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Indirect Spectrophotometric Determination of Nitrazepam by Charge Transfer Complex Formation Reaction Using *p-Bromanil* as π-Acceptor

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Abstract:

A sensitive spectrophotometric method for the determination of nitrazepam in pure as well as in dosage form is described. The method is based on the reaction of reduced nitrazepam (RNZ) with *p-bromanil* in the presence of borate buffer solution of pH9 to form a pink color charge transfer(CT) complex of maximum absorption at 340 nm. Under the optimized reaction conditions, Beer's law correlating the absorbance with nitrazepam concentration was obeyed in the range of 0.8-9.6 µg ml^{-1}. The molar absorptivity was 1.977×10^4 L $mol^{-1}cm^{-1}$. The limits of detection was 0.093µg ml^{-1}. The accuracy and precision of the method were satisfactory; the average recovery was 102% and values of relative standard deviations better than 1.5 %. The stoichiometry of the reaction was studied, and the reaction mechanism was postulated. The proposed CT complex formation method was successfully applied to the determination of nitrazepam in its pharmaceutical tablet with good accuracy and precision. The results obtained by the proposed method are compared with those obtained by the official method.

Keywords: Spectrophotometry; Charge transfer complex; nitrazepam; *p-bromanil*.

التقدير الطيفي غير المباشر للنايترازيبام بواسطة تفاعلات تكوين معقد الشحنة المنتقلة باستخدام الكاشف بارا- برومانيل

صبحي محسن جار الله المتيوتي

قسم الكيمياء, كلية التربية للعلوم الصرفة, جامعة الموصل, الموصل, العراق

الخلاصة

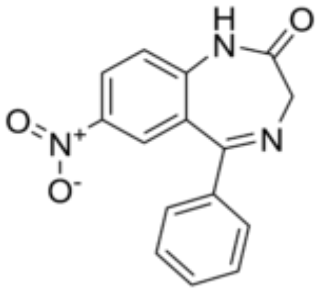
تم وصف طريقة طيفية حساسة لتقدير النايترازيبام بهيئته النقية وفي مستحضراته الصيدلانية كأقراص. تعتمد الطريقة على التفاعل بين النايترازيبام المختزل والكاشف بارا- برومانيل بوجود محلول البورات المنظم عند دالة حامضية 9 مكونا معقد الشحنة المنتقلة ذي لون ارجواني يمتلك طيفا امتصاصيا له أقصى امتصاص عند 340 نانوميتر. أمكن تطبيق قانون بير ضمن مدى التراكيز 0.8-9.6 مايكروغرام/مللتر في حين كانت الامتصاصية المولارية 1.977×10^4 لتر.مول$^{-1}$سم$^{-1}$. بحد كشف 0.093 مايكروغرام/مللتر ومعدل نسبة الاسترجاع 102% في حين كان ألانحراف القياسي النسبي 1.5%. كذلك تم دراسة طبيعة معقد

الشـحنة المنتقلة وميكانيكية التفاعل. طبقت الطريقة بنجاح في تقدير النايترازيبام في مسـتحضـراته الصـيدلانية بشـكل أقراص بدقة وتوافق جيدين. كما تم مقارنة الطريقة المقترحة مع الطريقة القياسية في الدستور البريطاني.

الكلمات المفتاحية : المطياف الضوئي ، معقدات الشحنة المنتقلة ، نايترازيبام ، برومانيل

Introduction

Nitrazepam, (NZ) chemically known as 1,3-dihydro-1-nitro-2-oxo-5-phenyl 2H-1,4-benzodiazepine-2-one (Figure 1), is a hypnotic agent that belongs to the benzodiazepine class, Nitrazepam used in the treatment of insomnia which has sedative and motor impairing properties [1], as well as anxiolytic , amnestic , anticonvulsant and skeletal muscle relaxant properties , and it has been used in the treatment of stress related disorders [2] .



$C_{15}H_{11}N_3O_3$ 281.3 g/mol

Figure 1: Structure of nitrazepam

Official spectrophotometric method for its determination in tablet has been reported in British Pharmacopoeia[1]. Various other spectrophotometric methods have been reported based on reduction of nitrazepam by using Zn/HCl and measuring the difference in their absorbance before and after reduction [3], coupling of the diazotized NZ with different reagents such as resorcinol [4] or N-(1-naphthyl) ethylene diamine) dihydrochloride [5], oxidation by ferric sulfate and coupling with pyrocatechol [6] and use of metol as π acceptor forming charge transfer complex with reduced NZ as n-donor [7]. Methods based on other techniques such as HPLC[8,9], polarography [10], complexometry [11], GC with electron capture [12] have been used for the determination of NZ in biological fluids and pharmaceutical samples.

The present investigation method is development of sensitive spectrophotometric procedure for the estimation of NZ in both pure and in pharmaceutical preparation as tablet based on the reduction of NZ by Zn powder / HCl and interaction as *n*-donor with *p-bromanil* as π-acceptor in aqueous solution to form charge transfer complex.

Experimental

Apparatus

Shimadzu UV-180 PC UV-Visible double-beam spectrophotometer equipped with a 1.0-cm path length quartz cell**,** Philips PW (9421) pH-meter with a combined glass electrode was used for pH measurements**.** Heating the solutions was carried out on water bath of frost instruments, LTD. All calculations in the computing process were performed in Microsoft Excel Windows

Chemicals

All reagents were of analytical-reagent grade which were provided by BDH and Fluka companies.

***Working standard solution of RNZ*:** Accurately 50 mg of NZ was weighed into a separate 100 ml beaker and dissolved NZ in 20ml absolute ethanol with heating. To this solution 5 ml of conc. hydrochloric acid and 1g of zinc dust were added and shaken thoroughly for 15 min and then diluted up to the mark with water in a 100 ml standard flasks (500 μg ml^{-1}), and filter through Whatmann no.41 filter paper , and then 20 ml from this solution was neutralize with 20% sodium carbonate solution to pH 6 and filtered using sintered glass crucible No.2 and diluted with water in 100 ml calibrated flask to obtain100 μg ml^{-1}.

***p-Bromanil (1×10^{-3}M)*:** Prepared by dissolving 0.0424 g of *p-bromanil* in 100 ml absolute ethanol.

***Borate buffer solution (0.05M pH 9)*:** Prepared by dissolving 1.904 g of disodium tetraborate in 100 ml distilled water and the pH was adjusted by pH measurement.

Sodium hydroxide (1x10^{-2}M) was prepared by dissolving 0.1 g in 250 ml of distilled water.

Recommended procedure

An aliquot of a standard solution containing 0.8-9.6 μg ml^{-1} of RNZ was transferred into a series of 25 ml calibrated flasks. A volume of 1 ml borate buffer solution was added to each flask followed by addition of 2.5 ml *p-bromanil* solution. The contents of the reaction mixture were diluted to the mark with distilled water and kept in a water bath adjusted at 35 °C for 40 min,. The absorbance of the colored complex was measured at 340nm against the reagent blank prepared in similar manner.

Procedure for NZ assay in tablets

Ten tablets (each tablet containing 5mg) were weighted, pulverized. A quantity of the powder equivalent to 25 mg of NZ was weighed accurately and dissolved in 10 ml absolute ethanol into a separate 50ml calibrated flask. Then, 5 ml of conc. hydrochloric acid and 1g of zinc dust were added and shaken thoroughly for about 30 min. The volume was diluted to the mark with distilled water, mixed well and filtered using a Whatman No.41 filter paper and 20 ml from this solution was neutralized with 20% sodium carbonate solution to pH 6 and filtered using sintered glass crucible No.2 and diluted with water in 100 ml calibrated flask to obtain100 μg ml^{-1}.

Results and discussion

Spectral characteristics

The proposed method involves the reduction of NZ and reaction with *p-bromanil* reagent in the presence of borate buffer solution of pH9 to form a pink colored charge transfer complex having maximum absorption at 340nm. This wavelength was used for all subsequent measurements. The absorption spectra of the reaction product are shown in Figure 2. The corresponding reagent blank have low absorbance at this wavelength .Where as the reagent blank has a maximum absorption at 317 nm versus distilled water.

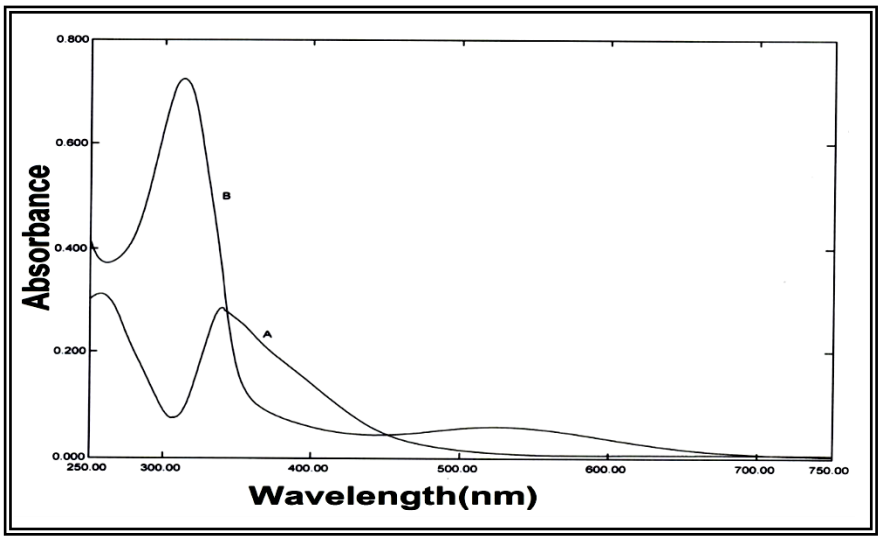


Figure 2: Absorption spectra of (A) RNZ (4 µg ml^{-1}) complex with *p-bromanil* reagent (1×10^{-2}M) against reagent blank and (B) reagent blank against distilled water under the optimum conditions

The optimum conditions for the color development of the *p-bromanil*-RNZ CT complex were established by for different parameters. The following experiments were conducted for this purpose and conditions so obtained were incorporated in general procedure.

Effect of solvents

Different solvents such as water, methanol, ethanol, acetonitrile, and acetone as medium for the reaction have been examined in order to achieve maximum sensitivity and complex stability. It was found that on using water as solvent for the drug, ethanol as solvent for *p-bromanil* and dilution with water gave maximum color intensity. So; this system of solvents have been recommended in this method.

Effect of pH, buffer solutions and bases

The effect of pH was studied in the range 6-10 using NaOH solution. The maximum absorbance value was obtained with pH9 in the presence of 0.5 ml of 0.01M NaOH (Figure 3). Therefore different buffers of pH9 were prepared by using phosphate, borate and carbonate buffers to investigate the sensitivity of the complex. As shown in Figure 4, it was found that borate buffer gave high absorbance value. So its selected in the subsequent experiments. However; the effect of borate buffer amount was studied and it was found that 1.0 ml gave maximum color intensity and chosen as the optimum amount, and beyond this amount, the absorbance would be decreased, (Figure 5).

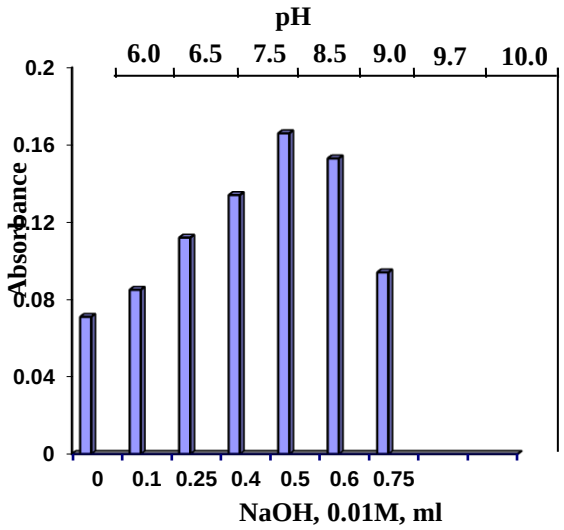


Figure 3: Effect of pH on the absorption of 4µgml^{-1} RNZ

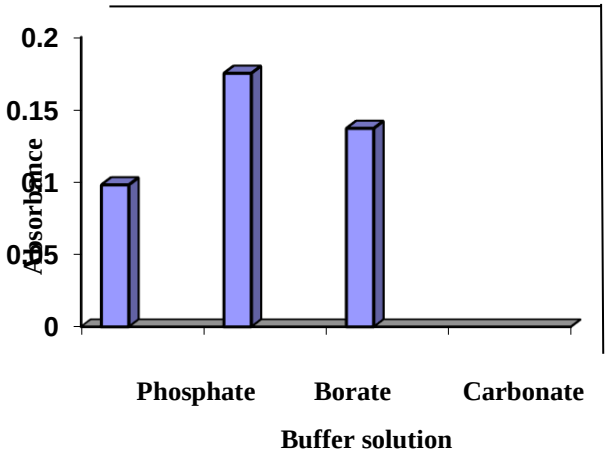


Figure 4: Effect of buffer solution on the absorption of 4µgml^{-1} RNZ

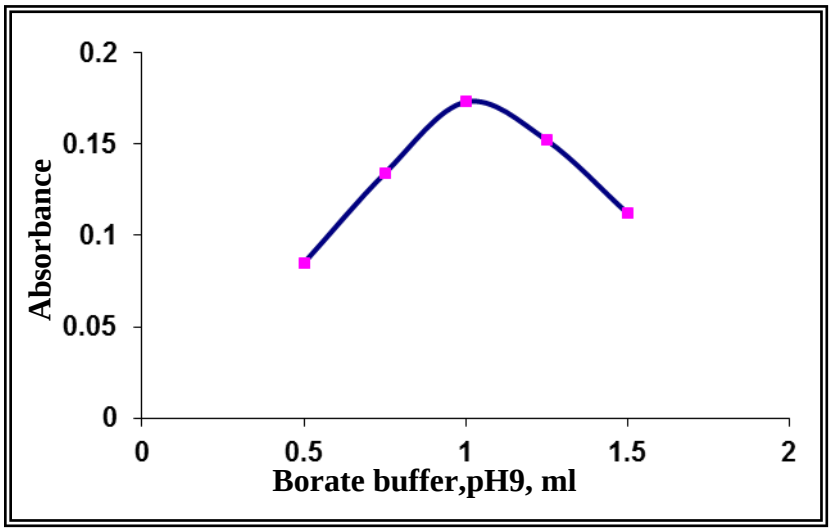


Figure 5: Effect of borate buffer solution amount on the absorption of 4µgml^{-1} RNZ

Effect of *p-bromanil* 1×10^{-2} concentration

The effect of changing the *p-bromanil* concentration on the absorbance of solution containing a fixed amount of the drug was studied. As shown in Figure 6, it was found that the absorbance increases with increasing *p-bromanil* concentration and reached maximum on using 2.5 ml which is selected in the subsequent work.

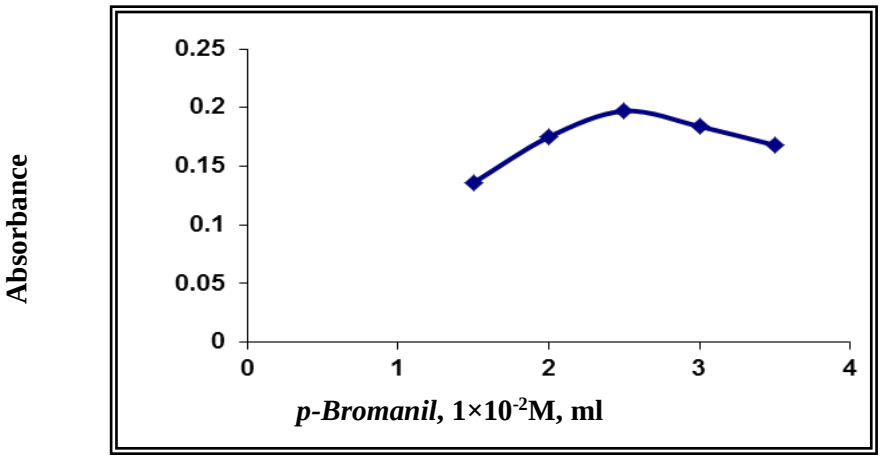


Figure 6: Effect of *p-bromanil* reagent concentration on the absorption of 4µgml^{-1} RNZ

Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature(20˚C) and in thermostatically controlled water-bath at different temperatures up to 45˚C. The absorbance was measured at 5 and 10 minutes intervals against reagent blank treated similarly. It was found that the color complex showed maximum absorbance after 40 min at 35°C and was stable for a further 60 min, (Figure 7). Above 35°C, the absorbance decreases, indicating dissociation. Hence, 40 min at 35°C are recommended for the proposed method.

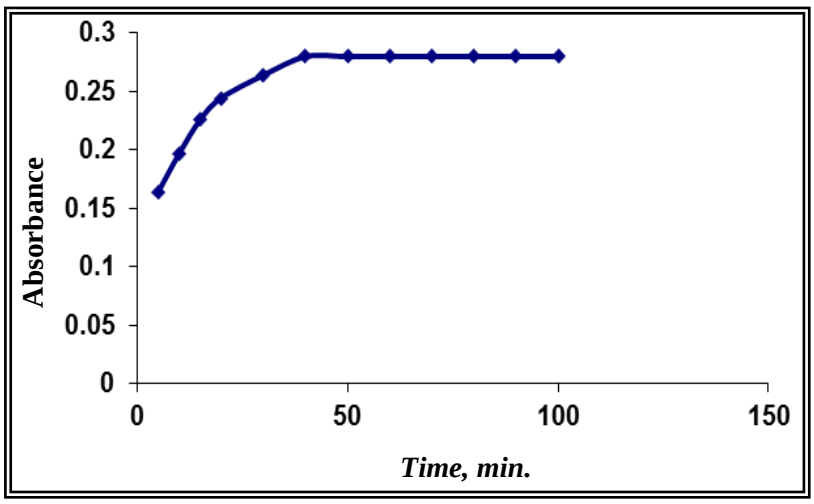


Figure 7: Effect of temperature on the absorbance of 4 µgml^{-1} RNZ with *p-bromanil* at 35°C

Effect of order of addition

To obtain optimum results the order of addition of reagents should be followed as given under the general procedure, otherwise a loss in color intensity was observed.

Quantification

In order to investigate the range in which the colored product adhere to Beer's law, the absorbance of the complex was measured at 340nm value after developing the color by following the suggested procedure for a series of solutions containing increasing amounts of RNZ (Figure8). The Beer's law limits and molar absorptivity values were evaluated and given in Table 1, indication that

the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for drug determined by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of six replicates of each three different concentrations for RNZ indicated that the method is precise and accurate. Limit of detection (LOD) is in the accepted range below the lower limit of Beer's law range.

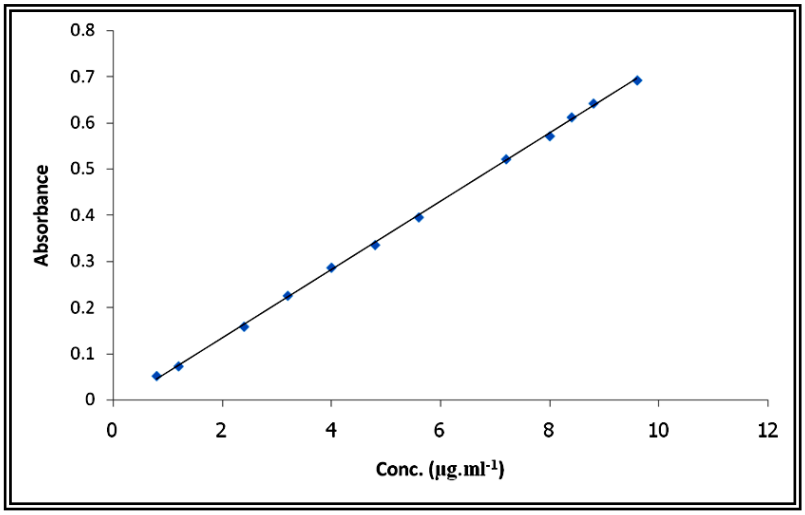


Figure8: Calibration graph for determination of nitrazepam

Table 1: Summary of optical characteristics and statistical data for the proposed method

Parameter values of method	
Beer's law limits (µg ml^{-1})	0.8 – 9.6
Molar absorptivity ($l.mol^{-1}. cm^{-1}$)	1.977×10^4
LOD (µg.ml^{-1})	0.093
LOQ (µg.ml^{-1})	0.309
Average recovery (%)**	102
Correlation coefficient	0.9995
Regression equation (*Y*) *	
Slope, *a*	0.073
Intercept, *b*	- 0.013
RSD**	≤1.5

* $Y = a X + b$, where X is the concentration of NZ in µg ml^{-1}.
** Average of six determinations.

Interference

The extent of interference by some excipients usually present in pharmaceutical preparations were studied by measuring the absorbance of solutions containing 4 µgml^{-1} of RNZ and various amounts of diverse species in a final volume of 25 ml. It was found that the studied excipients did not interfere seriously (Table 2). Slight positive interference was observed in the presence of large excess of excipients. However; an error of 5.0 % in the readings was considered tolerable. The results are given in table 2.

Table 2: Effect of excipients for assay of the studied drug .

Foreign Compound	Recovery of 4 µg ml^{-1} of NZ per µg/ml Foreign added				
	20	60	100	140	200
Glucose	99.43	98.56	101.23	100.85	103.35
Lactose	99.46	99.22	100.57	99.84	101.35
Arabic Gum	100.25	100.78	101.35	101.28	102.65
Sodium chloride	99.85	100.25	100.36	101.25	101.68
Starch	101.55	101.89	99.35	100.85	102.25

Stoichiometry and Stability constant

The molar ratio of the complex formed between the RNZ and *p-bromanil* reagent was investigated by applying the continuous variation (Job's) and mole ratio methods[13]. The results indicated that the complex was formed in the ratio of 1:1 for RNZ to the reagent (Figures 9). This finding supports that the n-π CT complex is formed through the amino group which produced from the reduction of the nitrazepam drug.

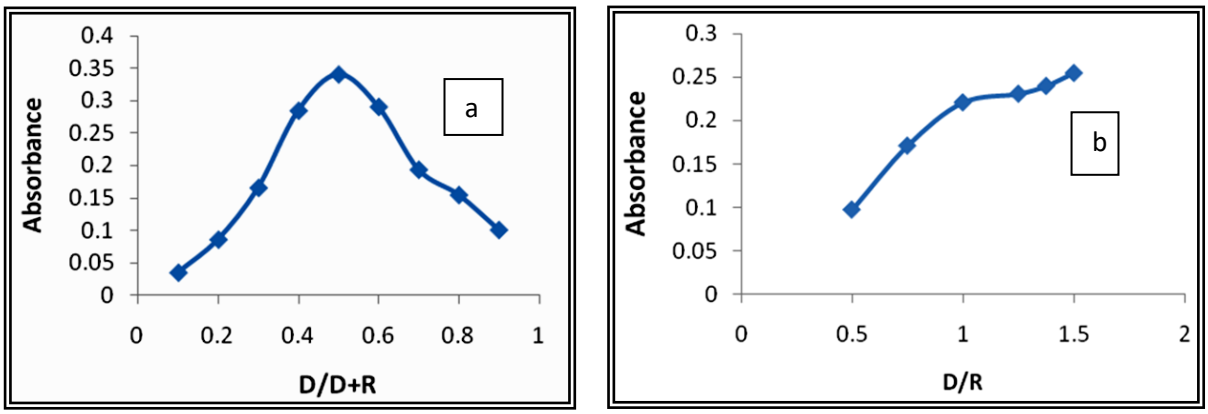


Figure 9: Continuous variation (a) and mole ratio (b) plots for complex of RNZ(3.98×10^{-4}M) and *p-bromanil* (3.98×10^{-4}M) under the optimu conditions.

The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the drug and *p-bromanil* (As) to one containing an excessive amount of p-*bromanil* reagent (Am). The average conditional stability constant of the complex was calculated, according to the 1:1 ratio, by the following equation:

$$Kc= (1-\alpha) / \alpha^2 C$$

$$\alpha= (Am-As) /Am$$

where Kc is the stability constant ($l.mol^{-1}$), α the dissociation degree and C the concentration of the product which is equal to the concentration of drug. However the average of stability constant for three different concentration was found 3.3×10^3 $l.mol^{-1}$ indicating high stability.

Reaction mechanism

The nature of the reaction between RNZ as n-donor with *p-bromanil* as π-acceptor in aqueous solution is not clearly understood. However, in the present work it was observed that the complex is formed in aqueous medium in the ratio 1:1 after addition of RNZ, which including primary amino group, to *p-bromanil* reagent at pH9 and new absorption spectrum appeared at 340 nm, which is not shown by either of the components present in solution. Also, it was found that *p-bromanil* reagent has an absorption spectrum at λ_{max} at 317 nm in the presence of pH9 which may be attributed to the formation of tribromohydroxy-p-benzoquinone [14] which is considered as a real complexing agent. However; complete electron transfer from the donor to the acceptor moiety took place with the formation of intensely colored radical ion with high molar absorptivity. On this basis, a suggested chemical reaction has been proposed in Illustration 1.

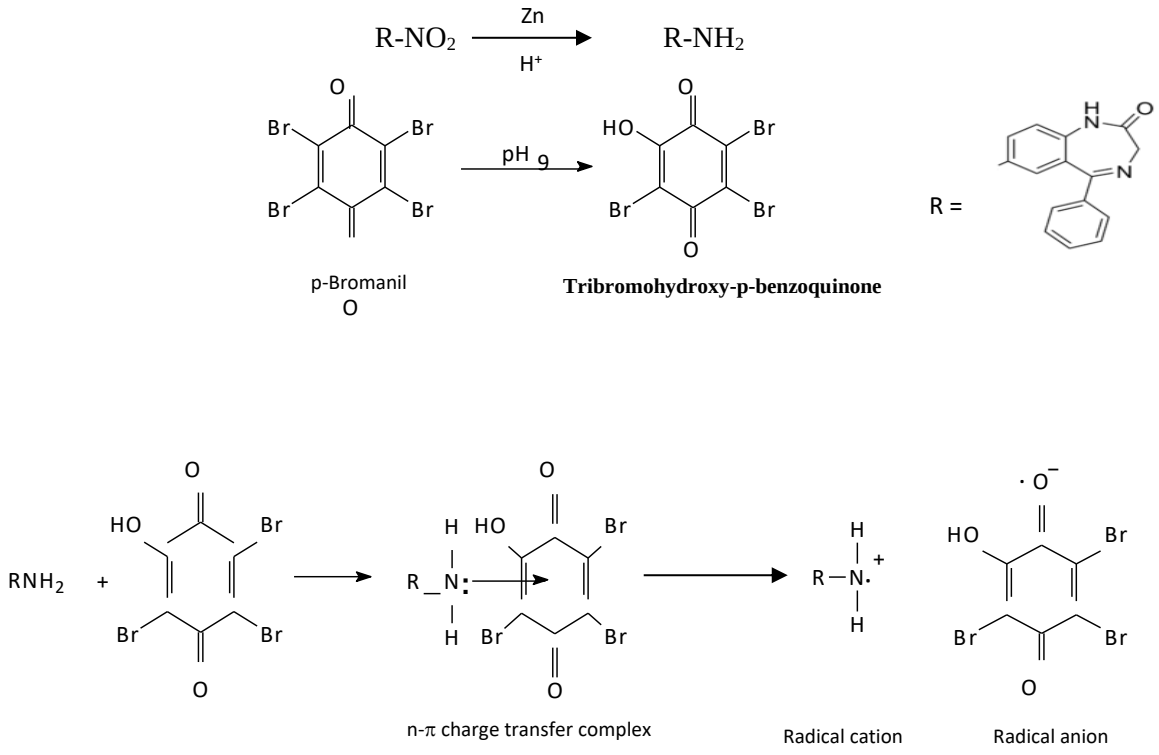


Illustration1:. Probable mechanism for RNZ- *p-bromanil* product formed.

Analytical application

The proposed method was successfully applied to determine nitrazepam in its pharmaceutical preparation as tablet. The obtained results were compared statistically by student′s t-test and F-test with the official method [1] at 95% confidence level with five degrees of freedom, as cited in Table 3. The results showed that the t- test and F-test were less than the tabulated value (t= 2.57, F= 5.05) [11], indicating that there was no significant difference between the proposed method and official method.

Table 3: Assay of nitrazepan in tablet using proposed method and comparison with the official method

Procedure applied	Pharmaceutical preparation	Drug amount present ($\mu g\ ml^{-1}$)	Recovery[a] (%)	Drug content found (mg)	Average recovery (mg)	Certified value (mg)	RSD[a]
Proposed method	Mogadon[c] Tablet	2 4 6	100.2 99 101	5.1 4.95 5.05	5.03 (1.47,2.94)[b]	5 mg	1.24
British Pharmacopoeia	Mogadon Tablet	5 (mg)	99 101 98.4	4.95 5.05 4.92	4.98	5 mg	1.38

a=Average of three determinations.
b=Figures in parenthesis are the calculated values for *t*, and *F* respectively.
c=Marketed by Roche Co. Switzerland

Conclusion

The proposed spectrophotometric method is sensitive (trace amounts can be determined), accurate (average recovery 102%), precise (RSD $\leq$ 1.5) and simple since it does not need neither organic medium nor solvent extraction step. The proposed method was applied successfully for the assay of the pharmaceutical preparation for the studied drug as tablet .

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