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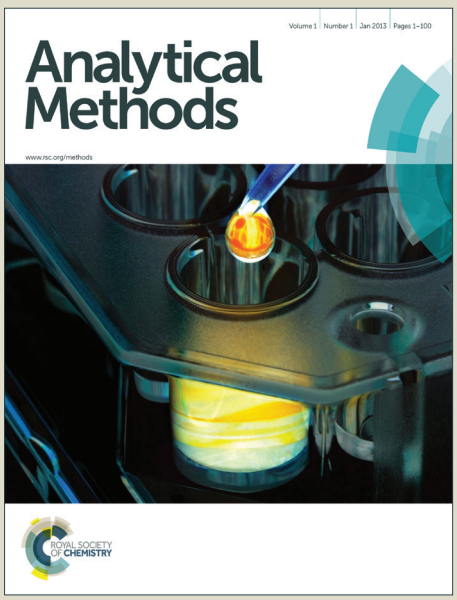

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Spectroscopic Studies on Naftazone and its Metal Complexes with Analytical Applications for Quality Control of Tablets

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Abstract

The spectroscopic characteristics of the hemostatic drug naftazone as well as its metal complexing ability were investigated spectrophotometrically in order to develop new, simple and sensitive spectrophotometric methods for its determination. The first method is based on measurement of the absorbance of the drug in NaOH ($2x10^{-3}$M) at 494 nm. The method showed excellent linearity over the concentration range of 0.5-10 µg mL^{-1}. The second method is based on complex formation between NFZ and different metal ions including Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+} in borate buffer. The absorbance of the formed complexes were measured at 512, 506, 498 and 502 nm for Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}, respectively. Beer's law is obeyed within the concentration ranges of 2.0-14.0, 2.0-10.0, 2.0-14.0 and 2.0-19.0 µg mL^{-1} for Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}complexes, respectively. The molar ratios and stability constants of the metal complexes are calculated and a proposal of the reaction pathway is consequently postulated. The proposed methods were validated according to ICH Guidelines and they were successfully applied for the determination of NFZ in its tablet dosage forms without interference from contaminant substances normally added to tablets. The results of the proposed methods were favorably compared with those obtained by the comparison method using student t-test and variance ratio F-test.

Keywords: Naftazone (NFZ); Spectrophotometry; Metal Complex; Molar ratio; Tablets.

Introduction

Naftazone (NFZ) is designated chemically as 1, 2-naphthoquinone-2-semicarbazone (Fig. 1). It is a haemostatic drug that is reported to increase venous tone and to have a capillary stabilizing effect. NFZ is used in venous insufficiency of the lower limbs and diabetic retinopathy.[1]

Literature survey revealed few analytical methods for determination of NFZ in raw material or human plasma including; TLC/simultaneous reflectance-transmittance spectrophotometry, [2] cathodic and adsorptive stripping voltammetry [3] and polarography. [4, 5] Only one HPLC method [6] was developed in our laboratory for its determination in pure form and tablet dosage forms. To the best of our knowledge up till now nothing has been reported regarding the spectrophotometric determination of NFZ either in pure form or in pharmaceutical preparations. Spectrophotometry continues to be a very popular technique in drug analysis because of its simplicity, low cost and availability in most laboratories. This encourages us to explore new simple, sensitive and rapid spectrophotometric methods for the assay of NFZ in pure form and tablets.

The present study describes two simple, rapid, sensitive and reproducible assays for the determination of NFZ in dosage forms. The first method is based on measurement of the absorbance of NFZ in NaOH solution ($2x10^{-3}M$), where the drug undergoes bathochromic and hyperchromic shifts exhibiting maximum absorbance at 494 nm. The second method is based on formation of complexes between the drug and some metal ions including Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}.

The proposed methods are the first spectrophotometric methods for NFZ; they have the advantages of being simple, rapid and convenient suggesting the applicability in quality control laboratories.

EXPERIMENTAL

Apparatus

- A Shimadzu UV-Visible 1601 PC spectrophotometer (Kyoto, Japan) was used for spectrophotometric measurements (P/N 206-67001). The recording range was 0-1.2.

- A consort NV P-901 digital pH meter (Turnhout, Belgium) calibrated with standard buffers was used for checking the pH of the buffer solutions used.

Materials and pharmaceutical formulations

- Naftazone pure sample (certified to have a potency of 99.9%), batch # 0301030075, was kindly provided by Alkan Pharma Co., 6th of October City, Egypt.
- Mediaven® tablets (labeled to contain 5 and 10 mg of NFZ/tablet, batches # 012 and 016, respectively), products of Alkan Pharma Co., 6th of October city, Egypt, under license of Drossapharm-Switzerland, were purchased from local pharmacy.

Chemicals and Reagents

All chemicals were of analytical reagents grade, solvents were of spectroscopic grade and distilled water was used throughout the study.

- $CuSO_4.5H_2O$, $NiSO_4$, $CoCl_2$, $ZnSO_4$, NaOH, boric acid, acetic acid, sodium acetate trihydrate and methanol were obtained from ADWIC Co. (Cairo, Egypt).
- Aqueous solutions of $CuSO_4.5H_2O$ ($1x10^{-3}$M), $ZnSO_4$ ($1x10^{-3}$M) $NiSO_4$ ($2x10^{-3}$M) and $CoCl_2$ ($2x10^{-3}$M) were freshly prepared in distilled water.

- 0.2M acetate buffer solutions were prepared by mixing appropriate volumes of 0.2M sodium acetate trihydrate and 0.2M acetic acid. [7]
- 0.2M borate buffer solutions were prepared by mixing appropriate volumes of 0.2M boric acid and 0.2M NaOH. [7]

Standard Solution

Standard solution of NFZ containing 100µg/mL was prepared in methanol. This solution was further diluted with the same solvent to obtain the appropriate concentration range. The solution was found to be stable for at least one weak when kept in the refrigerator at 4°C.

General procedures

Construction of calibration graph for method I

Accurately measured volumes of NFZ standard solution were transferred into a series of 10ml volumetric flasks to obtain final concentration range of 0.5-10.0 µg mL^{-1}. The volumes were completed to the mark with NaOH ($2x10^{-3}$M) solution and solutions were mixed well. The absorbance was measured at 494 nm against blank solution prepared simultaneously. The calibration graph was obtained by plotting the absorbance value *versus* concentration of the drug (µg mL^{-1}); alternatively the corresponding regression equation was derived.

Construction of calibration graph for method II

Accurately measured volumes of NFZ standard solution were transferred into a series of 10 mL volumetric flasks. The specified volumes of borate buffer solution of optimum pH value were added to each flask followed by the appropriate volume of metal ion solution (Table 1). The solutions were completed to the mark with distilled water

and mixed well. The absorbance of each solution was measured at the specific λ_{max} (Table1) against a reagent blank prepared simultaneously. The calibration graphs were obtained by plotting the absorbance values *versus* concentrations of the drug (µg mL^{-1}); alternatively the corresponding regression equations were derived.

Analysis of tablets

Ten tablets were accurately weighed, finely pulverized and thoroughly mixed. An accurately weighed quantity of the powdered tablets equivalent to 5.0 mg of the drug was transferred into a 50 mL volumetric flask and the volume was made up to the mark with methanol. The contents of the flask were sonicated for 30 min then filtered. Different volumes of the filtrate were accurately transferred into a series of 10 mL volumetric flasks. The procedures for calibration graph for each method was followed. The nominal contents of tablets were calculated either from the previously plotted calibration graphs or using the corresponding regression equations.

Procedure for Job's continuous variation method

Equimolar concentrations ($1x10^{-3}M$) of both NFZ and the metal ions ($CuSO_4.5H_2O$, $NiSO_4$, $CoCl_2$ and $ZnSO_4$) were prepared. Accurately measured volumes of NFZ standard solution and metal ion solutions were transferred into series of 10mL volumetric flasks in such a way to keep the total number of moles constant but the mole ratio of reactants varies systematically (0.1:0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6, 0.5:0.5, 0.6:0.4, 0.7:0.3, 0.8:0.2 & 0.9:0.1; NFZ: M^{2+}, respectively). The absorbance of each solution was then measured at the specified wavelength against blank

solution prepared simultaneously. The absorbance values were plotted against the volume fraction of metal ion.

Results and Discussion

Method I

Spectral shifts are among the most useful diagnostic features in drug molecules that possess ionisable groups. [8] A marked bathochromic shift to longer wavelengths in alkaline solution is observed for most of the phenolic drugs, hydroxypyridines, ketones, benzodiazepines, pyridones and nitro–compounds. [8]

Investigation of NFZ molecular structure revealed presence of a ketonic group which may contribute to spectral shifts. The Spectral characteristics of NFZ were investigated in different solvents including distilled water, methanol, sodium hydroxide ($2x10^{-3}$M) and hydrochloric acid ($2x10^{-3}$M). In methanol NFZ exhibits four absorption maxima peaking at 226, 271, 318 and 443 nm. On the other hand; NFZ exhibits three absorption maxima at 271, 319, 444 nm in water and in hydrochloric acid. In alkaline medium a great bathochromic shift accompanied by hyperchromic effect was achieved and the drug exhibit absorption maximum at 494 nm (Fig. 2). Therefore, a new simple and rapid methodology is developed for determination of NFZ through measurement of its absorbance in sodium hydroxide solution.

Optimization of experimental conditions

The experimental conditions affecting the color development and its stability were carefully studied.

Effect of molar concentration of NaOH

The influence of the concentration of NaOH on the absorption intensity was investigated over the range of $5x10^{-5}$ to $4x10^{-3}$M. It was found that; increasing in the concentration of NaOH produces corresponding increase in the absorption value up to a concentration of $1x10^{-3}$M after which the absorbance remained constant. So that, $2x10^{-3}$M was chosen as the optimum concentration of the NaOH in the present study.

Effect of different surfactants

The influence of different surfactants including sodium dodecyl sulfate (SDS), cetrimide and carboxy methyl cellulose on the absorption value was investigated at concentration of 100µg ml^{-1} of each surfactant. It was found that; these surfactants didn't enhance the absorption value of the formed chromophore.

Effect of time on the stability of the formed chromophore

The effect of the time on the absorption value was investigated and it was found to be stable for at least two hrs.

Mechanism of spectral shift

Spectral shift observed in NFZ absorption spectrum in alkaline medium is probably attributed to keto-enol tautomerism. [9] A proposal of the reaction mechanism is postulated in Scheme 1. The enol tautomer of NFZ is aromatic but the keto tautomer is not. Aromaticity and extended conjugation of the enol form contribute to hyperchromic and bathochromic shifts. [9]

Method II

The formation of metal complexes with organic compounds has long been recognized. However, the binary complexes of NFZ with metal ions have not been studied yet except for the copper (II) complex of NFZ that was studied voltammetrically, [5] although they may be an area of interest. This is because they may affect the bioavailability of NFZ as certain metal ions are present in relatively appreciable concentration in biological fluids. Studying the structural formula NFZ showed that it is promising in forming metal chelates between the nitrogen atom of the side chain together with the vicinal oxygen atom of the quinonic function to form a six-membered ring chelation structure [5] (Scheme 2).

- ***Optimization of experimental conditions***

The complexing ability of NFZ with different metal ions was investigated in an attempt to develop sensitive and specific analytical procedures for its determination. NFZ was found to form stable metal complexes with Cu^{2+} (method IIa), Ni^{2+} (method IIb), Co^{2+} (method IIc) and Zn^{2+} (method IId). Different experimental conditions affecting the complexes formation and their stability were carefully studied and optimized.

Absorption spectra

The absorption spectrum of NFZ in borate buffer exhibits three absorption maxima at 270, 320 and 448 nm. Up on complexation with Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+} ions, new absorption maxima were obtained at 512, 506, 498 and 502 nm, respectively, as illustrated in Fig. 3. For comparison, the spectra of metal ions are also illustrated in Fig. 3.

Effect of pH

The reaction between NFZ and the studied metal ions was investigated over the pH range of 3.6-5.6 using 0.2 M acetate buffer and 6.5-9.0 using 0.2 M borate buffer. Maximum absorption value was obtained at pH 7.6 for $Cu^{2+}$and Ni^{2+}, 8.0 for $Co^{2+}$and 7.4 for Zn^{2+} (Fig. 4).

Effect of metal ion concentration

The effect of metal ion concentration on the formation of NFZ-metal ion complexes was investigated using increasing volumes of Cu^{2+}, Zn^{2+} solutions ($1x10^{-3}$M) and Co^{2+}, Ni^{2+} solutions ($2x10^{-3}$M). The optimum volumes of metal ions for maximum absorbance were found to be 2.0 mL in case of Cu^{2+}, Co^{2+} and Zn^{2+} and 3.0 mL for Ni^{2+}.

Effect of volume of buffer

The influence of the volume of borate buffer on the formation of NFZ-metal ion complexes was also studied. The absorbance of the formed complexes increased up to 1.5 ml of the buffer, after that no further increase was obtained. So that 2.0 mL of the buffer solution was used for maximum and constant absorbance value of NFZ-metal ion complexes.

Effect of different surfactants

The effect of different surfactants (sodium dodecyl sulfate, cetrimide and carboxy methyl cellulose) was investigated at a final concentration of ($100\mu g\ mL^{-1}$) by addition to the reaction mixture containing NFZ ($10\mu g\ mL^{-1}$) prior measuring the absorbance value of the formed complex at corresponding λ_{max}. All tested surfactants had a

negligible or even have negative effect on the absorbance of the formed complexes.

Effect of time on the formation and stability of the formed complex

The effect of time on the absorbance of drug-metal ion complexes was investigated. It was found that the complex formation is instantaneous and the formed complexes are stable for at least two hrs.

Validation of the proposed methods

The validity of the proposed methods was tested regarding linearity, range, limit of quantitation, limit of detection, accuracy, precision, robustness and specificity according to ICHQ2 (R1) recommendations. [10]

Linearity and range

The calibration graphs obtained by plotting the values of the absorbance *versus* the final concentrations (μg mL^{-1}) were found to be rectilinear over the concentration ranges cited in Table 2. The validity of the proposed methods were proven by statistical analysis of the data [10] using the standard deviation of the residuals ($S_{y/x}$), the standard deviation of the intercept (S_a) and standard deviation of the slope (S_b). The results are abridged in Table 2. The small values of the figures indicate low scattering of the points around the calibration line proving linearity of the proposed methods.

Limits of quantitation and limits of detection

The limits of quantitation (LOQ) were determined according to ICH Q2 (R1) recommendation [10] by establishing the lowest concentrations that can be measured below which the calibration graphs are nonlinear. The limits of detection (LOD) were determined also by evaluating the lowest

concentrations of the analytes that can be readily detected. LOQ and LOD were calculated according to the following equations [10]:

$LOQ=10S_a/b$

$LOD=3.3S_a/b$

Where: S_a is the standard deviation of the intercept of regression line, and b is the slope of the regression line.

The results are summarized in Table 2.

Accuracy

To test the validity of the proposed methods they were applied to the determination of pure sample of NFZ over the concentration ranges cited in Table 3. The results obtained were in good agreement with those obtained using the comparison HPLC method. [6] Statistical evaluation of the proposed method using Student *t*-test and the variance ratio *F*-test [11] revealed no significance differences between the performance of the proposed and comparison methods regarding the accuracy and precision, respectively (Table 3). The comparison method [6] depends on determination of NFZ in pure form and in tablets by HPLC method with UV detection at 270 nm, using a mobile phase consisting of methanol: 0.02M sodium dihydrogen phosphate (60:40 v/v), at pH 6.

Precision

Repeatability

The repeatability of proposed methods was tested by applying them for the determination of three concentrations of NFZ in pure form for three successive times. The results are presented in Table 4.

Intermediate precision

Intermediate precision was tested by repeated analysis of NFZ in pure form using the concentrations over a period of three successive days. The results are also summarized in Table 4.

Good values of percentage recoveries and small values of %SD and %Er (Table 4) indicate high precision of the proposed methods.

Robustness

The robustness of the proposed methods is demonstrated by the constancy of the absorbance with the deliberated minor changes in the experimental parameters. For method I, these include minor change in molar concentration NaOH ($2x10^{-3}\pm1x10^{-3}$M), while in case of method II, changes include: change in pH (optimum pH ± 0.2), change in the volume of buffer (2.0 $\pm$ 0.5 mL), and change in the volume of metal ion (optimum volume ± 0.2 mL). These minor changes that may take place during the experimental operation didn't affect the absorbance value obtained by the proposed methods.

Specificity

The specificity of the proposed methods was investigated by observing any interference encountered from the common tablet excipients. These excipients did not interfere with the proposed methods.

Pharmaceutical applications

The proposed methods were successfully applied to determine NFZ in its pharmaceutical preparations. The results obtained were statistically compared with those of a reported method [6] by Student's *t*-test and variance ratio *F*-test as shown in Table 5. The experimental values of *t*

and *F* did not exceed the theoretical values, [11] indicating lack of significant difference between the compared methods.

Molar ratio and mechanism of complexaion reactions

The stoichiometry of the reaction between NFZ and metal ions was studied adopting Job's method of continues variation [12] and limiting logarithmic method. [13] Fig. 5 shows the continuous variation plots for NFZ and the four metal ions. Maxima occur at volume ratios V_M/V_L of 0.41/0.59, 0.42/0.58, 0.40/0.60, 0.41/0.59 for Cu^{2+}, Ni^{2+}, $Co^{2+}$and Zn^{2+} /NFZ, respectively, thus suggesting 1:1 ratio.

(Where V_M: volume fraction of metal ion, V_L: volume fraction of drug).

The results obtained by limiting logarithmic method (12) agreed with these results. Plots of log absorbance *versus* log [metal] and log [NFZ] gave straight lines, slopes of which were 0.8486/0.9409, 0.7662/0.8770, 0.7878/0.9869, and 0.9203/1.1578 for methods IIa, IIb, IIc and IId, respectively (Fig. 6).

Hence, it is concluded that the reaction proceeds in the ratio of 1:1. A proposal of the reaction mechanism based on the obtained molar reactivity between NFZ and metal ions is presented in Scheme 2.

Stability constants of the formed complexes

The stability constants of the formed complexes are calculated according to the following equation [14]:

$$K_f = \frac{A/A_m}{[(1- A/A_m)^{n+1}]C^n n^n}$$

Where:

A and A_m = the observed maximum absorbance and the absorbance obtained from the extrapolation of the two lines obtained from Job's continuous variation curves, respectively (Fig. 5).

C = the molar concentration of the drug corresponding to the maximum absorbance.

n = the mole fraction of the metal.

(Since the molar ratio is 1:1 for NFZ: M^{2+}, respectively, therefore n=0.5).

Using the above equation K_f values were found to be 69.0, 27.0, 38.0 and 33.0 for method IIa, IIb, IIc and IId, respectively.

Also, the Gibbs free energy changes (ΔG) of the reaction were calculated according to the following equation [14]:

$\Delta G = -2.303\ RT \log K_f$

Where: R = gas constant (8.3 Joule.degree^{-1}.mole^{-1}) and T = absolute temperature (ºC + 273).

Using the above equation ΔG was found to be -1.0×10^4, -8.2×10^3, -9.0×10^3 and -8.6×10^3 Joule. mole $^{-1}$ for methods IIa, IIb, IIc and IId, respectively. The negative values of ΔG indicate that the reaction is spontaneous.

Critical comparison of the developed methods

The critical comparison of the two developed spectrophotometric methods for the determination of NFZ leads to the following advantages/disadvantages:

1. All methods are sufficiently sensitive and selective for the determination of the analyte in its pharmaceutical formulations.
2. Method I was found to be the most sensitive method. Meanwhile, method IId offers the widest determination range (2.0-19.0 μg mL^{-1})
3. Methods I, IIa and IId exhibit best linearity (r=0.9999)

4. Both methods I and II employ simple diluting solvent ($2X10^{-3}$ M NaOH and distilled water, respectively) providing cost effectiveness. However, distilled water is more eco-friendly.

5. For application in quality control laboratories, method I is considered superior to other methods owing to its rapidness, minimum steps, simplicity and sensitivity providing high rate of sample throughput.

Other techniques such TLC, [2] voltametry [3-5] and HPLC [6] may also give good results but, because of the low cost and ease of carrying out the spectrophotometric methods, the proposed procedures are likely to be very suitable for the quality control of NFZ in tablet dosage form.

Conclusion

The proposed spectrophotometric methods provided sensitive, specific and inexpensive analytical procedures for determination of NFZ either in pure form or in its tablet dosage forms without interference from common excipients. Moreover, the developed methods are less time-consuming and do not require elaborate treatments and expensive solvents associated with chromatographic method. These attributes, in addition to the satisfactory sensitivity and reproducibility as well as the convenience and simplicity, make the proposed methods suitable for routine analysis in quality control laboratories.

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Table (1): Assay parameters for determination of NFZ by Method II

	Method IIa	Method IIb	Method IIc	Method IId
Metal	$CuSO_4.5H_2O$	$NiSO_4$	$CoCl_2$	$ZnSO_4$
Concentration of metal ion	$1x10^{-3}$ M	2×10^{-3} M	2×10^{-3} M	$1x10^{-3}$ M
Volume of metal ion (mL)	2.0	3.0	2.0	2.0
Borate buffer pH	7.6	7.6	8.0	7.4
Borate buffer volume (mL)	2.0			
λ_{max} (nm)	512	506	498	502

Table (2): Analytical performance data for the proposed Methods

Parameter	Method I	Method IIa	Method IIb	Method IIc	Method IId
Concentration range ($\mu g\ mL^{-1}$)	0.5-10.0	2.0-14.0	2.0-10.0	2.0-14.0	2.0-19.0
Limit of detection LOD ($\mu g\ mL^{-1}$)	0.07	0.08	0.21	0.45	0.27
Limit of Quantitation LOQ ($\mu g\ mL^{-1}$)	0.22	0.24	0.62	1.37	0.81
Regression equation *$\mathbf{Y= a+ bX}$	Y= -0.0106+0.1070X	Y= 0.0239+0.0676X	Y= 0.0212+0.0653X	Y= 0.0029+0.0501X	Y=-0.0366+0.0506X
Correlation coefficient (r)	0.9999	0.9999	0.9998	0.9995	0.9999
Standard deviation of the residuals ($S_{y/x}$)	3.7×10^{-3}	1.8×10^{-3}	4.5×10^{-3}	8.0×10^{-3}	5.5×10^{-3}
Standard deviation of the intercept (S_a)	2.4×10^{-3}	1.6×10^{-3}	4.1×10^{-3}	6.9×10^{-3}	4.1×10^{-3}
Standard deviation of the slope (S_b)	4.0×10^{-3}	2.0×10^{-4}	6.0×10^{-4}	8.0×10^{-4}	4.0×10^{-4}
%RSD	0.91	0.24	0.99	1.39	1.11
%Error (%RSD/√n)	0.37	0.10	0.40	0.57	0.45
$A^{\%}$ ($dl.gm^{-1}.cm^{-1}$)	1.1×10^{3}	6.8×10^{2}	6.5×10^{2}	5.0×10^{2}	5.1×10^{2}
Molar absorpitivity ($l.mol.^{-1}cm^{-1}$)	2.3×10^{4}	1.5×10^{4}	1.4×10^{4}	1.1×10^{4}	1.1×10^{4}

*Y: absorbance; a: intercept; X: Concentration ($\mu g\ ml^{-1}$); b: slope.

Table (3): Application of the proposed and comparison methods for the determination of NFZ in pure form

Parameter	Method I		Method IIa		Method IIb		Method IIc		Method IId		Comparison Method [6]	
	Conc. Taken ($\mu g.mL^{-1}$)	% found[a]	Conc. Taken ($\mu g.mL^{-1}$)	% found[a]	Conc. Taken ($\mu g.mL^{-1}$)	% found[a]	Conc. Taken ($\mu g.mL^{-1}$)	% found[a]	Conc. Taken ($\mu g.mL^{-1}$)	% found[a]	Conc. Taken ($\mu g.mL^{-1}$)	% found[a]
	0.5	100.18	2.0	99.93	2.0	100.16	2.0	99.90	2.0	101.39	2.0	100.25
	1.0	100.56	4.0	99.89	3.0	98.42	4.0	101.35	4.0	98.62	4.0	99.43
	2.0	98.41	8.0	100.43	4.0	100.23	6.0	100.83	6.0	100.99	8.0	99.61
	5.0	101.05	11.0	99.80	7.0	100.37	10.0	98.02	10.0	100.12		
	8.0	99.60	13.0	100.26	8.0	101.23	12.0	98.82	16.0	99.01		
	10.0	100.06	14.0	99.97	10.0	99.20	14.0	101.38	19.0	100.64		
Mean± SD	99.98±0.91		100.05±0.24		99.94±0.98		100.05±1.40		100.13±1.11		99.76±0.43	
t	0.376(2.365)*		1.296(2.365)*		0.281(2.365)*		0.338(2.365)*		0.536(2.365)*			
F	4.455(19.296)*		3.123(5.786)*		5.211(19.296)*		10.470(19.296)*		6.592(19.296)*			

[a] Each result is the average of three separate determinations.

*Values between brackets are the tabulated t and F values, at $p = 0.05$. [10]

Table (4): Precision data of the proposed methods for the determination of NFZ in pure form

Method	*Intra-day precision*					*Inter-day precision*				
	Conc. Taken (µg/ml)	Conc. found (µg/ml)	Mean %found ± SD	%RSD	%Er	Conc. Taken (µg/ml)	Conc. found (µg/ml)	**Mean %found + SD**	%RSD	%Er
I	2.0	1.971	98.57±0.97	0.99	0.57	2.0	1.968	98.41±1.40	1.42	0.82
	4.0	4.030	100.76±0.36	0.35	0.20	4.0	4.012	100.30±1.50	1.50	0.87
	8.0	8.046	100.58±0.36	0.35	0.20	8.0	7.984	99.80±1.29	1.29	0.75
IIa	2.0	2.013	100.67±0.74	0.74	0.42	2.0	2.023	101.16±0.85	0.84	0.48
	4.0	3.966	99.15±0.65	0.65	0.38	4.0	3.932	98.29±1.40	1.43	0.82
	8.0	7.975	99.69±0.49	0.49	0.29	10.0	9.839	98.39±1.80	1.83	1.05
IIb	2.0	2.008	100.40±1.59	1.59	0.92	3.0	2.973	99.10±1.64	1.66	0.96
	4.0	4.024	100.61±1.38	1.37	0.79	5.0	4.949	98.97±1.87	1.89	1.09
	8.0	7.981	99.76±0.77	0.78	0.45	9.0	9.145	101.61±0.84	0.83	0.48
IIc	2.0	2.011	100.56±1.52	1.52	0.88	2.0	1.985	99.23±1.53	1.54	0.89
	4.0	4.040	101.01±1.04	1.03	0.59	4.0	4.034	100.86±1.79	1.78	1.03
	8.0	8.059	100.74±1.28	1.27	0.74	10.0	9.795	97.95±1.42	1.45	0.84
IId	2.0	2.021	101.06±1.14	1.13	0.65	3.0	3.042	101.40±1.37	1.35	0.78
	4.0	3.978	99.44±1.24	1.25	0.72	5.0	5.078	101.55±1.50	1.47	0.85
	8.0	8.029	100.36±1.40	1.40	0.81	9.0	9.057	100.63±1.55	1.54	0.89

Table (5): Application of the proposed and comparison methods to the determination of NFZ in tablet dosage forms

Pharmaceutical preparation	*Conc. Taken (µg/ml)*	*Method I*	*Method IIa*	*Method IIb*	*Method IIc*	*Method IId*	*Comparison Method* [6]
		% found[a]	% found[a]	% found[a]	% found[a]	% found[a]	% found[a]
Mediaven tablets (10mg NFZ/tablet)	2.0	97.01	100.67	100.92	101.90	99.41	98.10
	4.0	98.27	97.30	99.46	102.34	101.09	98.40
	8.0	97.85	97.84	101.61	99.08	102.42	100.70
Mean± SD		97.71±0.64	98.06±1.81	100.66±1.1	101.11±1.77	100.97±1.51	99.10±1.4
t		1.506(2.776)*	0.349(2.776)*	1.539(2.776)*	1.557(2.776)*	1.593(2.776)*	
F		4.916(19.00)*	1.619 (19.00)*	1.679(19.00)*	1.546(19.00)*	1.124(19.00)*	
Mediaven tablets (5mg NFZ/tablet)	2.0	98.40	97.71	99.39	101.90	102.37	101.60
	4.0	98.97	100.63	100.61	101.35	99.60	100.20
	8.0	98.08	97.28	100.84	98.58	101.68	99.30
Mean ± SD		98.48±0.45	98. 54±1.82	100.28±0.78	100.61±1.78	101.22±1.44	100.40±1.10
t		2.623(2.776)*	1.465(2.776)*	0.107(2.776)*	0.198(2.776)*	0.796 (2.776)*	
F		12.717(19.00)*	2.473(19.00)*	2.212(19.00)*	2.357(19.00)*	1.548(19.00)*	

[a] Each result is the average of three separate determinations

*Values between brackets are the tabulated t and F values, at $P = 0.05$. [10]

List of Figures:

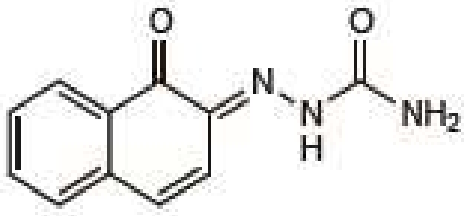


Figure 1. Structural formula of NFZ.

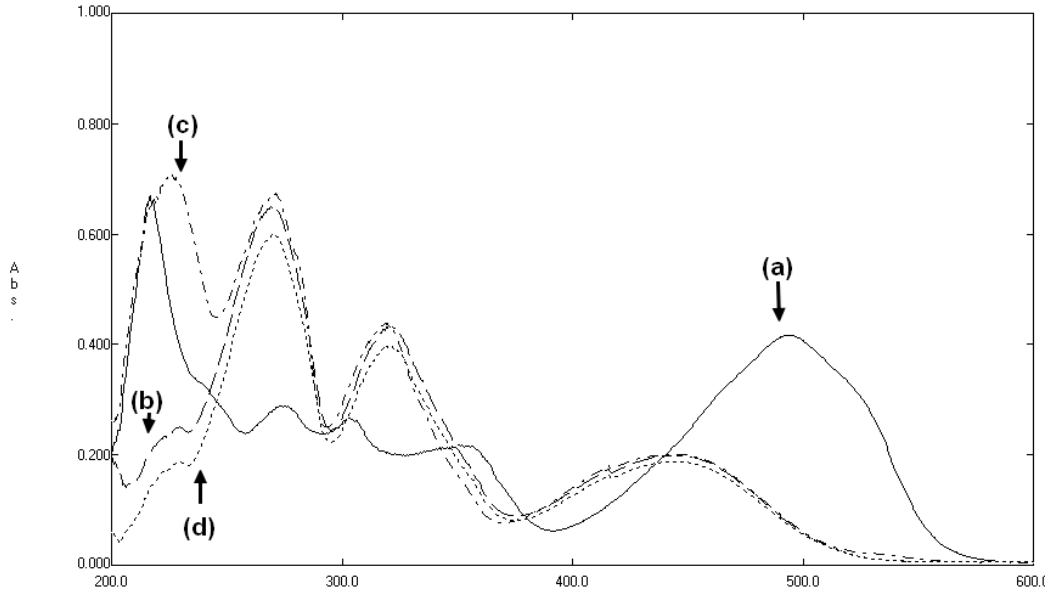


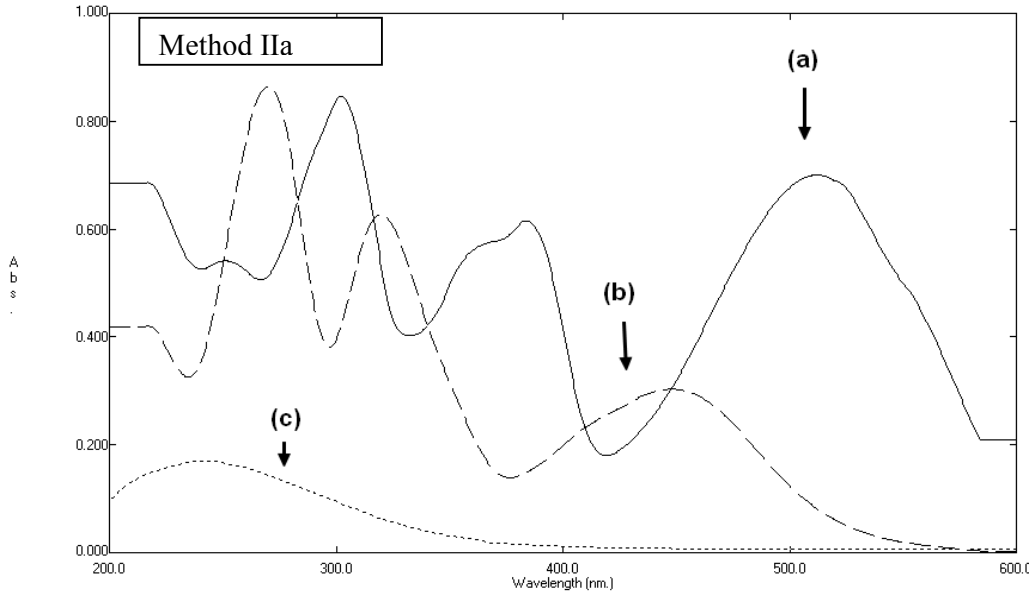
Figure 2. Absorption spectra of NFZ (5µg mL^{-1}) in:

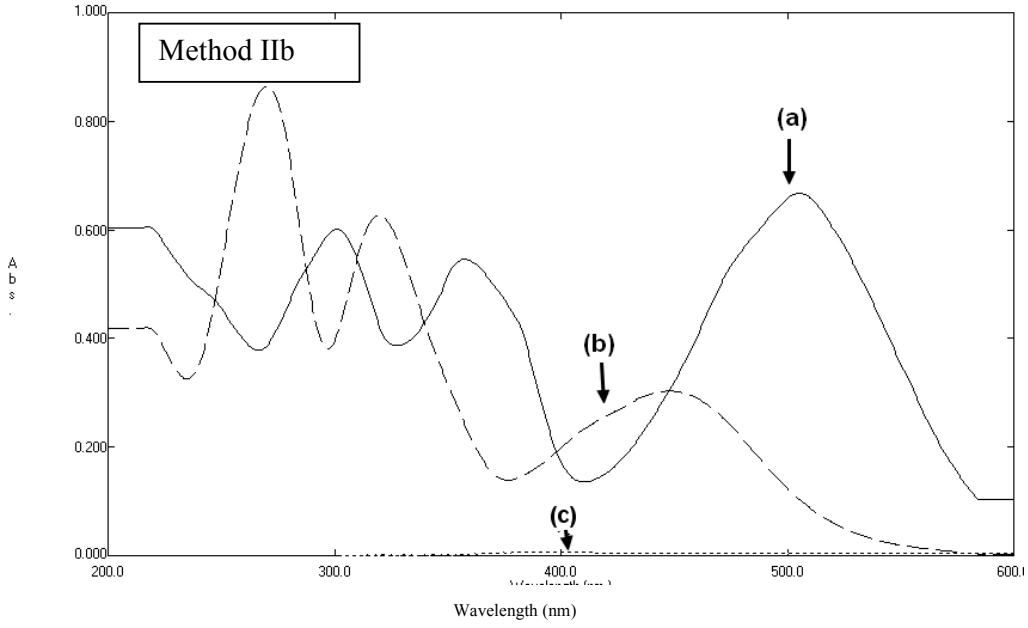
(a) NaOH ($2x10^{-3}$M)

(b) HCl ($2x10^{-3}$M)

(c) Methanol

(d) Distilled water.





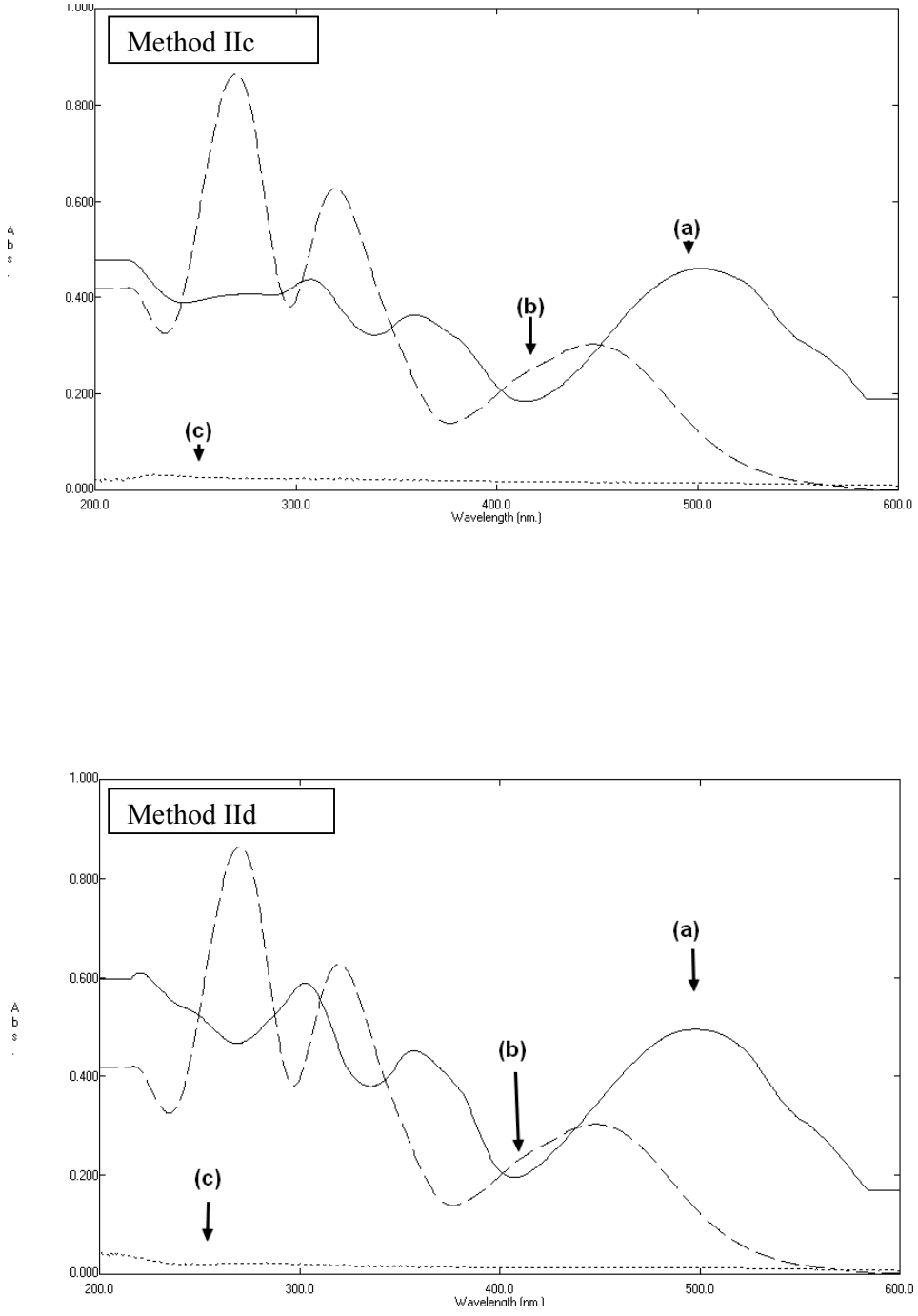


Figure 3. Absorption spectra of:

(d) NFZ (10µg mL^{-1}) complex with metal ion in borate buffer of optimum pH.

(e) NFZ (10µg mL^{-1}) in borate buffer of optimum pH.

(f) Metal ion in borate buffer of optimum pH.

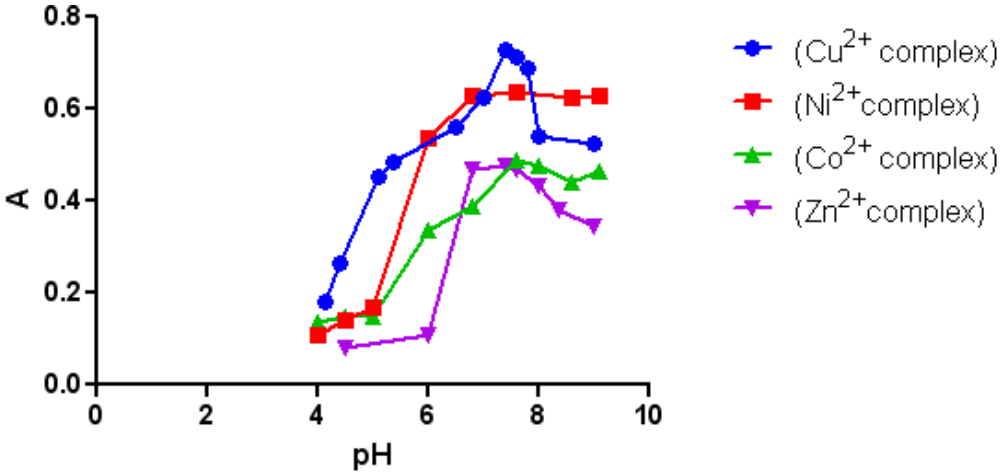


Figure 4. Effect of pH on absorption value of NFZ (10µg mL^{-1}) complex with $CuSO4.5H_2O$ (2mL of $1x10^{-3}$M), $NiSO_4$ (3mL of $2x10^{-3}$M) and $CoCl_2$ (2mL of $2x10^{-3}$M), and $ZnSO_4$ (2mL of $1x10^{-3}$M).

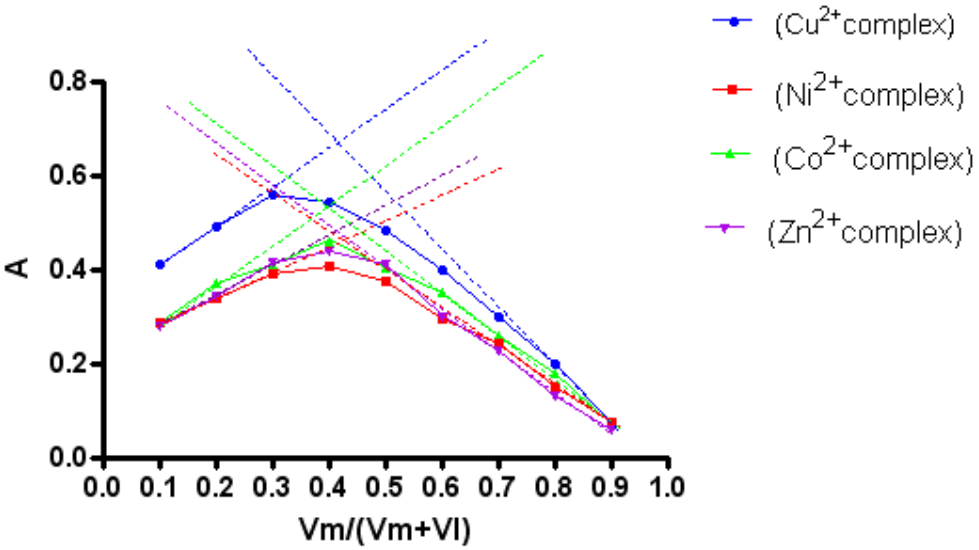
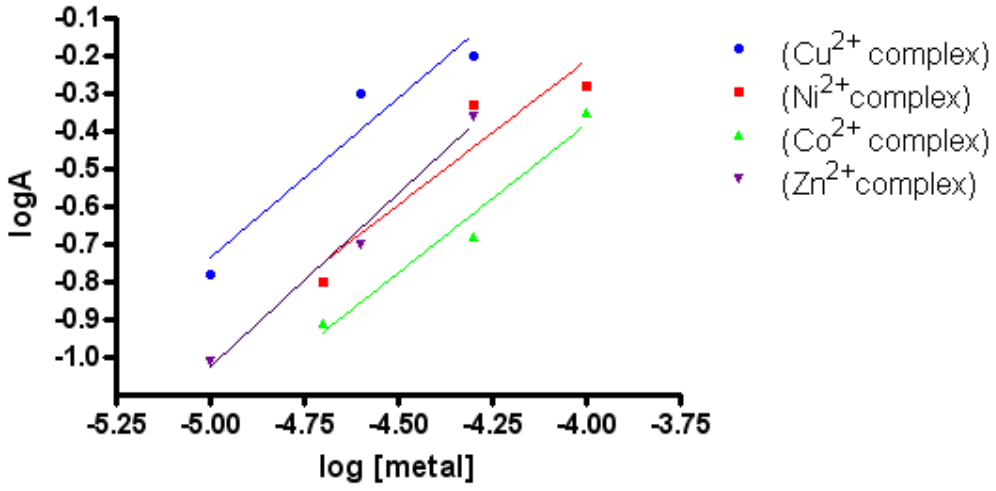


Figure 5. Continuous variation plot for NFZ ($1x10^{-3}$M) with Cu^{+2}, Ni^{+2}, Co^{+2} and Zn^{+2} ($1x10^{-3}$M)

(A)



(B)

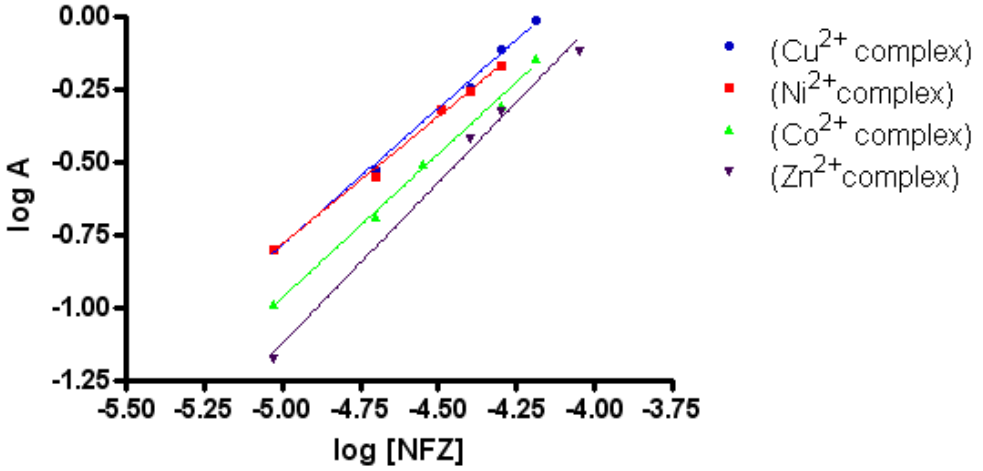
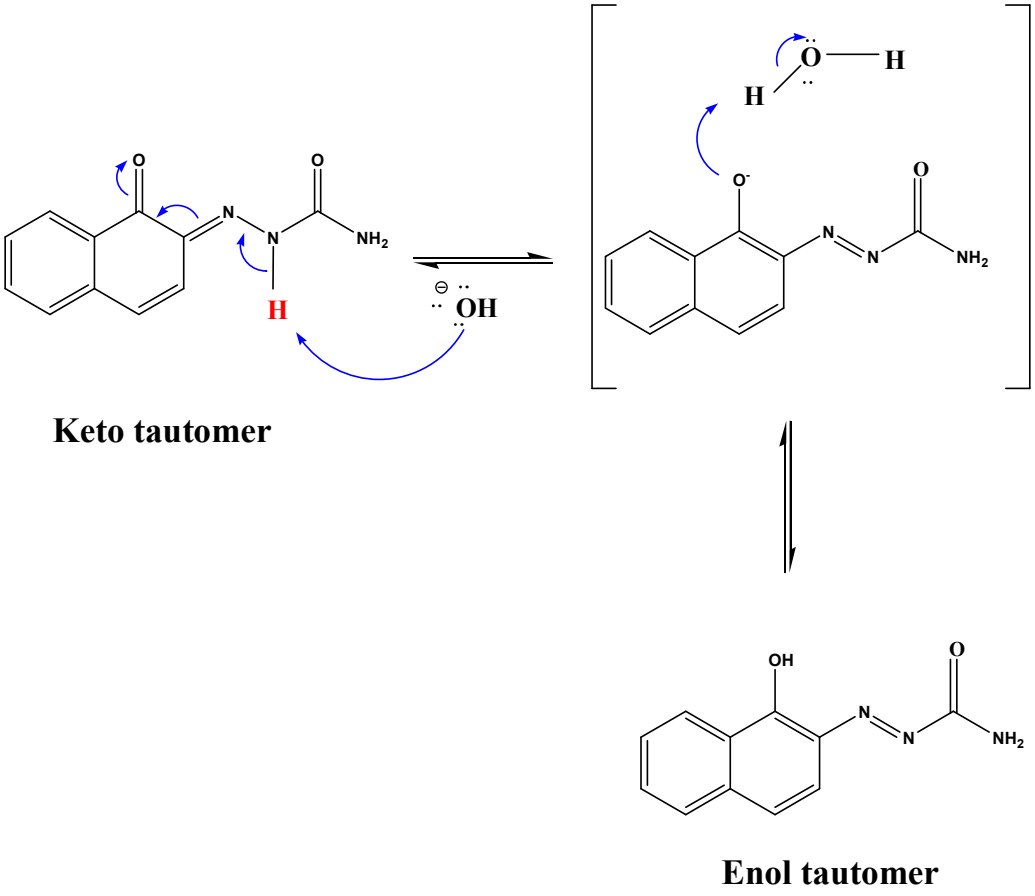
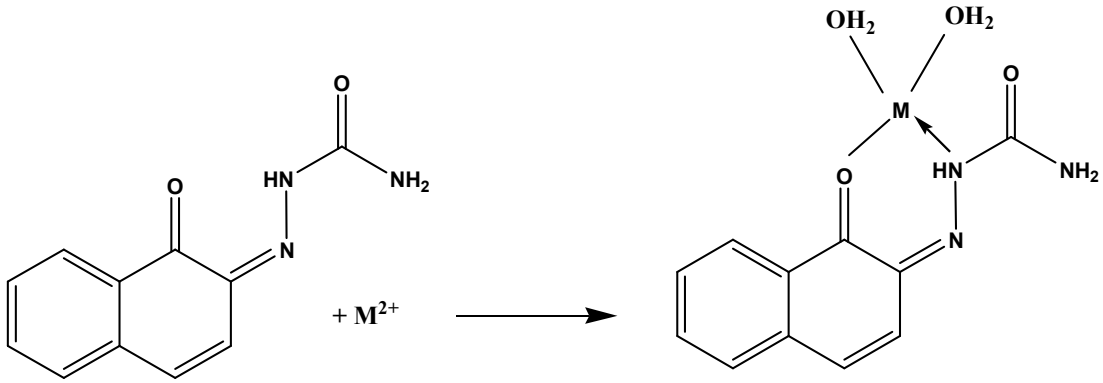


Figure 6. Limiting logarithmic plots for the molar reactivity of NFZ with the four investigated metals: (A) log A *vs.* log [metal] with [NFZ] kept constant; (B) log A *vs.* log [NFZ] with [metal] kept constant.



Scheme 1: Proposal for the keto-enol tautomerism of NFZ in alkaline medium.



NFZ NFZ-M^{2+}complex

M^{2+} = Cu^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+}

Scheme 2: Proposal for the complexation reaction between metal ions and NFZ.