the Prussian blue method had an ε value of 1.166×10^5(GLT) more sensitive. The reference method requires a rigorous anhydrous medium for accurate end point detection, as well as generation of a large quantity of organic solvents a waste. This creates a waste disposable problem. Therefore it is not a green method. Additionally ,the reference method is applicable to a macro scale (8mg,24mg500mg, .in contrast, the proposed method seldom employ organic solvents and are applicable over a micro range (µg/ml).these advantages ,coupled with the use of cheap and readily available chemicals and simple instrumentation, make the methods suitable for use in quality control laboratories in developing and under developed countries thet can ill-afford the expensive techniques such HPLC.

Acknowledgement

The gift samples of Raltgravir potassium and Galantamine hydrobromide by Aurabindo laboratories, Hyderabad is greatfully acknowledged.

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