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QUANTITATIVE ESTIMATION OF RAFOXANIDE IN POWDER FOR SUSPENSION BY SPECTROPHOTOMETRIC METHODS

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ABSTRACT

Two simple, rapid, cost effective spectrophotometric methods have been developed for the determination of Rafoxanide in its bulk form as well as pharmaceutical dosage form. Method M_1 is based on measurement of absorbance at its absorption maximum at about 280nm against a methanol solution as a blank. Method M_2 is based on oxidation of the drug followed by complexation with 2,2'-bipyridyl reagent to give blood red colored coordination complex which has shown maximum absorbance at the maximum at about 520 nm against its reagent blank. These methods were found linear within the concentration range of 5-25µg/ml and 4-28µg/ml for Method M_1 and Method M_2 respectively. The proposed methods show correlation coefficient of >0.999 for the given concentration ranges. These methods were validated according to ICH guidelines and the result of estimation of marketed powder formulation was found to be 99.50% and 99.77% for Method M_1 and Method M_2 respectively with their %RSD of precision less than 2.

INTRODUCTION

Rafoxanide (Fig.1) is the most frequently prescribed anthelmintic drug. It is chemically known as N-[3-chloro-4-(4-chlorophenoxy) phenyl]-2-hydroxy- 3, 5-diiodobenzamide. Its molecular formula is $C_{19}H_{11}Cl_2I_2NO_3$. Chemically it is a derivative of salicylanilide. It is a narrow-spectrum drug used to treat fluke, hookworm and other infestations. Rafoxanide has a residual effect, i.e. it not only kills the parasites present in the host at the time of treatment, but protects against re-infestation for a period of time (up to several weeks) that depends on the dose and the specific parasite it is an uncoupler of the oxidative phosphorylation. Rafoxanide show it selective and specific action in the cell mitochondria, by which it disturbs the production of ATP, the cellular "fuel". This seems to occur through suppression of the activity of succinate dehydrogenase and fumarate reductase which are the two enzymes involved in this process. This impairs the parasites motility and probably other processes as well [1-2].

Rafoxanide is official in Indian Pharmacopoeia which suggests titration method for the determination of RFX in bulk and pharmaceutical dosage forms. Several analytical techniques like LC-MS, HPLC, and HPLC –Flourescence Detection have been reported in the literature [3-7]. These instruments are expensive and their maintenance and operation are costly. The methods applied using these techniques are tedious and laborious. UV-visible spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories because of their simplicity, accuracy, precision and low cost. In the literature, none of the spectrophotometry procedures have been reported for the determination of Rafoxanide in pharmaceutical dosage forms. This paper describes for the first time the application of UV-spectroscopy for the quantitative analysis of Rafoxanide in pharmaceutical bulk and dosage forms.

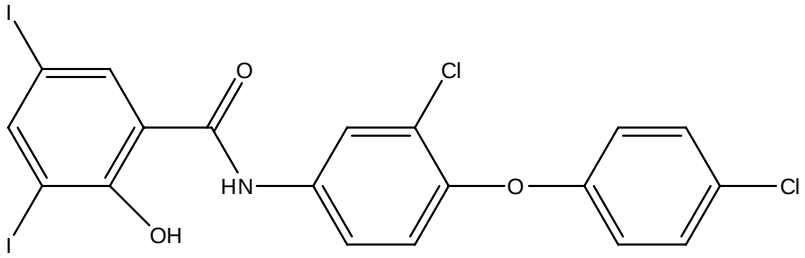

Figure 1: Chemical structure of Rafoxanide

MATERIALS AND METHODS

Materials and instruments

All the chemicals and reagents 2, 2'-bipyridyl (Qualigens), hydrochloric acid (Qualigens) and methanol (Qualigens) used were of analytical grade and solutions were prepared in double distilled water. Systronics UV/Visible spectrophotometer model -2203 with 10mm matched quartz cells was used for all spectral measurements. All chemicals used were of analytical reagent grade. Rafoxanide was supplied as gift sample by Hetero labs, Hyderabad. Dosage form containing Rafoxanide was obtained from commercial sources in the local market in the form of powder for reconstitution.

Preparation of reagents:

2,2' bipyridyl solution (Qualigens, 0.156% w/v, 1.0 x 10^{-2} M) : Prepared by dissolving 156 mg of 2, 2'-bipyridyl in 100 ml methanol.

$FeCl_3$ stock solution (Qualigens, 0.162 % w/v, 1M): About 162 mg of anhydrous ferric chloride was accurately weighed and dissolved in 100 ml of distilled methanol.

Dilute $FeCl_3$: 33.3 ml of above stock solution was further diluted to 100 ml with water.

Orthophosphoric acid solution (Qualigens, 2.0 x 10^{-1} M) : 1.3 ml of orthophosphoric acid was diluted 100 ml with distilled water.

Preparation of standard drug solution for methods M_1 and M_2:

100mg of Rafoxanide pure drug was accurately weighed, transferred into a 100ml volumetric flask containing 40ml of methanol and sonicated for about 10 minutes. The volume was made up to the mark with methanol to get the stock solution (1mg/ml). This solution was further diluted with same solvent to get the working standard solution of concentration of 100µg/ml.

Spectral Characteristics:

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}), the spectra were scanned in the wavelength region of 200-400 and 400-800 nm against corresponding reagent blanks for Method M_1 and Method M_2 respectively. The reagent blank absorption spectrum of each method was recorded against solvent employed in each method. The results were graphically presented and the absorption curves of colored species formed in each method shows characteristic absorption maximum whereas the blank in each method has low or no absorption in this region.

Recommended procedure for construction of calibration curve and assay of Rafoxanide in powder for suspension:

Method M_1:

To a series of 10ml volumetric flasks, carefully transferred aliquots of standard drug solutions [0.5-2.5ml (100µg/ml)]. The volume was made up to mark with methanol. The absorbance of each solution was recorded at 280nm against methanol as blank. A calibration curve was plotted by taking concentration of each standard on x-axis and absorbance of each standard on y-axis (Figure.2).

About 0.5g of the powder equivalent to 0.1g of Rafoxanide was weighed, dispersed in 50ml of methanol and then diluted up to the mark with the same solvent to 100ml, sonicated for 10minutes and filtered. Through a cotton wool and the filtrate was suitably diluted to get a sample solution.

The absorbance of each solution was recorded at 280nm against methanol as blank. The amount of Rafoxanide present in the dosage form was computed from its calibration curve by regression analysis.

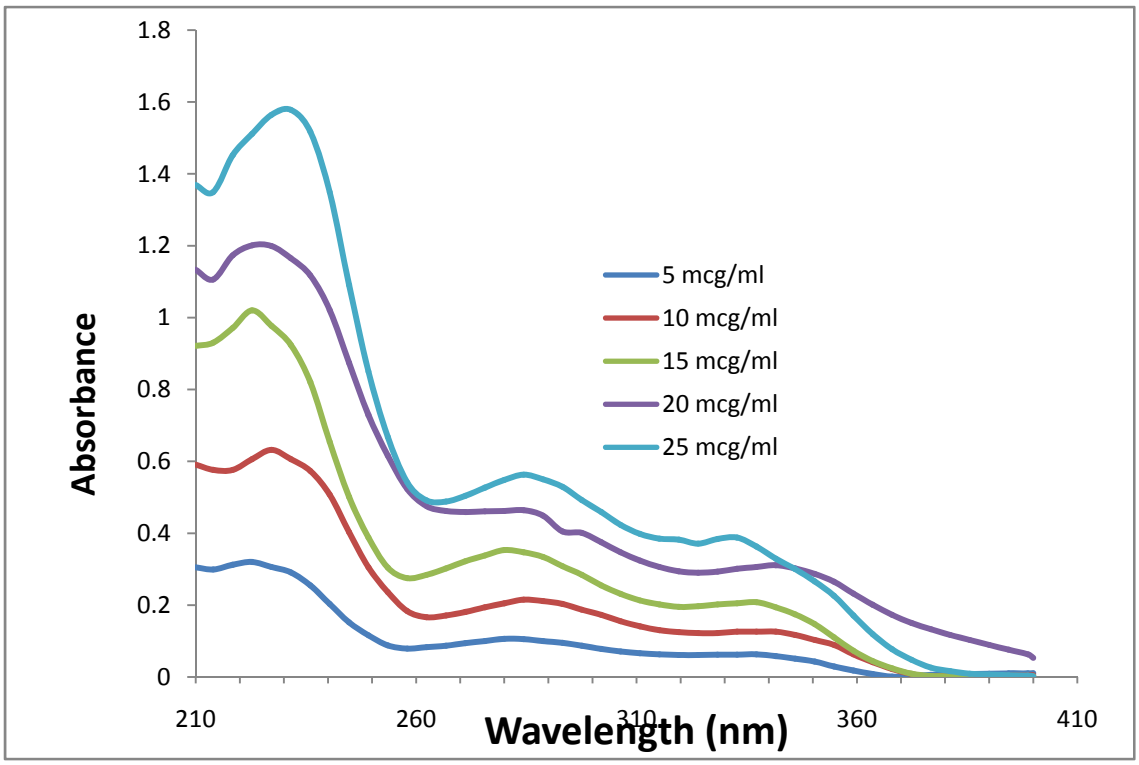

Figure. 2: Overlay Absorption spectra of Rafoxanide in methanol

Method M_2:

Aliquots of methanolic standard drug [0.4-2.8ml (100µg/ml)] solution were transferred into a series of 10ml volumetric flasks. To each flask, added 2 ml of $FeCl_3$ and 1ml of 2, 2'-bipyridyl 0.1M solution shaken well and the reaction mixture was heated at 50° C on a boiling water bath for about 15 min. The solution was then cooled to room temperature and 2ml of orthophosphoric acid was added in order to prevent further oxidation of $FeCl_3$ and the volume of the resulted solution was made up to the mark with methanol. The blood-red colored complexes thus formed were estimated at 520 nm. The amount of RFN present in the formulation was computed from its calibration plot (Figure.3)

About 0.5g of the powder equivalent to 0.1g of Rafoxanide was weighed, dispersed in 50ml of methanol and then diluted up to the mark with the same solvent to 100ml, sonicated for 15minutes and filtered. Through a cotton wool and the filtrate was suitably diluted with methanol to get a working sample solution procedure as described under standard drug solution. The amount of Rafoxanide present in the formulation was computed from its calibration plot.

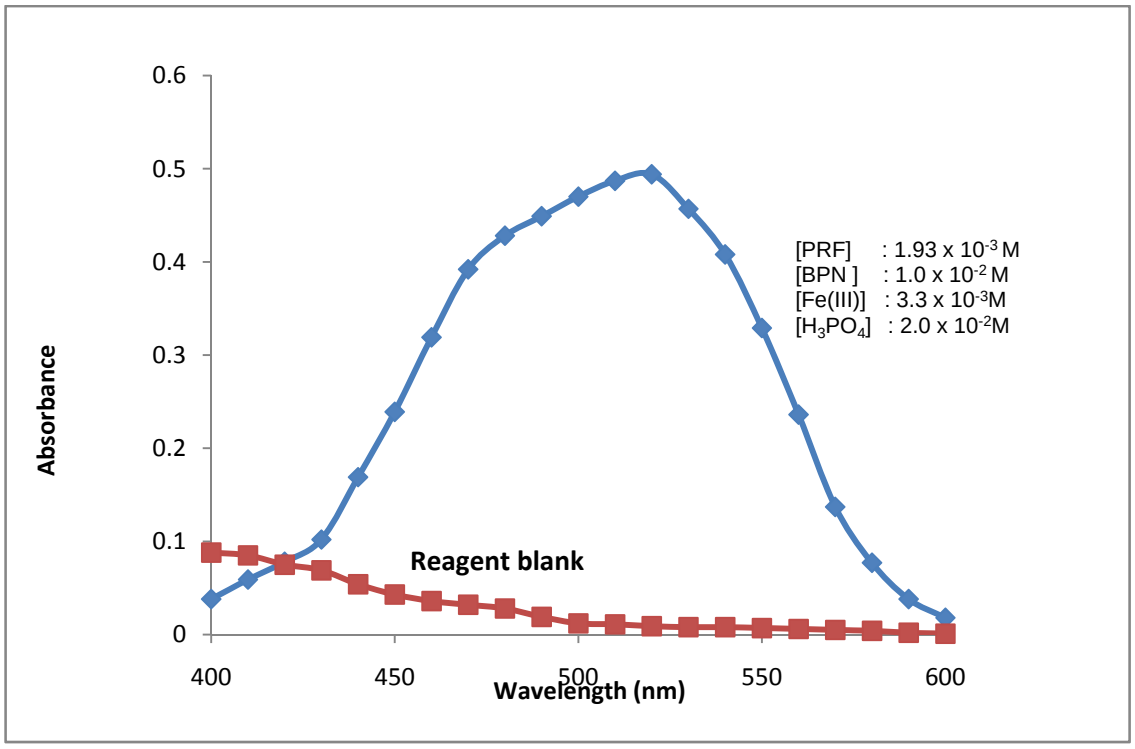

Figure.3: Absorption spectra of Rafoxanide with 2,2' bipyridyl system and its reagent blank

Validation of analytical methods [8]:

The UV spectrophotometric methods developed for the estimation of the drug was validated by observing the following parameters

Specificity:

To observe the interference of excipients in the estimation of selected drug, a placebo was prepared by using common excipients like lactose, magnesium stearate, and talc. The powder was dissolved in the solvent system and filtered. The absorbance of the resulting solution was measured at 280nm and 520nm shown in Table-1.

Table 1: Absorbance data of placebo of the proposed methods for rafoxanide

Trials	M_1	M_2
1	0.003	0.002
2	0.002	0.004
3	0.004	0.003

Linearity and range:

Calibration curve for Rafoxanide was constructed by plotting a graph in between the concentration and absorbance. The concentration of the solution was maintained in between 5-25µg/ml and 4-28 µg/ml. Accurately measured standard working solutions of rafoxanide (0.5, 1, 1.5, 2 & 2.5ml for Method 1 and 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8ml for Method 2) were transferred to a series of 10ml volumetric flasks and diluted up to the mark with suitable solvents. The absorbance values were determined at 280nm and 520nm. (Figure.4 and 5)

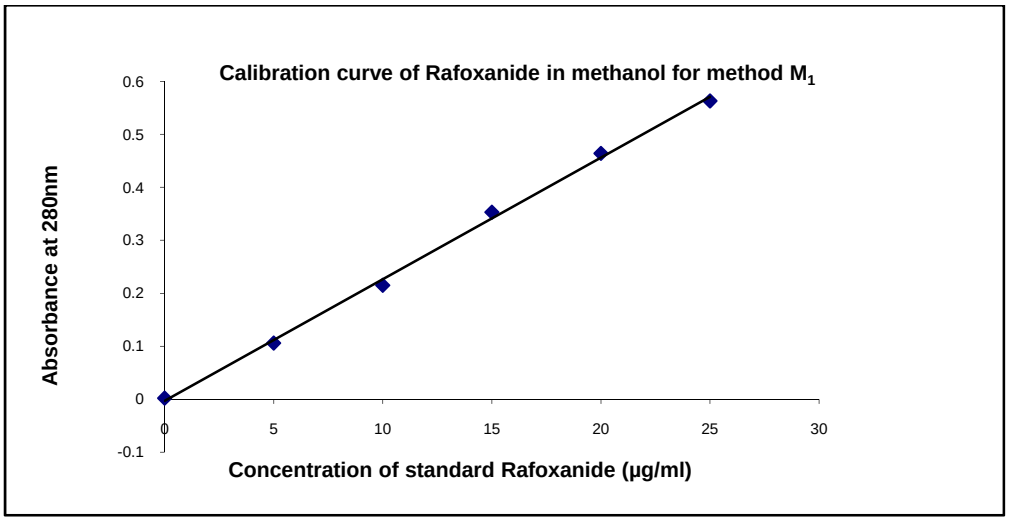

Figure.4: Calibration curve of Rafoxanide for Method (M_1)

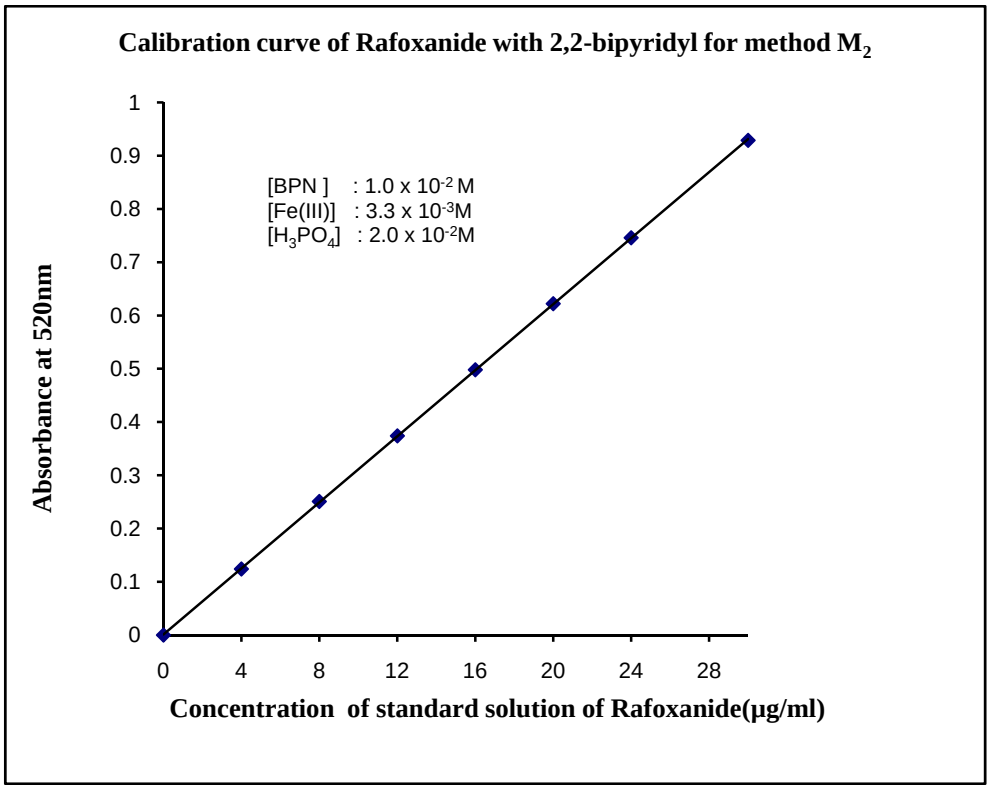

Figure 5: Calibration curve of Rafoxanide for Method (M_2)

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines [8]. The LOD and LOQ are calculated by using following formulas

$$\mathbf{LOD = 3\sigma / S}$$

$$\mathbf{LOQ = 10\sigma / S}$$

Where s- the standard deviation of the y-intercept and S- the slope of regression line

Precision:

The following precision analysis was carried out.

1) Repeatability:

Sample drug solution of rafoxanide was prepared by using suitable solvents (methanol and 2,2'-bipyridyl) and observed the absorbances at 280nm and 520nm repeatedly for six times and the %RSD were calculated.

2) Intermediate precision/ ruggedness

The intraday and inter day precision of the proposed method was performed by analyzing the corresponding responses for 6 times on the same day and 6 different days of sample drug solutions. The absorbances of the resulting solution were determined at 280nm and 520nm. The results were reported in terms of %RSD and shown in Table-2.

Table 2: Precision (Repeatability) Data of Rafoxanide

Trials	Concentration found by method M_1 (µg/ml)	Concentration found by method M_2 (µg/ml)
1	15.02	15.06
2	14.97	14.97
3	14.99	14.98
4	14.98	15.08
5	14.99	14.98
6	15.09	15.09
Mean	15.00	15.02
Standard deviation	0.0130	0.0132
% RSD	0.090	0.091

Accuracy (Recovery Test)

Recovery studies were carried out by using standard addition method; known amount of standard drug (80, 100, and 120% of labeled claim of a powder) rafoxanide was added to pre-analyzed sample and subjected to proposed UV/Visible methods.

Results and Discussion:

Optical characteristics and regression data of the proposed methods were calculated from the observed data and shown in table 3. The low % RSD values indicates the method is precise Recovery studies were carried out for both the developed methods by addition of known amount of standard drug solution of rafoxanide to pre-analyzed powder sample solution at three different concentration levels. The recovery was in the range of 99.5±0.2% for M_1 and 99.77±0.019 for M_2. The optical characteristics and the data concerning to the proposed methods are incorporated in table 4 and 5.

Table 3: Optical characteristics and regression data of the proposed methods for Rafoxanide

* Average of six determinations.

Parameter	M_1	M_2
λ_{max} (nm)	280	520
Beer's law limits (µg / ml)	5-25	4-28
Molar absorptivity (L. mole^{-1} cm^{-1})	1.452x 10^4	1.430x 10^4
Detection limits (µg / ml)	1.106	0.101
Sandell's sensitivity (µg /cm ^{2}/0.001 absorbance unit)	0.043103	0.040
Optimum photometric range (µg / ml)	10-20	8-20
Regression equation (Y = a+ bc): Slope (b)	0.023	0.03099
Standard deviation of slope (S_b)	5.04 x 10^{-4}	5.54x 10^{-5}
Intercept (a)	0.003	0.00133
Standard deviation of intercept (S_a)	7.69x 10^{-3}	9.51x 10^{-4}
Standard error of estimation (S_e)	1.06 x 10^{-2}	4.28x 10^{-3}
Correlation coefficient (r)	0.999	0.9999
% Relative standard deviation*	0.4121	0.8492
%Range of Error (Confidence limits)*		
0.05 level	0.4325	0.8913
0.01 level	0.6783	1.3978

Table 4: Assay of Rafoxanide in dosage forms

Method	Labeled Amount (%w/v)	Proposed Methods			Found by reference method[1] ± S.D
		Amount found* (%w/v) ± S.D	T (value)	F (Value)	
M_1	20%w/v	19.98± 0.011	0.572	1.893	19.88± 0.011
M_1	20%w/v	20.10± 0.013	0.457	2.193	20.08± 0.013
M_1	20%w/v	19.99± 0.009	0.564	1.759	20.15± 0.009
M_2	20%w/v	20.09± 0.009	0.541	1.233	20.12± 0.009
M_2	20%w/v	20.06± 0.017	1.023	1.651	20.06± 0.017
M_2	20%w/v	20.02± 0.021	0.936	2.825	20.02± 0.021

*Average ± standard deviation of three determinations, the t and F- values refer to comparison of the proposed method with reference method.

Theoretical values at 95 % confidence limits t = 2.571 and F = 5.05.

Table 5: Recovery (Accuracy) Data of Rafoxanide at 280nm in methanol

Method	Formulation Amount (µg/ml)	Level of Addition (%)	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Average %Recovery N=3
M_1	10	80	8	17.89	99.3	99.50±0.022
	10	100	10	19.91	99.5	
	10	120	12	21.94	99.7	
M_2	10	80	8	17.99	99.94	99.77±0.019
	10	100	10	19.95	99.75	
	10	120	12	21.92	99.63	

The chemistry involved in the above-proposed method M_2 to give various colored chromogens can be explained by the following.

RFN exhibits reducing property due to the presence of functional moieties (one or more) vulnerable to oxidation selectively with oxidizing agents such as Fe (III) under controlled experimental conditions. When treated with known excess of oxidant, RFN 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 colorimetrically, which is equivalent to either the reacted oxidant or reduced form of oxidant formed. The reduced form of Fe III (Fe II) has a tendency to give colored complex on treatment with 2,2' bipyridyl [9,10].

The first step in the methods mentioned above is the oxidation of RFN with the oxidant.

RFN + Fe (III) → Oxidation products + Fe (II) + Fe (III)
(Excess) (Reduced form of Oxidant) (Unreacted)

In this method, as Fe (III) interferes, even though to a little extent in the determination of Fe (II), the reactivity of the interfering entity has to be made insignificant by complexing it with o-phosphoric acid.

Fe (III) + o-phosphoric acid → Complex (unreactive)

The second step concerns with the estimation of the reduced form of oxidant with appropriate chromogenic agent as 2,2' bipyridyl. The complex formation was illustrated in the Figure 6

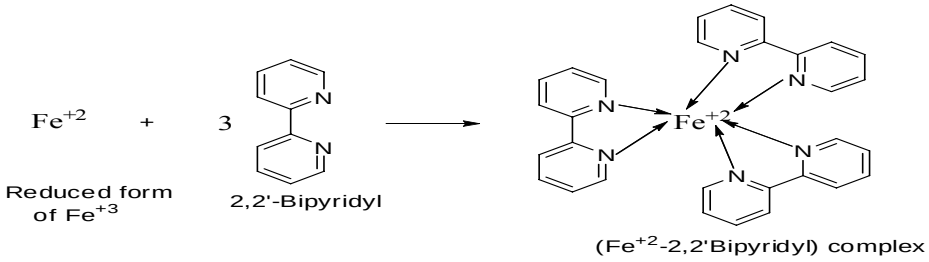

Figure 6: Reduction of Ferrous with 2,2'-Bipyridyl reagent

CONCLUSION

There was no UV/Visible spectrophotometric methods were reported for the estimation of Rafoxanide in either bulk or pharmaceutical formulations. The author developed two spectrophotometric methods based on the reactivity of different structural units such as phenyl and diiodobenzamide in Rafoxanide. Each method uses a specific reagent and the ε_{max} values of each method are different. The sensitivity order of various proposed methods is $M_2 > M_1$.

Statistical analysis of the results shows that the proposed procedures have good precision and accuracy. Results of analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations. These methods can be adopted for routine quality control of PRF in bulk and pharmaceutical preparations.

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