CHAPTER – IV

NEW SPECTROSCOPIC METHODS FOR THE ASSAY OF RIVASTIGIMINE USING VARIOUS CHROMOGENIC REAGENTS.

4.01. Drug Profile:

Exelon®(rivastigmine tartrate)(RIV)[196], a reversible cholinesterase inhibitor and is known chemically as (S)-N-Ethyl-N-methyl-3-[1-(dimethyl amino)ethyl]-phenyl carbamate hydrogen-(2R,3R)- tartrate used for the treatment of Alzheimer's disease (AD) has been selected for the present investigation. It's official status has been presented in **Table1.01,P.7.** The characteristics, therapeutic importance, chemical names, structure, analytically useful functional groups and commercially available formulations of RIV are presented in **Tables 4.01, P.202; 4.02,P. 203** & **4.03,P.204** respectively. Pharmacopoeia does not give any official determination method for RIV. Few chromatographic methods for the determination of rivastigmine (RIV) have been described [197-200]. LC-MS method has been used to quantitative analysis of RIV and its major metabolite in biological fluids [201].

Existing analytical methods reveal that little attention was paid in developing visible spectrophotometric methods by exploring thoroughly the analytically useful functional groups in RIV. Hence there is a need to develop sensitive and flexible visible spectrophotometric methods, which prompted the author to carry out in this accord. The author developed five simple and sensitive visible spectrophotometric methods for the determination of RIV in pure or pharmaceutical formulations. The proposed methods will be very useful in the field of drug analysis due to their simplicity, low cost and relatively short analysis time when compared with other techniques.

Table - 4.01

Structural features of Rivastigmine

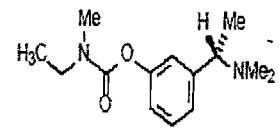
SI. No	Generic Name	Category	Chemical Name, Molecular formula& Molecular weight	Structure	Analytical important moieties/functional groups
3	**Rivastigmine**	Cholinergic Agent	2, 6-dioxo-4-phenyl-piperidine-3-carbonitrile.		Aliphatic tertary amine and keto moiety are present

Table - 4.02

Physico chemical characteristic and therapeutic importance of Rivastigmine

Category	Characteristics	Mode of action and therapeutic use
Nootropic Agents Cholinergic Agents Parasympathomimetics	**Molecular formula**: $C_{14}H_{22}N_2O_2$. **Formula Weight**: 250.337 g/mol. **Appearance** : White powder **Solubility**: Practically insoluble in water, and is soluble in dimethylformide, ethanol, acetonitrile and acetone.	Rivastigmine is a para sympathomimetic and a reversible cholinesterase inhibitor.Rivastigmine is indicated for the treatment of mild to moderate dementia of the Alzheimer's type. Rivastigmine increases the concentration of acetylcholine through reversible inhibition of its hydrolysis by cholinesterase. This acts to enhance cholinergic function.

Table - 4.03

Commercially available formulations

S.No.	Proprietary name	Pharmaceutical concerned	Formulations	Other ingredients usually present (active)	Inactive
1)	EXELON	NOVARITIS	Tab – 1.5 mg ; 3.0mg	---------	Sodium starch,romellose, microcrystalline cellulose, magnesium stearate snd colorants

4.02. Experimental:

i. Instruments used:

An Elico digital UV-Visible spectrophotometer SL-159 with 1cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

ii. Preparation of standard drug solution:

An 1mg/ml solution was prepared by dissolving 100mg of pure RIV in 100ml of distilled water and this stock solution was diluted step wise with distilled water to get the working standard solution of concentration of 250µg/ml (M_{1a}), 250µg/ml (M_{1b}), 200µg/ml (M_8), 200µg/ml (M_9), 200µg/ml (M_{10}) and 200 µg/ml (M_{13}).

Method M_{1a}

Solution of various reagents such as TPooo (Fluka; 0.2%, 5.70 x 10^{-3}M), HCl (E. Merck, 0.1M), Chloroform (Qualigens) was prepared in the same way as described under PLZ in chapter – II (**P.62**).

Method M_{1b}

Solution of various reagents such as ARS (0.2%, 5.84 x 10^{-3}M), HCI solution (0.1M) were prepared in the same way as described under PLZ in chapter – II (**P. 62**).

Method M_8:

Solution of various reagents such as CTC solution (2.5 x 10^{-1}M), Buffer solution (pH 2.0) were prepared in the same way as described under PLZ in chapter – II (**P. 68**).

Method M_9

Solution of Citric acid - acetic anhydride reagent, (E.Merck; 1.2%, 6.245 x 10^{-2}M), Methanol (Qualigens), Acetic anhydride (Qualigens) was prepared in the same way as described under PLZ in chapter – II (**P. 68**).

Method M_{10}:

Solution of various reagents such as SNP solution (E. Merck; 5%, 1.678 x 10^{-2}M) NH_2OH Solution (Fluka; 5%, 7.095 x 10^{-2}M) and Na_2CO_3 solution (Loba; 10%, 9.43 x 10^{-1}M) were prepared in the same way as described under PLZ in Chapter –II (**P. 69**).

Method M_{13}:

Solution of various reagents such as DDQ Solution (Loba; 0.1%, 4.40 x 10^{-3}M) were prepared in the same way as described under GLP in Chapter –III (**P. 147**).

4.03. Proposed procedures:

After systematic and detailed study of the various parameters involved, as described under results and discussions the following procedures [M_{1a} (TPooo) , M_{1b} (ARS), M_8 (CTC), M_9 (Citric Acid-AC_2O), M_{10} (SNP-HA) and M_{13} (DDQ)] were recommended for the assay of RIV in bulk samples and pharmaceutical formulations.

Method $M_{1a}$and M_{1b}

Into a series of 125ml separating funnels containing aliquots of standard RIV solution (0.5 - 3.0ml, 250μg.ml^{-1} for M_{1a} and 250μg.ml^{-1} for M_{1b}) 6.0ml of 0.1 M HCI solution and 2.0ml of dye (TPooo M_{1a} or ARS M_{1b}) solution were added successively. The

total volume of aqueous phase in each separating funnel was adjusted to 15.0ml with distilled water. To each separating funnel 10.0ml of chloroform was added and the contents were shaken for 2min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 480nm or 430nm against a similar reagent blank. The amount of RIV was deduced from the calibration curve (**Fig. 4.07**(M_{1a}), **P. 220**, **Fig. 4.08** (M_{1b}), **P. 220**).

Method M_8

Into a series of 125ml of separating funnels, aliquots of standard RIV solution (0.5 - 3.0ml, 200μg.ml^{-1}) were taken. Then 2.0ml of buffer (pH 2.0) and 5.0 ml (2.5 x 10^{-1}M) of CTC solutions were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0ml with distilled water. To each separating funnel, 10.0ml of nitrobenzene was added and the contents were shaken for 2min. The two phases were allowed to separate and the absorbance of the separated nitrobenzene layer was measured at 620nm against a similar reagent blank. The amount of RIV was computed from its calibration graph (**Fig. 4.09, P. 220**).

Method M_9

Aliquots of standard solution (0.5 - 3.0ml, 200μg.ml^{-1}) were taken into a series of 25ml graduated tubes and gently evaporated on a boiling water bath to dryness. To this 10ml (6.245 x 10^{-2}M) citric acid – acetic anhydride reagent was added and the flasks were immersed in a boiling water bath for 30min. The tubes were cooled to room temperature and made up to the mark with acetic anhydride. The absorbance of the colored solutions was measured after 15min

at 580nm against a reagent blank. The amount of RIV was calculated from the calibration graph (**Fig. 4.10, P. 220**).

Method M_{10}

Aliquots of standard RIV solution (1.0 - 5.0ml, 200μg.ml^{-1}) were transferred into a series of 25ml calibrated tubes. Then 1.0ml (1.678 x 10^{-2}M) of SNP and 1.0ml (7.195 x 10^{-2}M) of HA were added successively and kept aside for 5min. Then 1.0ml (9.43 x 10^{-1}M) of Na_2CO_3 solution was added and shaken for 15min. The volume was made up to the mark with distilled water. The absorbance was measured after 10min at 440nm against a similar reagent blank. The amount of RIV was computed from its calibration graph (**Fig. 4.11, P. 220**).

Method - M_{13}

Aliquots of standard drug solution (0.5 - 2.5ml, 200μg.ml^{-1}) were prepared by using DMF-dioxane (1:9) and delivered in to a series of 10 ml calibrated tube and the volume was adjusted to 3.0ml with dioxane. Then 1.0ml of DDQ (1.618 x 10^{-2} M) was added to each tube and the volume was made up to 10 ml with dioxane. The absorbances were measured at 460nm against a reagent blank during the stability period. The amount of drug was computed from to appropriate calibration graph (**Fig. 4.12, P. 220**).

For pharmaceutical formulations:

An accurately weighed portion of tablet powder equivalent to about 100mg of RIV was transferred into a 100ml volumetric flask. Added about 80ml of warm chloroform and shaken well for about 20min. The contents were diluted with chloroform up to the mark and mixed thoroughly. The solution was filtered the filtrate was

evaporated to dryness. The residue was used for the preparation of standard solution as shown under standard solution preparation. These solutions were analyzed as under procedures described for bulk solutions.

4.04. Results and Discussions:

i. Spectral Characteristics:

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of RIV were taken and colors were developed separately by following the above procedures.The absorption spectra were scanned on a spectrophotometer in the wave length region of 340 to 900nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results were graphically represented in **fig.4.01- 4.06, P. 217-219**. The absorption curves of the colored species in each method show characteristics absorption maxima. Where as the blank in each method has low or no absorption in this region.

ii. Optimum conditions fixation in procedures:

The optimum conditions for the color development of methods (M_{1a}, M_{1b}, M_8, M_9, M_{10} and M_{13}) were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.

Methods (M_{1a}, M_{1b}, M_8, M_9, M_{10}, and M_{13})

The optimum conditions established for methods (M_{1a}, M_{1b}, M_8, M_9, M_{10}, and M_{13}) were found to be same as described in Chapter II & Chapter III and is given in **Table. 4.04, P. 211 & 212** ;Table **4.05, P. 213**; **Table 4.06, P. 214**; **Table 4.07, P. 215** and **Table 4.08, P. 216** respectively.

iii. Optical Characteristics:

In order to test whether the colored species formed in above methods adhere to Beer's law, the absorbance at appropriate wavelength of a set of solutions containing varying amounts of RIV and specified of amounts of reagents were recorded against the corresponding reagent blanks. The Beer's law plots of these recorded graphically (**fig. 4.07 – 4.12, P.220**). Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range for RIV in each method were calculated. Least square regression analysis was carried out for getting the slope, intercept and the correlation coefficient values (**Table 4.09a & 4.09b, P. 224-225**).

Precision:

The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of RIV in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (**Table 4.09a & 4.09b, P. 224-225**).

Table 4.04

Optimum conditions established in method M_{1a} and M_{1b} for RIV

Parameter		Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	TPooo	480 - 490	490	
λ_{max} (nm)	ARS	410 - 450	430	
Effect of acid or buffer on color development.		0.08 - 0.12 HCl for M_{1a} & M_{1b}	0.1M HCl for M_{1a},M_{1b}.	Variation of concentration or pH of acid beyond the upper and lower limits resulted in low absorbance values.
Effect volume of (5.70×10^{-3}M) TPooo, and ARS, (3.20×10^{-3}M)		1.0 - 3.0ml (M_{1a}, M_{1b})	2.0ml ($M_{1a,1b}$)	2.0ml dye solution of was necessary for covering broad range of Beer's law limits.
Choice of organic solvent for extraction of the colored complex.		Chloroform for (M_{1a}, M_{1b})	Chloroform for (M_{1a}, $M_{1b,}$)	The water immiscible solvents tested for the extraction of the colored complex into organic phase, which include (chlorobenzene, carbontetrachloride, benzene, n-butanol and chloroform). Chloroform was preferred for its selective extraction of the colored drug-dye complex from the aqueous phase.

Table 4.04

Optimum conditions established in method M_{1a} and M_{1b} for RIV

Parameter	Optimum range	Conditions in procedure	Remarks
Effect of shaking time on extraction.	1- 5min	2min	Constant absorbance values were obtained for shaking periods between 1-5min.
Effect of temperature on the colored species.	Laboratory temperature (28 ± 3^0C)	Laboratory temperature	At low temperature ($<20^0$C) the extraction of colored species was found to be improper. At high temperature ($>35^0$C) the stability of the colored species was found to be less.
Stability of the colored species in organic solvent.	1 - 60min 1 - 60min	5min 5min	

Table 4.05

Optimum conditions established in method M_8 for RIV

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	610 - 630	620	
Effect of buffer pH on color.		pH - 2.0	Variation of pH of the buffer beyond the upper and lower limits resulted in low absorbance values.
Vol. of buffer required for maximum intensity of color.	1.5 - 2.5ml	2ml	2.0ml of buffer pH 2.0 was found to be necessary in aqueous phase for maximum color development in nitrobenzene layer.
Effect of vol. of (2.50×10^{-1} M) of CTC solution required for complex formation.	4 - 6ml	5.0ml	5.0ml of CTC was found to be necessary for complex formation and to cover broad range of Beer's law limits. No added advantage was observed with more than 5.0ml.
Effect of the ratio of organic to aqueous phase on extraction.			A ratio of 2:3 organic to aqueous phases was required for the efficient extraction of the complex.

Table 4.06

Optimum conditions established in method M_9 for RIV

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	575 - 585	580	
Effect of volume (6.245 x 10^{-2}M) of citric acid acetic anhydride reagent.	10.0 - 15. ml	10.0ml	Experiments were carried out to study the optimum requirement of citric acid – acetic anhydride reagent 10.0ml of the reagent. 10.0ml of the reagent was necessary for optimum color development.
Effect of heating time on color development.	25 - 35min in boiling water bath	30min in boiling water bath	A heating time of 30min was necessary for maximum color development. Below 25min the absorbance was found to be less.
Dilution of chromogen with different solvents.	Acetic anhydride	Acetic anhydride	Different solvents like acetic acid, methanol, ethanol, isopropanol, chloroform, methyl isobutyl ketone, benzene, dichloromethane were used as diluents, but acetic anhydride was found to be ideal for final dilution.
Stability of the colored species after final dilution.	15min - 2hrs	15min	The color was stable for 2hrs, after this time the absorbance started to decrease.

Table 4.07

Optimum conditions established in method M_{10} for RIV

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	560 – 580	580	
Effect of volume of hydroxylamine HCl (7.09×10^{-2}M) on the colored product.	0.8 - 1.5ml	1.0ml	Addition of more than 1.5ml of hydroxylamine HCl (7.09×10^{-2}M) resulted in irregular color production.
Effect of volume of SNP (1.6×10^{-1}M) solution on colored species.	0.75 -1.25ml	1.0ml	Addition of SNP (1.6×10^{-1}M) beyond the optimum range resulted in non-linear color production.
Effect of volume of Na_2CO_3 solution (9.43×10^{-1}M) on color production.	0.5 - 1.5ml	1.0ml	Addition of 1.0ml of the alkali (9.43×10^{-1}M) is enough to maintain the required alkalinity.
Reaction time.	10 - 20min	15min	A minimum of 10min was found to be necessary to cease the effervescence and for maximum color development and minimum blank reading.
Stability of the colored species after final dilution.	5 - 60min	10min	The intensity of the colored product begins to decrease slowly after 60min.

Table 4.08

Optimum conditions established in method M_{13} for RIV

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	450 - 470	460	
Effect of volume of (4.40×10^{-3}M) of DDQ in CH_3CN.	1.0 - 1.5ml.	1.0ml	Beyond upper and lower limits, the absorbance values were not constant and beyond 2.5ml leads to higher blank values
Solvent for final dilution.	Dioxane.	Dioxane	Dioxane has been found to be suitable for final dilution to give better absorbance values.
Stability of the colored species after final dilution.	10 - 60min.	10min.	The intensity of the colored product begins to decrease slowly after 60 min.

Fig. 4.01: Absorption spectrum of RIV with TPooo (M_{1a})

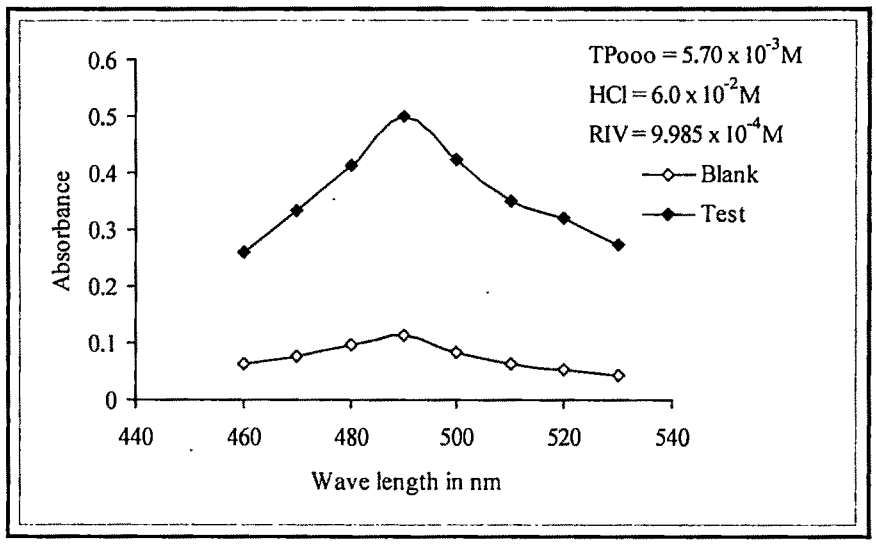


Fig. 4.02: Absorption spectrum of RIV with ARS (M_{1b})

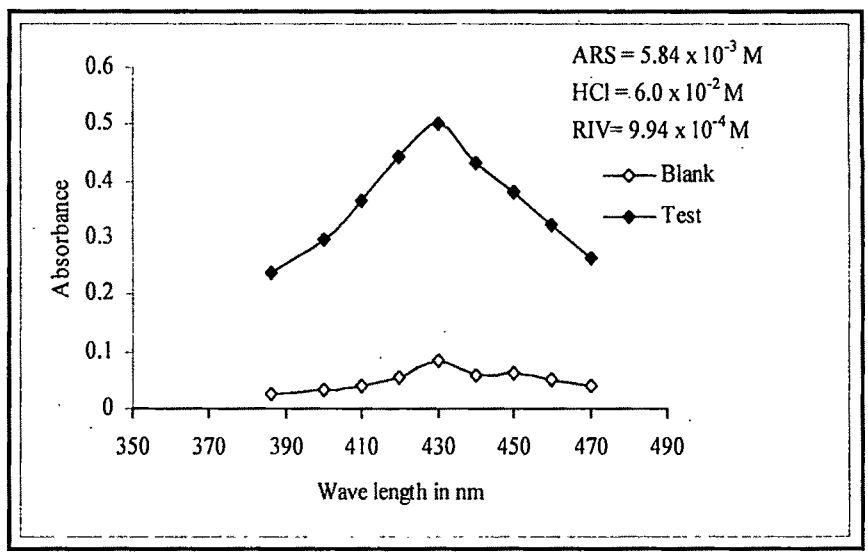


Fig. 4.03: Absorption spectrum of RIV with CTC (M_8)

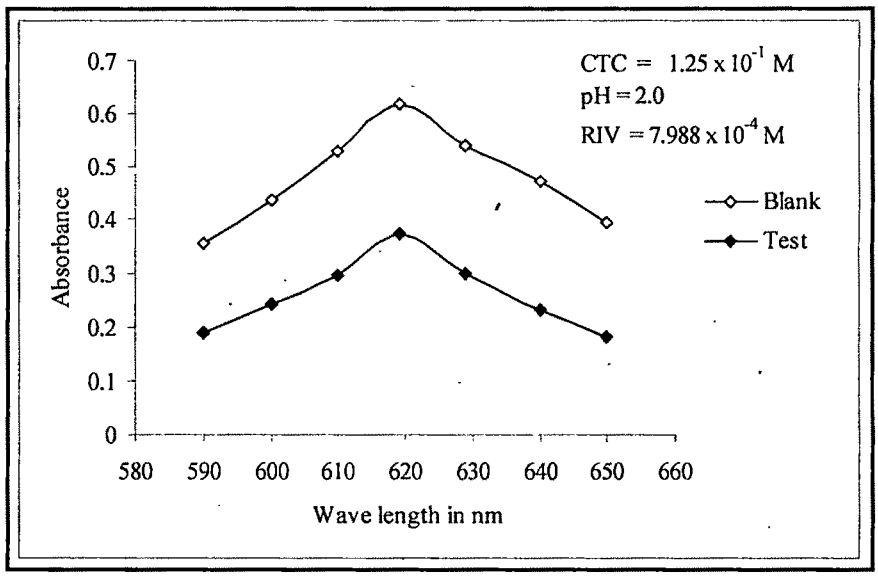


Fig. 4.04: Absorption spectrum of RIV with CiA – AC_2OH (M_9)

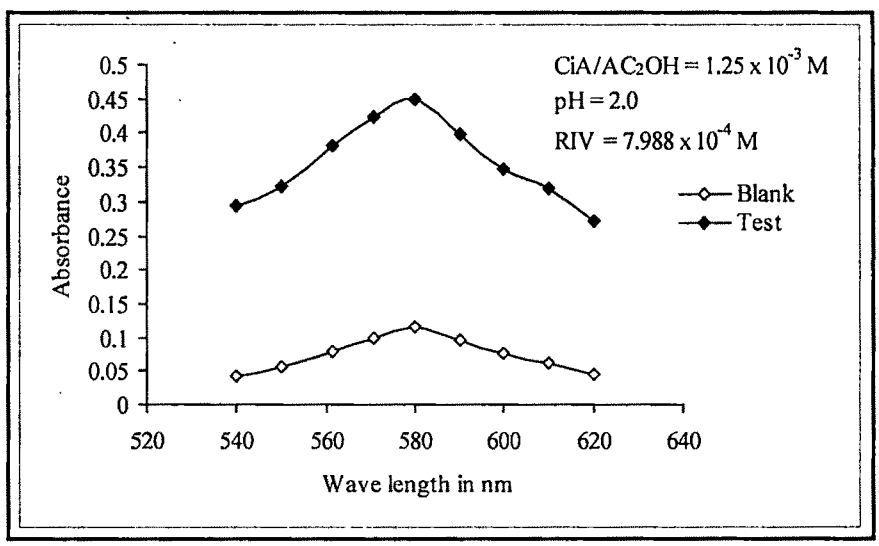


Fig. 4.05: Absorption spectrum of RIV with SNP-HA (M_{10})

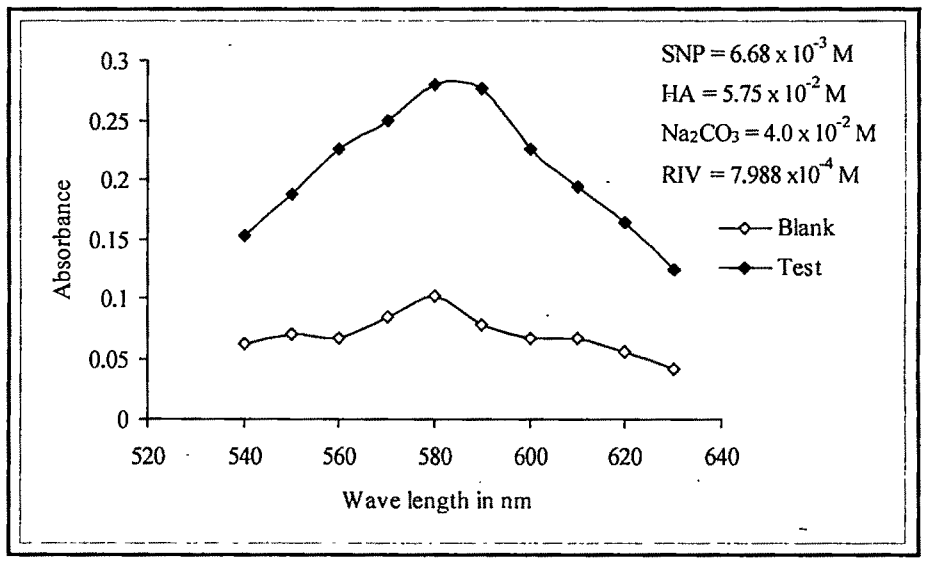


Fig. 4.06: Absorption spectrum of RIV with DDQ (M_{13})

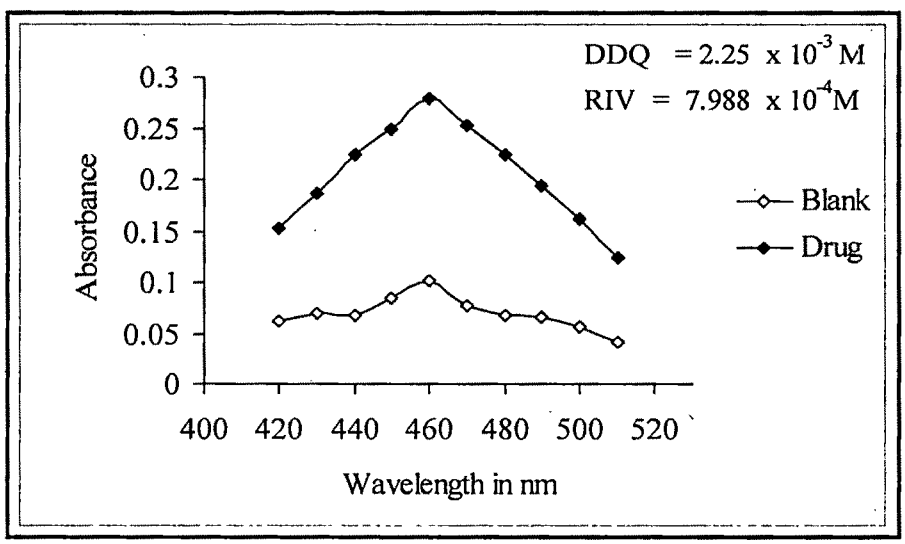


Fig. 4.07: Beer's Law plot of RIV with TPooo (M_{1a})

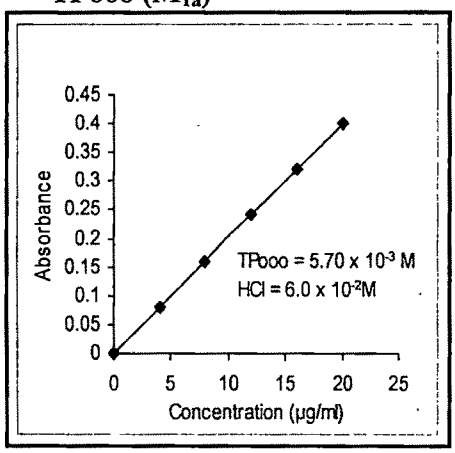


Fig. 4.08: Beer's Law plot of RIV with ARS (M_{1b})

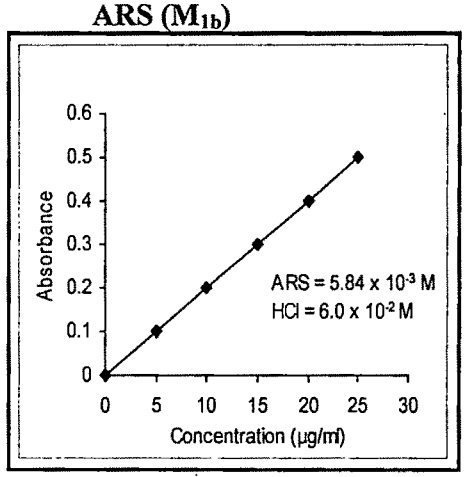


Fig. 4.09: Beer's Law plot of RIV with CTC (M_8)

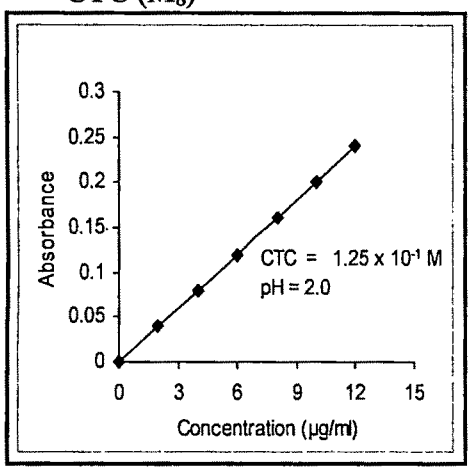


Fig. 4.10: Beer's Law plot of RIV with CiA – AC_2OH (M_9)

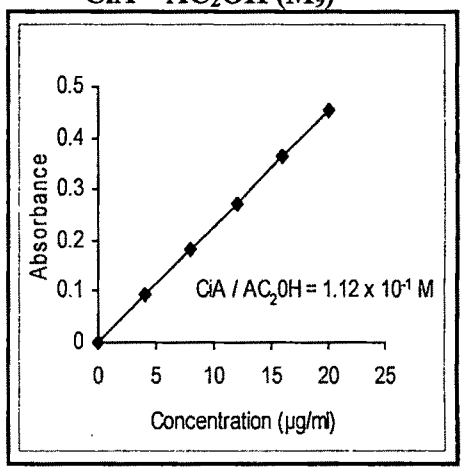


Fig. 4.11: Beer's Law plot of RIV with SNP-HA (M_{10})

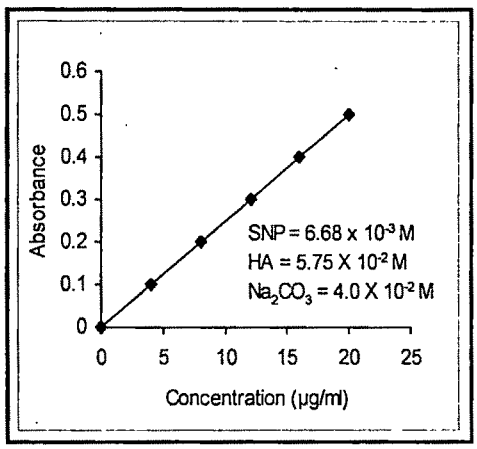


Fig. 4.12: Beer's Law plot of RIV with DDQ (M_{13})

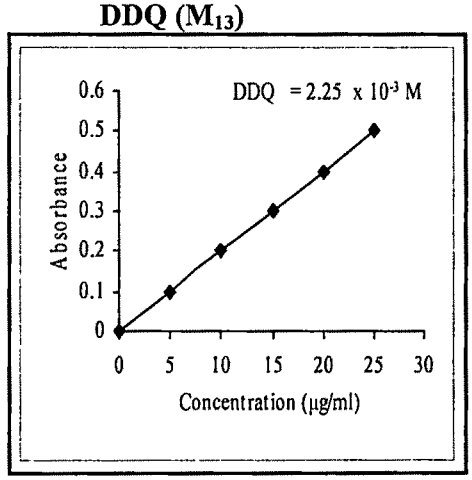


Fig: 4.13.Ringbom plot of RIV - TPooo(M_{1a})

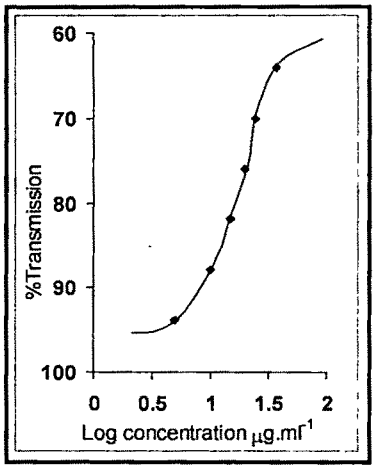


Fig:4.14.Ringbom plot of RIV - ARS(M_{1b})

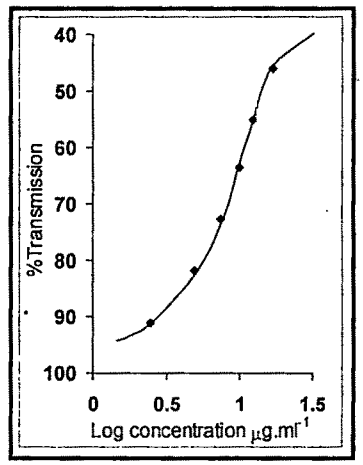


Fig: 4.15.Ringbom plot of RIV - CTC(M_8)

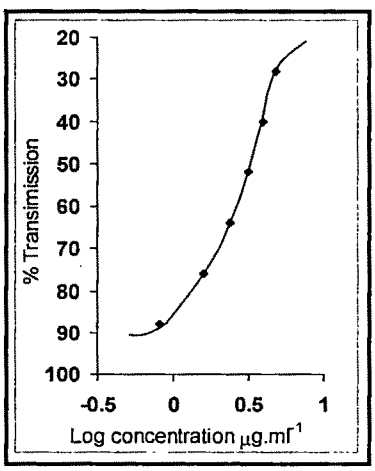


Fig:4.16.Ringbom plot of RIV - CiA (M_9)

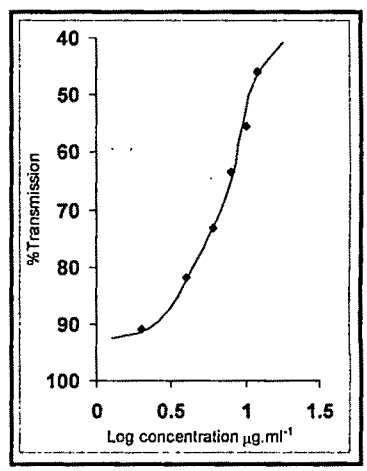


Fig: 4.17.Ringbom plot of RIV - SNP-HA(M_{10})

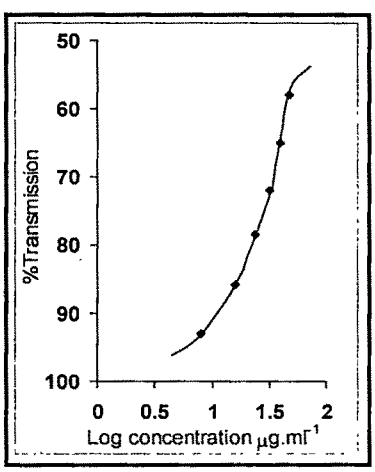
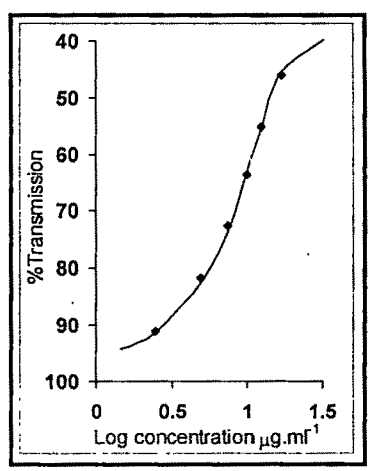


Fig:4.18.Ringbom plot of RIV - DDQ (M_{13})



Accuracy:

To determine the accuracy of each proposed method, different amounts of bulk samples of RIV within the Beer's law limits were taken and analyzed by the proposed method. The results (percent error) are recorded in **Table 4.09a & 4.09b, P. 224-225.**

Interference studies:

The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of RIV (in methods M_{1a}, M_{1b}, M_8, M_9, M_{10}, and M_{13}) under optimum conditions were investigated. The commonly used excipients and other active ingredients usually present in formulations did not interfere even if they are present in excess amount than they usually exist in formulations.

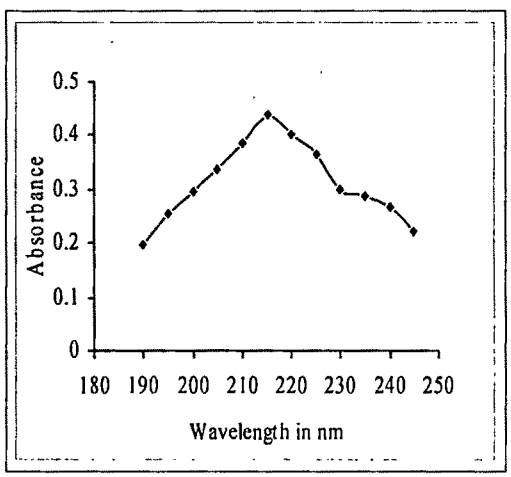
Analysis of formulations:

Commercial formulations (tablets) containing RIV were successfully analysed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with F and t tests and found not to different significantly. The results were summarized in **Table 4.10a & 4.10b, P. 226 - 227**. Percent recoveries were determined by adding standard drug to preanalyzed formulations. The results of the recovery experiments by the proposed methods are also listed in **Table 4.10a & 4.10b, P. 226 - 227.**

Procedure for the assay of RIV in formulations

An accurately weighed portion of tablet content equivalent to about 100mg of RIV was transferred into a 100ml volumetric flask. Added about 80ml of chloroform and shaken well for about 20min. The contents were diluted with chloroform upto the mark and mixed thoroughly. The solution was filtered and the filtrate was diluted to 100ml with chloroform to get 1mg/ml of drug in formulations .5.0ml of this solution was further diluted to 200ml to get 25µg/ml solution. The absorbance of the solution was determined at λ_{max} 215nm. The quantity of the drug was computed from the Beer's law plot of the standard drug in isopropyl alcohol.

Absorption spectra of RIV in $CHCl_3$ (UV reference method)



Beer's law plot of RIV in $CHCl_3$ (UV reference method)

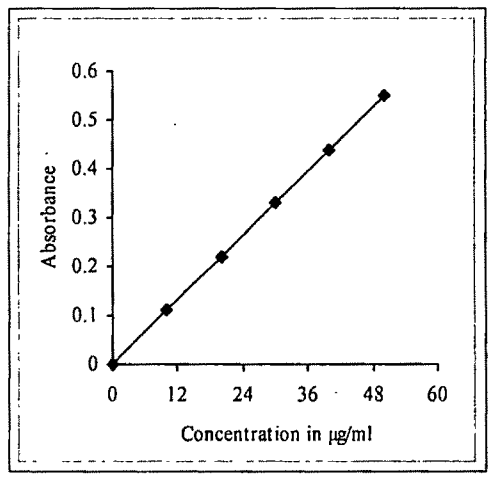


Table 4.09 a

Optical and regression characteristics, precision and accuracy of the proposed methods for RIV

Parameter	M_{1a}	M_{1b}	M_8	M_9
λ_{max} (nm)	490	430	620	580
Beer's law limits (μg/ml)	2.0 - 20.0	4.0 - 24.0	5.0 - 15.0	4.0 - 20.0
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	1.77×10^4	1.35×10^4	8.63×10^2	9.00×10^3
Sandell's sensitivity (μg.cm^{-2}/0.001 absorbance unit)	0.037	0.1032	0.769	0.0742
Optimum photometric range (μg/ml)	12.0 – 20.0	8.4 – 20.0	2.5 - 10.0	4.0 - 16.0
Regression equation (Y=a+bc) slope (b)	0.026	0.02799	0.001	0.013
Standard deviation on slope (S_b)	1.49×10^{-4}	2.115×10^{-2}	1.33×10^{-4}	6.88×10^{-4}
Intercept (a)	9.0×10^{-4}	2.25×10^{-3}	-5.0×10^{-4}	3.1×10^{-3}
Standard deviation on intercept (S_a)	1.98×10^{-3}	2.806×10^{-1}	3.32×10^{-4}	1.82×10^{-3}
Standard error on estimation (S_e)	1.89×10^{-3}	2.676×10^{-1}	3.16×10^{-4}	1.74×10^{-3}
Correlation coefficient (r)	0.9999	0.9998	0.9998	0.9999
Relative standard deviation (%)*	0.724	0.8094	0.258	0.451
% Range of error (confidence limits)				
0.05 level	0.760	0.9306	0.271	0.474
0.01 level	1.19	1.459	0.425	0.743
% error in Bulk samples **	0.311	0.139	-0.343	0.306

* Average of six determinations considered

** Average of three determinations

Table 4.09 b

Optical and regression characteristics, precision and accuracy of the proposed methods for RIV

Parameter	M_{10}	M_{13}
λ_{max} (nm)	580	460
Beer's law limits (μg/ml)	5.0 - 25.0	5.0 - 25.0
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	9.50×10^3	2.45×10^3
Sandell's sensitivity (μg.cm^{-2}/0.001 absorbance unit)	0.0792	0.270
Optimum photometric range (μg/ml)	5.0 - 20.0	10.0 – 20.0
Regression equation (Y=a+bc) slope (b)	0.1743	0.0036
Standard deviation on slope (S_b)	1.6900×10^{-4}	9.18×10^{-4}
Intercept (a)	2.500×10^{-3}	6.0×10^{-4}
Standard deviation on intercept (S_a)	2.2420×10^{-3}	7.12×10^{-4}
Standard error on estimation (S_e)	2.138×10^{-3}	7.65×10^{-4}
Correlation coefficient (r)	0.9999	0.9999
Relative standard deviation (%)*	0.4165	0.305
% Range of error (confidence limits)		
0.05 level	0.4812	0.320
0.01 level	0.7510	0.502
% error in Bulk samples **	0.164	0341

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

Table 4.10a

Assay of RIV in Pharmaceutical Formulations

Formulations*	Amount taken (mg)	Amount found by proposed Methods**				Reference method	Percentage recovery by proposed methods***			
		M_{1a}	M_{1b}	M_8	M_9		M_{1a}	M_{1b}	M_8	M_9
Tablet I	3.0	2.92±0.08 F=1.30 t=1.61	2.96±0.04 F=3.06 t=0.94	2.94±0.06 F=1.36 t=1.33	2.95±0.05 F=1.95 t=1.15	2.99±0.07	99.32±0.68	98.99±0.98	98.30±0.68	98.60±0.48

* Tablets from four different pharmaceutical companies

** Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the Reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.262

*** Recovery of 10mg added to the preanalysed pharmaceutical formulations (average of three determinations).

Table 4.10b

Assay of RIV in Pharmaceutical Formulations

Formulations*	Amount taken (mg)	Amount found by proposed Methods**		Reference method	Percentage recovery by proposed methods***	
		M_{10}	M_{13}		M_{10}	M_{13}
Tablet I	3.0	2.91±0.09 F=1.65 t =1.73	2.89±0.11 F=2.46 t=1.92	2.99±0.07	97.32±0.61	96..68±0.92

* Tablets from four different pharmaceutical companies

** Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.262

*** Recovery of 10mg added to the preanalysed pharmaceutical formulations (average of three determinations).

4.05. Conclusions:

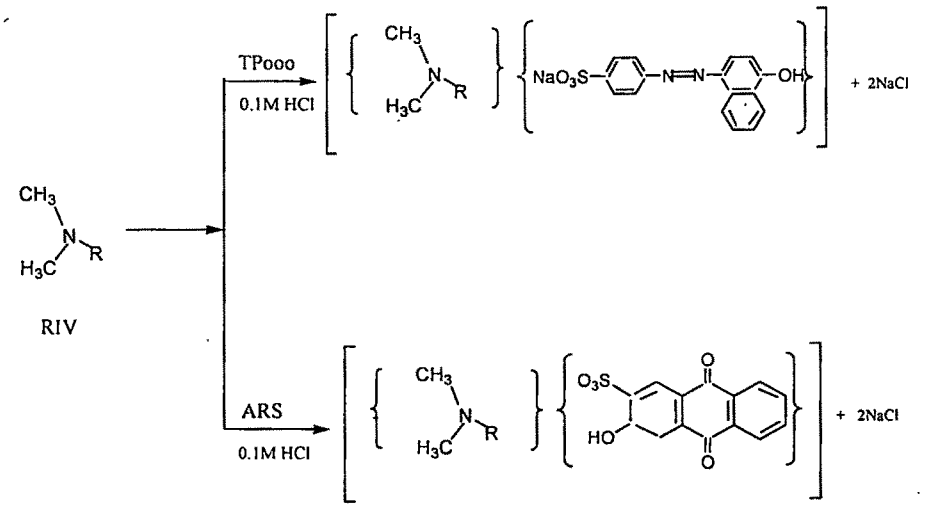
The proposed methods exploit the various functional groups in RIV molecule. The decreasing order of sensitivity (ϵ_{max}) among the proposed methods is $\mathbf{M_{1a} > M_{1b} > M_{10} > M_9 > M_8 > M_{13}}$; the concomitants which do not contain the functional groups chosen in the present investigation do not interfere in the color development by proposed methods. Thus the proposed methods are simple, sensitive or selective with reasonable precision and accuracy and constitute better alternatives to the reported ones in the assay of RIV in bulk form and pharmaceutical formulations.

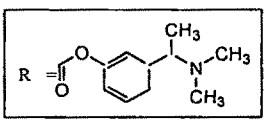
4.06. Nature of colored species:

As Rivastigmine possesses aliphatic tertiary nitrogen tertiary nitrogen and keto groups the author has attempted in developing the color species for the proposed methods basing on the reactivity of the functional moieties with the chromgenic reagents.

Method-M_{1a} & M_{1b}:

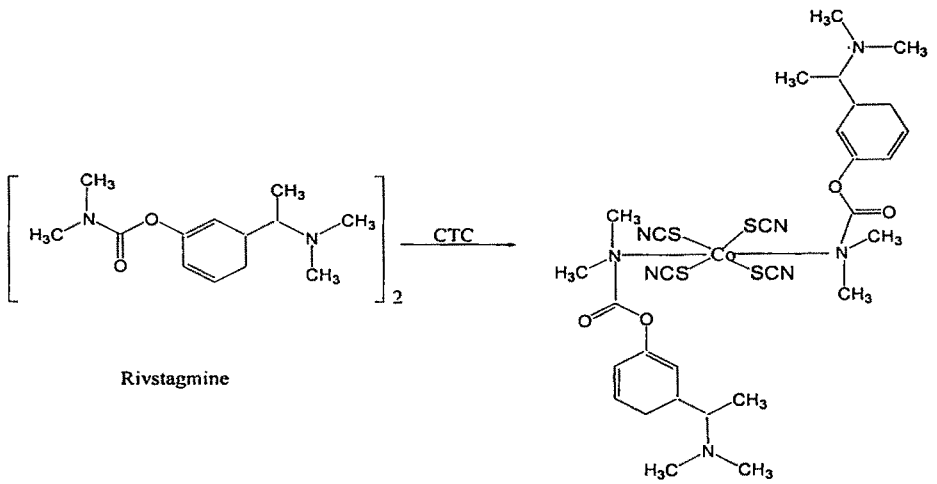
RIV being a base forms an ion association complex with an acidic dye (TPooo, ARS) which is extractable into chloroform from the aqueous phase. The protonated nitrogen (positive charge) of the drug molecule in acid medium is expected to attract the oppositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction. Based on the analogy, the structures of ion association complexes are shown in scheme





Method M_8:

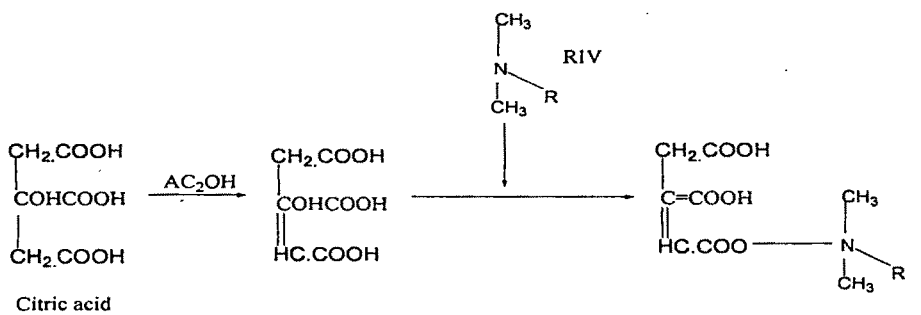
The colored complex formed between RIV and CTC can be attributed to aliphatic tertiary nitrogen in RIV. The probable sequence of reaction based on analogy is presented in scheme.



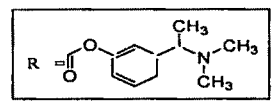
Rivstagmine

Method M_9:

Aconitic acid followed by its anhydride formation takes place from citric acid and acetic anhydride through dehydration. Formation of an internal salt of aconite anhydride with tertiary nitrogen in RIV is responsible for violet color formation and the probable sequence of reaction is presented in scheme.

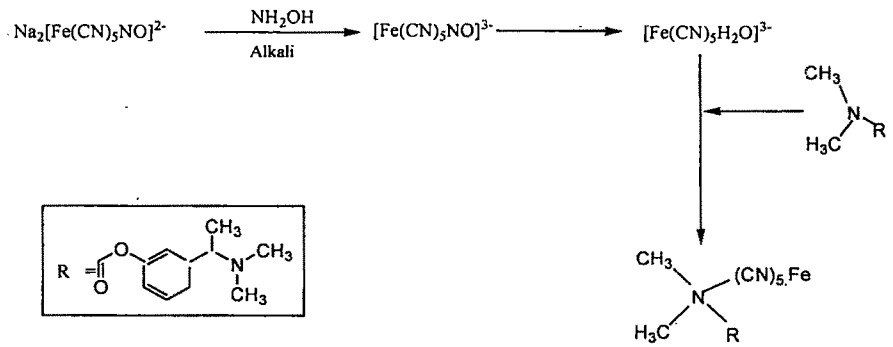


Citric acid



Method M_{10}:

In this method Rivastigmine acts as an electron donor SNP in presence of hydroxylamine and alkali exists as aqua ferri cyanide $[Fe(CN)_5H_2O]^{3-}$. The color obtained may be due to the formation of $[Fe(CN)_5M]^{3-}$. Where M is a compound exhibiting legating properties (M = Drug). Based on the analogy, the probable sequence of reactions is presented in scheme.



Method M_{13}:

RIV possesses aliphatic tertiary nitrogen and functions as electron donor and participates in charge transfer interaction with DDQ. The color species formation in the method appears to be due to the formation of radical anion. Based on analogy the sequence of reaction is given in the scheme.