Research Article

Newer Method to Estimate Rizatriptan in Bulk and Pharmaceutical Formulation by Using Colorimetric Method

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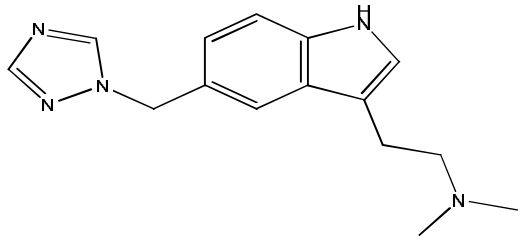
ABSTRACT

A simple colorimetric method was developed and validated for the estimation of Rizatriptan this method based on the formation of red colored complex between Rizatriptan and chromogenic agent 4-Amino phenazone in the presence of potassium ferricyanide and sodium carbonate which obeyed Beer's law in the concentration range of 20 to 100 µg/ml and shows a absorption maxima at 531 nm.

Keywords: Rizatriptan, colorimetric method.

INTRODUCTION

Rizatriptan (RITZA) chemically N,N-dimethyl-2-[5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethanamine is a selective serotonin (5-HT_1) agonist in cranial arteries responsible for vasoconstriction and reduction of inflammation associated with antidromic neuronal transmission and it is used for the treatment of migraine headaches.



In, the present study methanol. is used as solvent for estimation of the drugs by using UV-Spectrophotometry. The present method is relying on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC & HPTLC.

METHOD AND MATERIAL

Rizatriptan was determined colorimetrically in bulk and dosage forms by using the 4-Amino Phenazone in presence of Pottasium Ferricyanide and Sodium Carbonate.Systematic Investigations were carried out to optimize the proposed method.

Preparation of reagents

1) **4- Amino Phenazone**
   Prepared by Dissolving 3 gm of 4-Amino Phenazone in100ml methanol.
2) **Potassium Ferricyanide**
   Prepared by Dissolving 3 gm of Potassium Ferricyanide in 100 ml distilled water.
3) **Sodium Carbonate**
   Prepared by Dissolving 1gm of Sodium Carbonate in 100ml distilled water.
4) **0.1 N H_2SO_4**
   Dissolve 1 ml conc. sulphuric acid in 350 ml of distilled water.

Instrumentation

All the experiments were carried out on Jasco V530 series UV spectrophotometer using 1cm matched quartz cuvettes**.**

Preparation of standard stock solution of Rizatriptan (1mg/ml)

Stock solution of Rizatriptan was prepared by dissolving 100 mg in 0.1 N H_2SO_4 and make up to 100 ml with 0.1 N H_2SO_4 so as to get a conc of 1mg/ml.

Preparation of working standard solution of Rizatriptan (100µg/ml)

The working standard solution of Rizatriptan was prepared by further diluting the stock solution suitably with 0.1 N H_2SO_4 to get a concentration of 100µg / ml.

Systematic Study

The systematic study of the effect of the following variables was considered.

Preliminary investigation

To 1 ml of the drug solution containing 100µg/ ml, 1 ml of 3% of 4-Amino phenazone ,followed by 3% of 1 ml of Pottasium ferricyanide and 1 ml of 1% of Sodium carbonate was added. The reaction mixture was kept aside for 10 min for development of colour and finally make up the volume with 0.1 N H_2SO_4. A red coloured chromogen was obtained. A blank solution was prepared in similar way omitting the drug. This solution was scanned in the range 400-800 nm to fix the λmax of coloured complex.

PARAMETER FIXATION

Determination of λmax

An absorption maxima (or) λmax are the wavelength at which maximum absorption takes place.

- λmax of blank sample: (figure No. 1)
- λmax of coloured sample: (figure No. 2)

Stability of colour

1 ml of the working standard solution was taken into a 10 ml volumetric flask to this 1ml of 3% solution of 4-Amino phenazone, followed by 1ml of 3% potassium ferricyanide and 1ml of 1% Sodium carbonate was added and Reaction mixture was kept aside for 10 min for completion of the reaction Then the volume made up to the mark with 0.1 N H_2SO_4 and the absorbance was taken at 531nm against a reagent blank.

The stability of color was found to be more then 80 min. as shown following table no 1 and Figure No- 3.

Effect of order of addition

Series of experiments were carried out to test whether the variation in the order of addition of the reagents affected the absorbance of the coloured species and stability. The result showed that there was development of colour only when 4- Aminophenazone and potassium ferricyanide anhydrous was added first and then Sodium Carbonate and absorbance was taken at 531 nm and effect of conc. was evaluated. There was no development of color when Sodium Carbonate was added first and then 4-Aminophenazone and potassium ferricyanide anhydrous.

Effect of concentration of reagents

Experiments were carried out to ascertain the optimum concentrations of reagents needed for rapid and quantitative formation of red colored species by measuring the absorbance of series of solutions in which one parameter was varied and others fixed.

Procedure

1ml of the drug solution was placed in 7 different 10 ml volumetric flasks. To each volumetric flask 1ml of varying concentration of 4-Aminophenazone (0.5%, 1%, 1.5%, 2%, 2.5%,3 %, 3.5 %) were added, followed by 1 ml of the 3% Potassium ferricyanide followed by 1% of Sodium Carbonate were added. Reaction mixture was kept aside for 10 min for complete development of colour.Then the volume was made up to 10 ml with 0.1 N H_2SO_4 , and absorbance was measured against corresponding reagent blank in each case. The results were recorded in Table no.2 and graph was given in Fig. no. 4

Effect of volume of reagent (2.5 %4-aminophenazone)

To 1.0 ml of the drug solution was taken into 5 volumetric flasks of 10ml capacity and then 0.5, 1, 1.5, 2,and 2.5 ml of 2.5 % 4-Aminophenazone, followed by 1 ml of 3% potassium ferricyanide and 1 ml of 1 % sodium carbonate was added and the reaction mixtures were kept aside for 10 min for complete development of color then the volume was made up to 10ml with 0.1 N H_2SO_4. The absorbance was measured with corresponding reagent blank. The results were given in table No.3 and the graph is given in figure 5.

Effect of concentration of reagent (Potassium Ferricyanide)

Procedure

1ml of the drug solution was placed in 5 different 10 ml volumetric flasks, to which 1.5 ml of 2.5 % 4-Aminophenazone, followed by different concentrations of Potassium Ferricyanide and 1 ml of 1% of Sodium Carbonate was added and the reaction mixtures were kept aside for 10 min for complete development of color then the volume was made up to 10ml with 0.1 N H_2SO_4. The absorbance was measured against corresponding reagent blank. The results were given is Table No.4 and the graph was given in figure No.6.

Effect of volume of Potassium ferricyanide reagent

Procedure

To 1ml of the drug solution, placed in 4 different 10ml volumetric flasks, 1.5 ml of the 2.5 % 4-Aminophenazone was added to the volumetric flasks, followed by 0.5, 1, 1.5, 2 ml of 3 % Potassium ferricyanide was added and followed by 1% Sodium Carbonate, and the

reaction mixtures were kept aside for 10 min for complete development of color then the volume was made up to 10ml with 0.1 N H_2SO_4.The absorbance was measured against corresponding reagent blank. The results were given in table No .5 and graph was given in fig No.7.

Effect of Concentration of Reducing agent (Sodium Carbonate)

Procedure

1ml of the drug solution was placed in 6 different 10 ml volumetric flasks, to which 1.5 ml of 2.5 % 4-Aminophenazone, followed by 1.5 ml of 3 % Potassium Ferricyanide was added, and then 1 ml of different concentrations of 1 ml Sodium Carbonate was added and the reaction mixtures were kept aside for 10 min for complete development of color then the volume was made up to 10ml with 0.1 N H_2SO_4. The absorbance was measured against corresponding reagent blank. The results were given is Table No.6 and the graph was given in figure No.8.

Effect of volume of Sodium carbonate reagent

Procedure

To 1ml of the drug solution, taken in 7 different 10ml volumetric flasks, 1.5 ml of the 2.5 % 4-Aminophenazone and 1.5 ml of 3% Potassium ferricyanide and different volumes of 1% Sodium Carbonate was added, and. the reaction mixtures were kept aside for 10 min for complete development of color then the volume was made up to 10ml with 0.1 N H_2SO_4. The absorbance was measured against corresponding reagent blank. The results were given in table No .7 and graph was given in fig No.9.

Optical characters

Determination of Concentration range

For spectrophotometric analysis determination of the concentration range which obeys the Beer- Lambert's law is necessary for accuracy and reproducibility.

Preparation of standard curve

A standard curve was prepared by using pure Rizatriptan in the concentration of 20-100µg/ml by this method and selecting the absorption maxima at 531 nm.

Procedure

From the stock solution 0.2, 0.4, 0.6, 0.8, 1.0 ml (which gives 20 -100 .g/ml) drug solution were placed in 5 different 10 ml volumetric flasks. To this 1.5 ml of 2.5 % 4-Aminophenazone and 1.5 mi of 3% Potassium ferricyanide and 1ml of 1% Sodium carbonate was added. The reaction mixtures were kept aside for 10 min for complete development of color then make up to 10ml with 0.1 N H_2SO_4. The blank was also prepared simultaneously in the same way omitting the drug. The absorbance of the resulting solutions was measured at 531 nm against reagent blank using UV Spectrophotometer and the results were recorded in table No: 8 and the graph was given in the figure No: 10.

The six such linearities were taken for regression co-efficient and eight linearities were taken for standard deviation separately

Analysis of formulation

Rizatriptan was procured from the local market as tablets of strength 5 & 10 mg and marketed with brand name of RITZA and it was manufactured by Natco pharma ltd.

Preparation of sample solution

20 tablets were weighed and crushed properly using a mortar and pestle. Then Powder weight equivalent to 100mg was weighed and transferred to 100ml of volumetric flask and dissolved in 0.1 N H_2SO_4 and filter through whatmann filter paper in to another 100ml volumetric flask and make up to mark with same diluent which gives the solution of 1mg/ml conc., Further dilution were preformed to get a concentration of 100µg/ml.

Recovery experiments

To check the accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different concentration like 40, 60 and 80µg/ml were prepared in case of both pure drug solution and formulation extract solution and these solutions were subjected to analysis by above developed method as mentioned in 8.0. The six such samples were prepared and average of that readings taken for calculation of % recovery. This is reported in table no.5.

Statistical Evaluation

The precision of each proposed method was ascertained by analyzing the same concentration in freshly prepared sample solution of Rizatriptan 3 times, of three different sample solutions. The set absorbance values obtained were then used to calculate the drug content in vials and this was used to obtain standard deviation (s), Standard Error (S.E), precision (P) and ± value.

Table 1: Stability of Colour

S. No.	Volum of drug solution (100µg/ml)	Time in Min	Absorbance at 531 nm
1	1 ml	0	0.9734
2	1 ml	10	0.9731
3	1 ml	20	0.9725
4	1 ml	30	0.9719
5	1 ml	40	0.9709
6	1 ml	50	0.9703
7	1 ml	60	0.9677
8	1 ml	70	0.9642
9	1 ml	80	0.9614
10	1 ml	90	0.9531
11	1 ml	100	0.9465
12	1 ml	110	0.9402
13	1 ml	120	0.9389
14	1 ml	130	0.9328

Table 2: Effect of concentration of reagent

S. No.	Vol. of Drug (100µg/ml)	Concentration of 4-Amino Phenazone	Vol. of 4-Amino Phenazone	Vol. of Potassium Ferricyanide (3 %)	Vol. of Sodium Carbonate (1%)	Abs. at 531 nm
1	1 ml	0.5 %	1 ml	1 ml	1 ml	0.0867
2	1 ml	1 %	1 ml	1 ml	1 ml	0.1537
3	1 ml	1.5 %	1 ml	1 ml	1 ml	0.2828
4	1 ml	2 %	1 ml	1 ml	1 ml	0.4232
5	1 ml	2.5 %	1 ml	1 ml	1 ml	0.5764
6	1 ml	3 %	1 ml	1 ml	1 ml	0.3346
7	1 ml	3.5 %	1 ml	1 ml	1 ml	0.2584

Table 3: Effect of volume of 4- amino phenazone

S. No.	Vol. of Drug (100µg/ml)	Concentration of 4-Amino Phenazone	Vol. of 4-Amino Phenazone	Vol. of Potassium Ferricyanide (3 %)	Vol. of Sodium Carbonate (1%)	Abs. at 531 nm
1	1 ml	2.5 %	0.5ml	1 ml	1 ml	0.3116
2	1 ml	2.5 %	1 ml	1 ml	1 ml	0.5668
3	1 ml	2.5 %	1.5 ml	1 ml	1 ml	0.6643
4	1 ml	2.5 %	2 ml	1 ml	1 ml	0.4555
5	1 ml	2.5 %	2.5 ml	1 ml	1 ml	0.2849

Table 4: Effect of concentration of potassium ferricyanide

S. No.	Vol. of Drug (100µg/ml)	Volume of 4-Amino Phenazone (2.5 %)	Concentration of Potassium Ferricyanide	Vol. of Potassium Ferricyanide	Vol. of Sodium Carbonate (1%)	Abs. at 531 nm
1	1 ml	1.5 ml	1 %	1 ml	1 ml	0.2909
2	1 ml	1.5 ml	2 %	1 ml	1 ml	0.4955
3	1 ml	1.5 ml	3 %	1 ml	1 ml	0.6575
4	1 ml	1.5 ml	4 %	1 ml	1 ml	0.4258
5	1 ml	1.5 ml	5 %	1 ml	1 ml	0.3009

Table 5: Effect of Volume of Potassium Ferricyanide

S. No.	Vol. of Drug (100µg/ml)	Volume of 4-Amino Phenazone (2.5 %)	Concentration of Potassium Ferricyanide	Vol. of Potassium Ferricyanide	Vol. of Sodium Carbonate (1%)	Abs. at 531 nm
1	1 ml	1.5 ml	3 %	0.5 ml	1 ml	0.2797
2	1 ml	1.5 ml	3 %	1 ml	1 ml	0.6674
3	1 ml	1.5 ml	3 %	1.5 ml	1 ml	0.8316
4	1 ml	1.5 ml	3 %	2 ml	1 ml	0.5160

Table 6: Effect of concentration of sodium carbonate

S. No.	Volume of Drug (100 µg/ml)	Volume of 4-Amino Phenazone (2.5 %)	Volume of Potassium Ferricyanide (3 %)	Concentration of Sodium Carbonate	Volume of Sodium Carbonate	Abs. at 531 nm
1	1 ml	1.5 ml	1.5 ml	0.2 %	1 ml	0.1012
2	1 ml	1.5 ml	1.5 ml	0.4 %	1 ml	0.3515
3	1 ml	1.5 ml	1.5 ml	0.6 %	1 ml	0.5362
4	1 ml	1.5 ml	1.5 ml	0.8 %	1 ml	0.7104
5	1 ml	1.5 ml	1.5 ml	1 %	1 ml	0.8209
6	1 ml	1.5 ml	1.5 ml	1.2 %	1 ml	0.6948

Table 7: Effect of volume of sodium carbonate

S. No.	Volume of drug (100 µg/ml)	Volume of 4-Amino Phenazone (2.5 %)	Volume of Potassium Ferricyanide (3 %)	Volume of Sodium Carbonate (1 %)	Absorbance at 531 nm
1	1 ml	1.5 ml	1.5 ml	0.2 ml	0.1926
2	1 ml	1.5 ml	1.5 ml	0.4 ml	0.3462
3	1 ml	1.5 ml	1.5 ml	0.6 ml	0.5178
4	1 ml	1.5 ml	1.5 ml	0.8 ml	0.6933
5	1 ml	1.5 ml	1.5 ml	1 ml	0.8194
6	1 ml	1.5 ml	1.5 ml	1.2 ml	0.7341
7	1 ml	1.5 ml	1.5 ml	1.4 ml	0.6578

Table 8: Absorbance of Different concentration of Rizatriptan Obeying beer's law

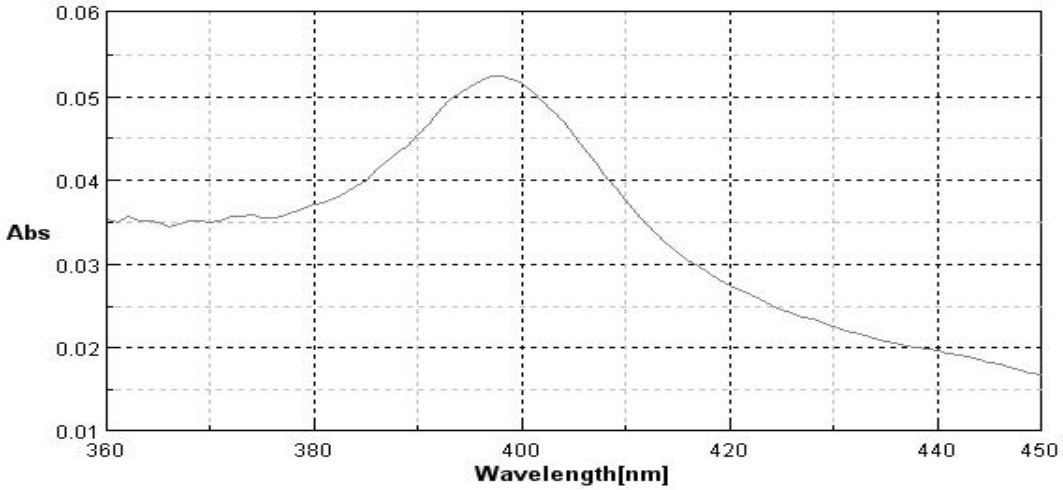
S. No.	Volume of drug 1 mg/ml	Concentration of drug taken in µg/ml	Absorbance At 531nm
1.	0.2 ml	20 µg	0.1786
2.	0.4 ml	40 µg	0.3502
3.	0.6 ml	60 µg	0.5235
4.	0.8 ml	80 µg	0.6889
5.	1 ml	100 µg	0.8437

Table 9: Optical characteristics and precision of the proposed method for rizatriptan

Parameter	Result
λmax (nm)	531
Beer's law limits (µg/ml)	20-100
Molar absorptivity (1/mol.cm)	3.3858×10^4
Sandell's sensitivity (µg.cm^2/0.001 Au)	0.1148
Regression equation (y=a+bc) Slope (b) Intercept (a)	 0.008 0.007
Correlation coefficient (r)	0.999
Relative standard deviation (%)	0.3444
% Range of error 0.05 level 0.01 level	 0.2879 0.4260
confidence limit (µ) 0.05 level 0.01 level	 0.6096±0.0017 0.6096±0.0025

Table 10: Assay and recovery of rizatriptan in dosage forms

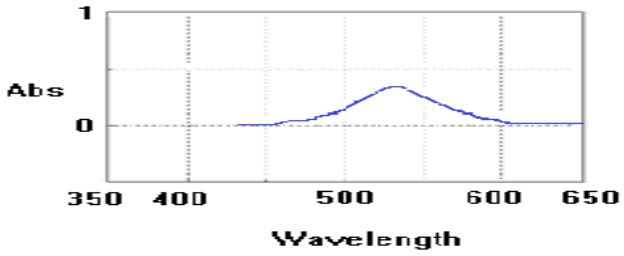
Method	Sample	Labeled Amount (mg)	Amount Found mg/Tablet	% Recovery
1	F-1	10	8.8032	95.86
2	F-2	5	4.7392	97.45



Model : V-530.
Band width : 2nm.
Response : Medium.
Measurement : 450-360 nm
No. of cycle : 1.
Sample : Blank.
λmax : 380nm

Conclusion: The λmax of the blank was found to be 380nm.

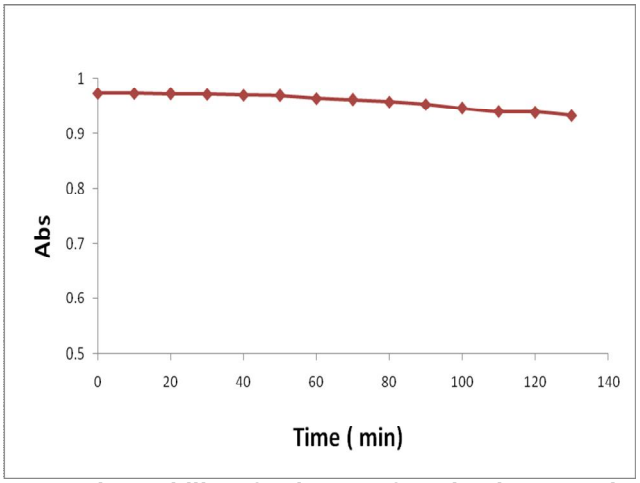
Fig. 1:



λmax detrmination of coloured sample
Model : V-530.
Band width : 2nm.
Response : Medium.
Measurement : 650-350nm.
No. of cycle : 1.
Sample : Rizatriptan.
λmax : 531nm

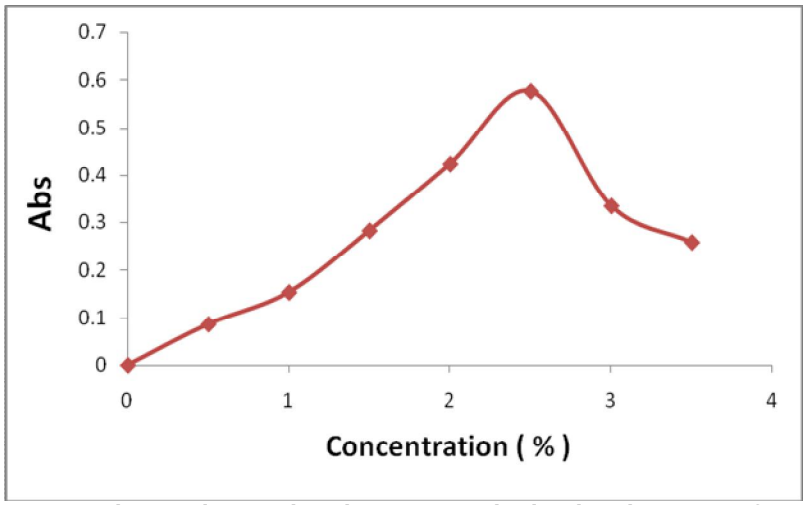
CONCLUSION: The λmax of the blank was found to be 380nm and no impurity peak found at the peak of the colored product which is 531nm.

Fig. 2:



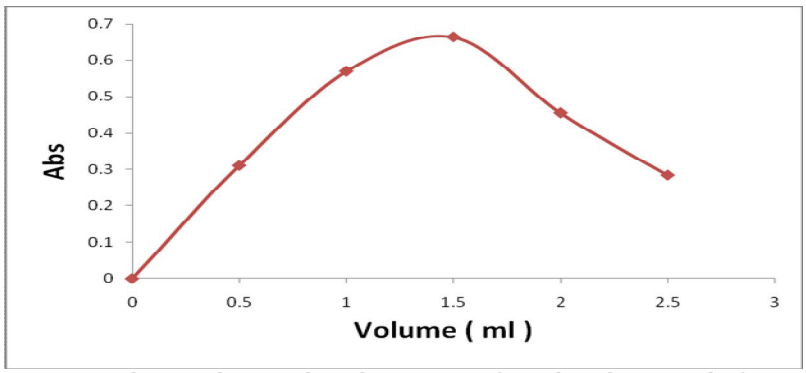
CONCLUSION: The stability of color was found to be more then 80 min

Fig. 3: Stability of colour



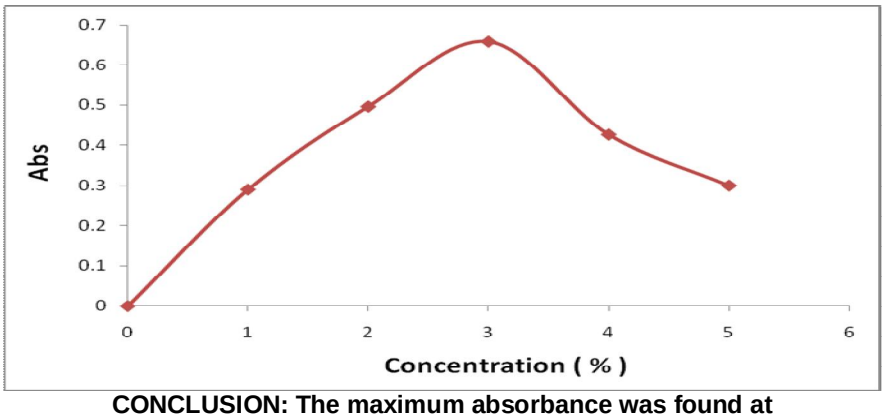
CONCLUSION: The maximum absorbance was obtained at the conc. of 2.5 % of 4-Aminophenazone.

Fig. 4: Effect of Concntration of 4-Amino Phenazone



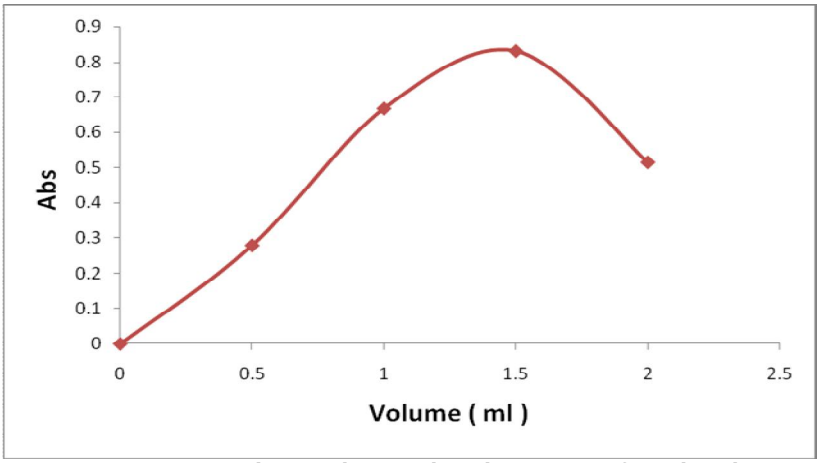
CONCLUSION: The maximum absorbance was found at the 1.5 ml of 2.5 % of 4-Aminophenazone.

Fig. 5: Effect of volume of 4-amino phenazone



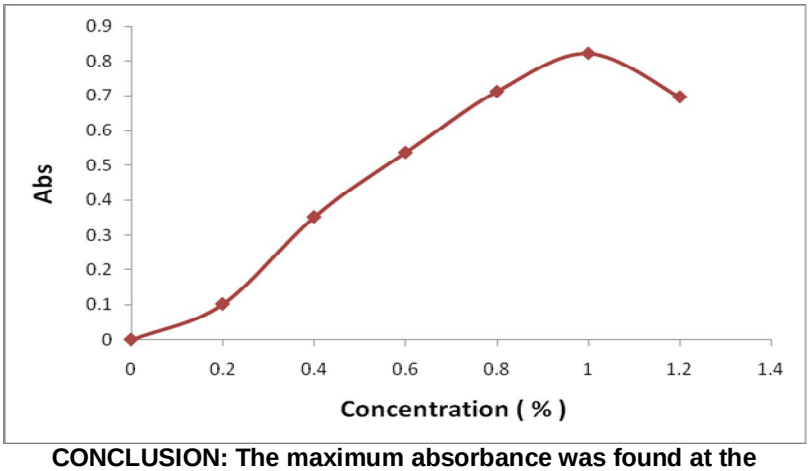
CONCLUSION: The maximum absorbance was found at the 3 % of Potassium ferricyanide.

Fig. 6: Effect of concentration of potassium ferricyanide



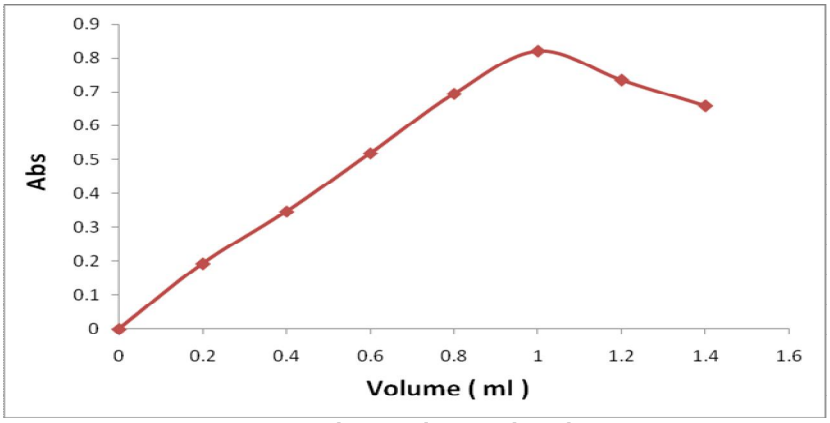
CONCLUSION: The maximum absorbance was found at the 1.5 ml of 3 % of Potassium ferricyanide.

Fig. 7: Effect of volume of potassium ferricyanide



CONCLUSION: The maximum absorbance was found at the 1 % of Sodium Carbonate.

Fig. 8: Effect of concentration of sodium carbonate



CONCLUSION: The maximum absorbance was found at 1 ml of the 1 % of Sodium Carbonate.

Fig. 9: Effect of volume of sodium carbonate

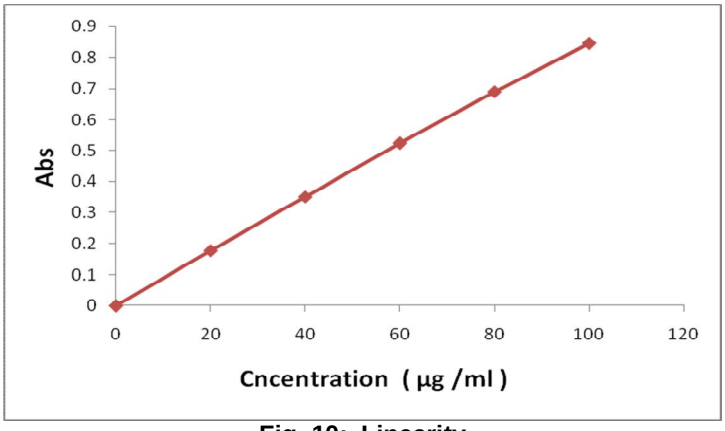


Fig. 10: Linearity

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