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Spectrophotometric Determination of Nitrazepam by Coupling of Diazotized Reduced Nitrazepam with N-(1-naphthyl)ethyl-enediamine Dihydrochloride

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ABSTRACT

A simple, rapid, accurate and precise spectrophotometric method is proposed for the determination of nitrazepam in pure form. The method is based on the reduction of nitrazepam by zinc powder in concentrated hydrochloric acid medium followed by diazotization of reduced nitrazepam and coupling with N-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) to give a pink coloured product which is stable, water-soluble and has a maximum absorption at 564 nm with a molar absorptivity of 1.633×10^3 $l.mol^{-1}.cm^{-1}$. Beer's law is obeyed in the concentration range of 10 to 500 µg of nitrazepam in a final volume of 25 ml.

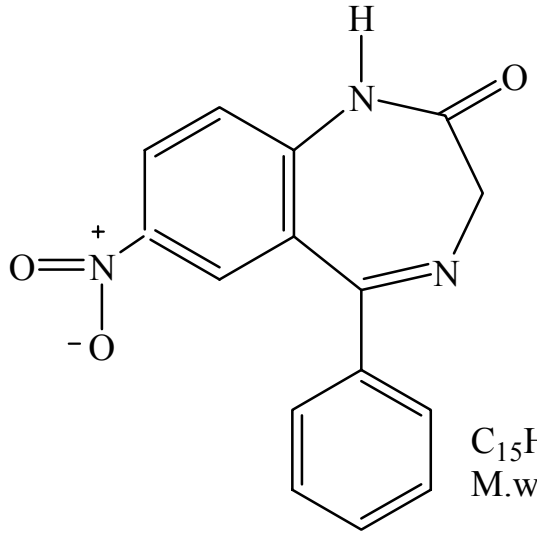
التقدير الطيفي للنايترازيبام بتفاعل الاقتران الآزوتي للنايترازيبام المختزل مع الكاشف ن-(1-نفثيل) اثلين ثنائي الامين ثنائي الهيدروكلوريد

الملخص

تم اقتراح طريقة طيفية بسيطة، سريعة، دقيقة ومتوافقة لتقدير النايترازيبام بـشكله النقـي اعتمـدت الطريقة على اختزال النايترازيبام بواسطة مسحوق الخارصين في وسط حامض الهيدروكلوريك ثم ازوتـة ناتج الاختزال يتبعها اقتران الناتج المختزل المؤزوت مـع الكاشـف ن-ثنـائي امـين (1-نفثيـل) ثنـائي الهيدروكلوريد ليعطي معقد ذا لون وردي مستقر وذائب بالمحلول المائي، تقاس شدة امتصاصه عند الطـول الموجي 564 نانوميتر وكانت قيمة الامتصاصية المولارية 1.633×10^3 لتر.مول$^{-1}$.سم$^{-1}$. وانطبق قـانون بير في مدى التركيز بين 10 و500 مايكروغرام من النايترازيبام في حجم نهائي 25 مللتر.

INTRODUCTION

Six benzodiazepines play prominent roles in the therapy of epilepsy. Although benzodiazepines are chemically quite similar with a suitable structural, there are differences in their activities. They have two different mechanisms of antiseizure action. Nitrazepam is used for infantile spasms and myoclonic seizures (Katzung, 1998). Nitrazepam is 7-nitro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (British pharmacopeia, 2000).



$C_{15}H_{11}N_3O_3$
M.wt. = 281.3 g/mol

Nitrazepam is a yellow, crystalline powder and it is classified to benzodiazepines group. Many analytical methods are used for its determination such as a spectrophotometric methods. These include reduction of nitrazepam with other 1,4- benzodiazepines compounds by using Zn/HCl and measuring the difference in their absorbance before and after reduction (Elbrashy, 1993). Another spectrophotometric method was described for the determination nitrazepam in the drug dosage forms (Davidson, 2005). Another colorimetric method is also described for the determination of nitrazepam by its reaction with tertabutylammonium hydroxide in dimethylformamide to form an orange colour (Walash, 1993). A kinetic method for the determination of nitrazepam by the continuous addition of reagent (CAR) technique is reported. The method involves the formation of an azo dye between 1-naphthol and a diazonium salt in turn obtained by reaction between the drug and nitrous acid. The absorbance of the azo dye is measured at 600 nm (Carmona, 1992). A method involving high performance liquid chromatography with dual electrode electrochemical detection in there doxmode (LC-DED) has been developed for the determination of nitrazepam benzodiazepine and tranquilizer in serum (Honeychurch, 2006). Also, HPLC technique is used to separate and determine fifteen benzodiazepines in human blood (Yokchue, 2010). Direct quantitative densitometry of nitrazepam and its main metabolites in urine is developed. These compounds are separated by thin-layer chromatography (Inoue, 1985).

EXPERIMENTAL

Instruments

All spectrophotometric measurements are performed on Shimadzu UV-160A, UV-Visible recording spectropho-tometer.

Reagents

All chemicals used are of analytical reagent grade.

Standard reduced form of nitrazepam solution, 100 µg.ml^{-1}.

This solution is prepared by dissolving 0.025 g of nitrazepam (NDI-Iraq) in 5 ml of ethanol by heating and the volume is diluted to 25 ml with ethanol in a volumetric flask, a 5 ml of this solution is taken and a 0.4 g of zinc powder, 2 ml of 36% concentrated HCl are added then the reaction mixture is shaked occasionally for 30 min. The solution is filtered and the filtrate is completed with distilled water to 50 ml in a volumetric flask. This solution is stable for at least one week.

Hydrochloric acid, 1N.

This solution is prepared by appropriate dilution of 36% concentrated hydrochloric acid solution to 250 ml with distilled water in a volumetric flask.

Sodium nitrite solution, 0.5%.

This solution is prepared by dissolving 0.5 g of sodium nitrite in 100 ml distilled water in a volumetric flask.

Sulphamic acid solution, 1%.

This solution is prepared by dissolving 1 g of sulphamic acid in 100 ml distilled water in a volumetric flask.

N-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) reagent solution, 0.1%.

This solution is prepared by dissolving 0.1 g of (NEDA) in 100 ml distilled water in a volumetric flask.

Procedure and calibration graph

To a series of 25 ml calibrated flasks, an increasing volume covering the concentration range (1-500) µg .25ml^{-1} of reducing form of nitrazepam solution are transferred, followed by the addition of 1 ml of 1N of hydrochloric acid and 1 ml of 0.5% sodium nitrite solution and, shaking occasionally for 3 min, then a 3 ml of 1% sulphamic acid is added, with occasional shaking and standing for 3 min. to remove the excess of nitrite ions, a 2.5 ml of 0.1% (NEDA) reagent is added and the flasks are diluted with distilled water. The absorbances are measured at 564 nm against the reagent blank. Beer's law is obeyed over the range of concentration 1 to 200 µg nitrazepam in 25 ml and a concentration above 200 µg in 25 ml gives a negative deviation (Fig.1). The apparent molar absorptivity referred to nitrazepam has been found to be 1.633×10^{3} l.mol^{-1}.cm^{-1}.

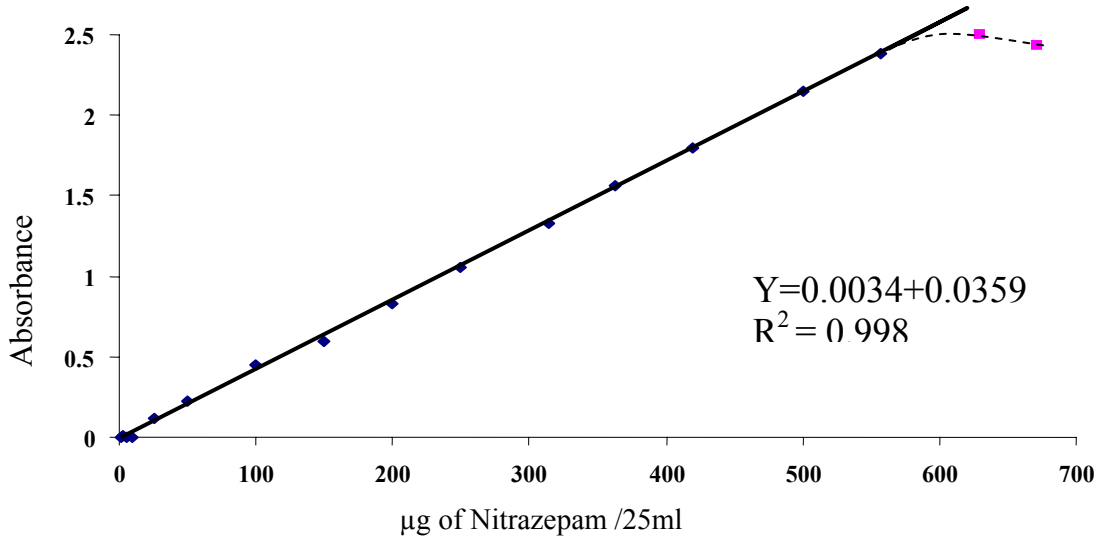


Fig. 1. Calibration graph for Nitrazepam determination

RESULTS AND DISCUSSION

During the investigation, 100 µg of reducing nitrazepam is taken and the final volumes are brought to 25 ml with distilled water.

Optimization of variables

The effect of various parameters on the absorption intensity of the colored complex is studied and the reaction conditions have been optimized.

Effect of quality and quantity of acid used for diazotization reaction

The effect of quality and quantity of acid on intensity of the coloured complex is examined. Different volumes (0.5-7.0) ml of 1N of different acid solutions are added to an aliquot of solution containing 100 µg of reducing form of nitrazepam. The intensities of absorption are read against the reagent blank. The results are shown in Table 1.

Table 1: Effect of quality and quantity of acid.

Acid 1N	Absorbance / ml of acid									
	0.5	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
HCl	0.548	0.552	0.532	0.530	0.498	0.481	0.476	0.461	0.457	0.462
H_2SO_4	0.527	0.537	0.530	0.525	0.537	0.535	0.521	0.505	0.510	0.505
HNO_3	0.491	0.497	0.506	0.501	0.504	0.494	0.481	0.453	0.434	0.440
CH_3COOH	0.509	0.518	0.522	0.532	0.520	0.527	0.533	0.541	0.521	0.527
HCOOH	0.500	0.512	0.502	0.501	0.492	0.469	0.469	0.444	0.456	0.462
H_3PO_4	0.531	0.538	0.533	0.529	0.527	0.521	0.517	0.507	0.501	0.449
$HClO_4$	0.528	0.539	0.537	0.535	0.537	0.501	0.497	0.497	0.469	0.471

The results shown in Table 1 indicate that 1.0 ml of 1N HCl is considered as an optimum value therefore it is recommended for subsequent experiments.

Effect of sodium nitrite amount with the time

Different amounts of the 0.5% NaNO2 solution are added and the time needed to complete the diazotization of reducing form of nitrazepam is studied by standing of the solutions after adding sodium nitrite solution for different times, with occasional shaking, then the other reagents are added and the absorbance is measured against the reagent blank .The results indicate that complete diazotization of nitrazepam occurs after 3 min. when 1 ml of 0.5% $NaNO_2$ solution is added because it gives the higher sensitivity, therefore it has been selected for subsequent experiments.

Effect of sulphamic acid amount with the time

The effect of the amount of 1% sulphamic acid solution for removing the excess sodium nitrite with the standing time with occasional shaking are investigated. The results indicate that complete reaction of sulphamic acid with sodium nitrite occurs after 3 min. when 3 ml of 1% sulphamic acid solution are added and the intensity decreased above 3 min. because the intensity of reagent blank increased. Therefore, the standing time 3 min. is recommended for the subsequent experiments.

Effect of the (NEDA) reagent amount

The effect of the amount of 0.1% (NEDA) reagent on a maximum formation of the coloured complex is investigated. The results are shown in Table 2.

Table 2: Effect of the amount of (NEDA) reagent amount on absorbance.

ml of 1% N-NED	Absorbance of nitrazepam µg/ml						R
		10	25	50	100	200	
0.5	B	0.006					0.999413
	S	0.061	0.118	0.232	0.434	0.909	
1.0	B	0.007					0.998425
	S	0.063	0.119	0.245	0.487	1.075	
1.5	B	0.008					0.999666
	S	0.061	0.117	0.234	0.498	1.058	
2.0	B	0.006					0.999393
	S	0.062	0.155	0.267	0.532	1.112	
2.5	B	0.008					0.999924
	S	0.065	0.153	0.278	0.565	1.126	
3.0	B	0.008					0.999666
	S	0.064	0.149	0.262	0.546	1.123	

The results shown in Table 2 indicated that 2.5 ml of (NEDA) reagent solution give the higher sensitivity value of correlation coefficient, therefore it has been selected for the subsequent experiments.

Effect of acidity

The effect of two different amount of 1N HCl on intensity of the coloured complex formed when added to an aliquot of solution containing 100 μg of reducing form of nitrazepam is examined. The intensities of absorption are read against the reagent blank. The results are shown in Table 3.

Table 3: Effect of the acidity on absorbance.

With out addition of HCl After N-NED	**1 ml of 1N HCl is added after addition N-NED.**	**2 ml of 1N HCl is added after addition N-NED**
0.558	0.554	0.556

The results shown in Table 3 indicate that the addition of the acid after the formation of coloured complex does not increase the intensity of absorption of sample, therefore, the addition of an acid is not selected for subsequent investigation.

Effect of surfactants

The effect of surfactants were studied by the addition of 3 ml of various types of surfactant (cationic, anionic and neutral) to the medium of reaction with different orders of addition. The results are shown in Table 4. The selected surfactants are :
Cetylpyridinium chloride monohydride (CPC), (cationic).
Sodium dodecyl sulphate (SDS), (anionic).
Polyoxyethylene (20) (Tween 80), (neutral).

Table 4: Effect of surfactants and the order of addition.

Surfactant type	I		II		III		IV		V	
	A	λ_{max}	A	λ_{max}	A	λ_{max}	A	λ_{max}	A	λ_{max}
CPC 1×10^{-3} M	0.558	564	0.556	564	0.478	550	0.554	565	0.551	560
SDS 1×10^{-3} M	0.553	565	0.549	564	0.561	567	0.552	571	0.553	571
Tween 80 1×10^{-3} M	0.491	581	-----	-----	0.481	581	0.515	562	0.441	561

- absorbance without surfactant = 0.565

I: Sample +Surfactant+HCl+$NaNO_2$+Sulphamic acid + Reagent (NEDA).
II: Sample + HCl + Surfactant + $NaNO_2$ + Sulphamic acid + NEDA.
III: Sample + HCl + $NaNO_2$ + Surfactant + Sulphamic acid + NEDA.
IV: Sample + HCl + $NaNO_2$ + Sulphamic acid + Surfactant + NEDA.
V: Sample + HCl + $NaNO_2$ + Sulphamic acid + NEDA+ Surfactant.
VI: Sample + HCl + $NaNO_2$ + Sulphamic acid + NEDA (A=0.0554 and λ_{max}=564) .

The results in Table 4 indicate that there is no change in the intensity of coloured product. Therefore, surfactants have been omitted in the subsequent experiments.

Effect of time

The effect of time on the development and stability of the colored complex for different amounts of reducing form of nitrazepam is investigated under the optimum experimental conditions is established. Complete color formation occurs immediately after all reaction mixture are added and the absorbance of the complex remains constant for at least 60 minutes (Table 5).

Table 5: Effect of time on the absorbance of complex.

µg of [nitrazepum per 25 ml]	**Absorbance / min**							
	Direct addition	**5**	**10**	**20**	**30**	**40**	**50**	**60**
5	0.023	0.039	0.041	0.041	0.040	0.040	0.041	0.041
50	0.197	0.268	0.271	0.272	0.272	0.271	0.272	0.273
100	0.210	0.469	0.529	0.556	0.556	0.555	0.557	0.556
200	0.439	0.989	1.098	1.120	1.121	1.122	1.122	1.122

Absorption spectra

Absorption spectra of the coloured complex formed from the reaction between diazotized reducing form of nitrazepam and (NEDA) reagent in acidic medium against its corresponding reagent blank shows maximum absorption at 564 nm in contrast to the (NEDA) reagent blank which have less absorption at 564 nm (Fig.2).

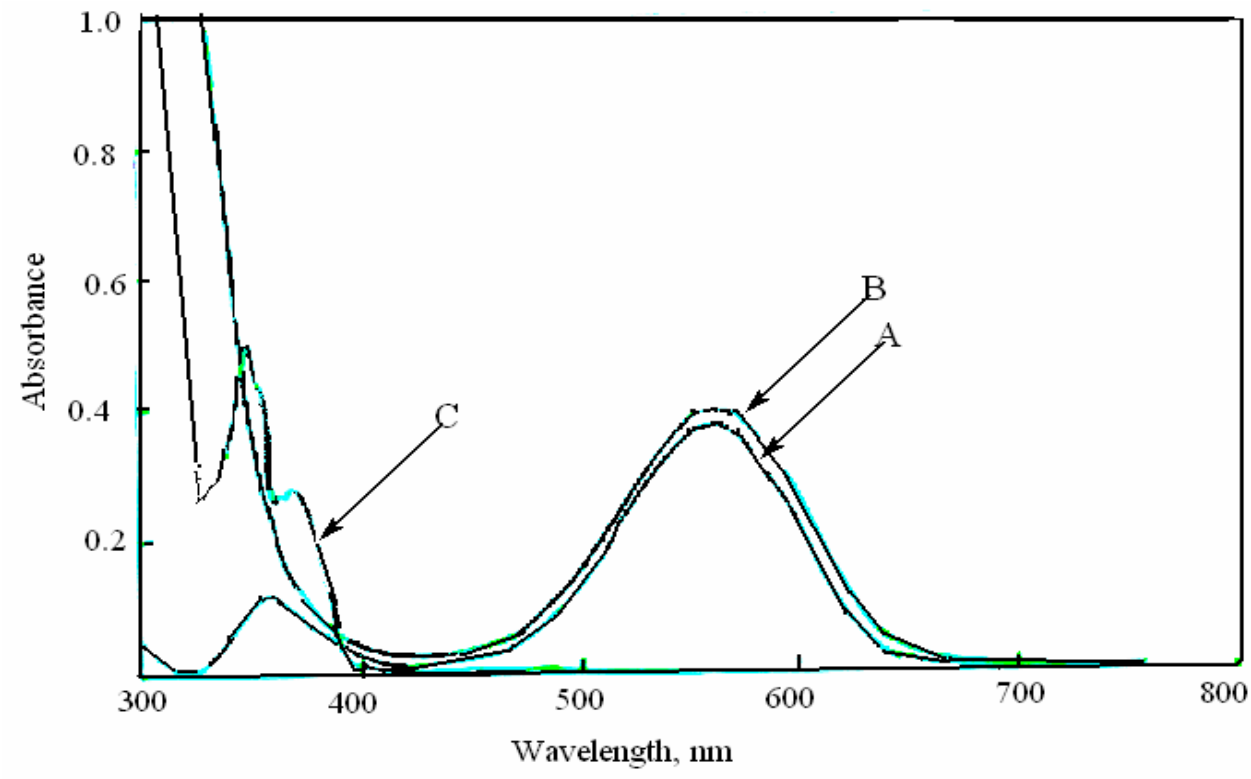


Fig. 2: Absorption spectra of 100 µg of reducing form of nitrazepam / 25 ml treated according to the optimum conditions and measured against (A) blank, (B) distilled water, and (C) blank measured against distilled water.

Accuracy and precision

To check the accuracy and precision of the calibration graph, nitrazepam is determined at three different concentrations. The results shown in Table 6 indicated that the calibration graph is satisfactory.

Table 6: Accuracy and precision.

Amount of nitrazepam taken, µg/25ml	Relative error*, %	Relative standard deviation*, %
10	- 2.45	1.07
25	+ 0.67	1.00
50	- 0.72	1.00

* Average of five determinations.

Nature of nitrazepam-(NEDA) reagent complex.

Job's method (Delevie, 1997) and mole ratio method have been used in the determination of the reaction ratio of nitrazepam with (NEDA) reagent. The obtained results (Fig.3 & Fig.4) showed that 1:1 nitrazepam to (NEDA) reagent ratio is obtained.

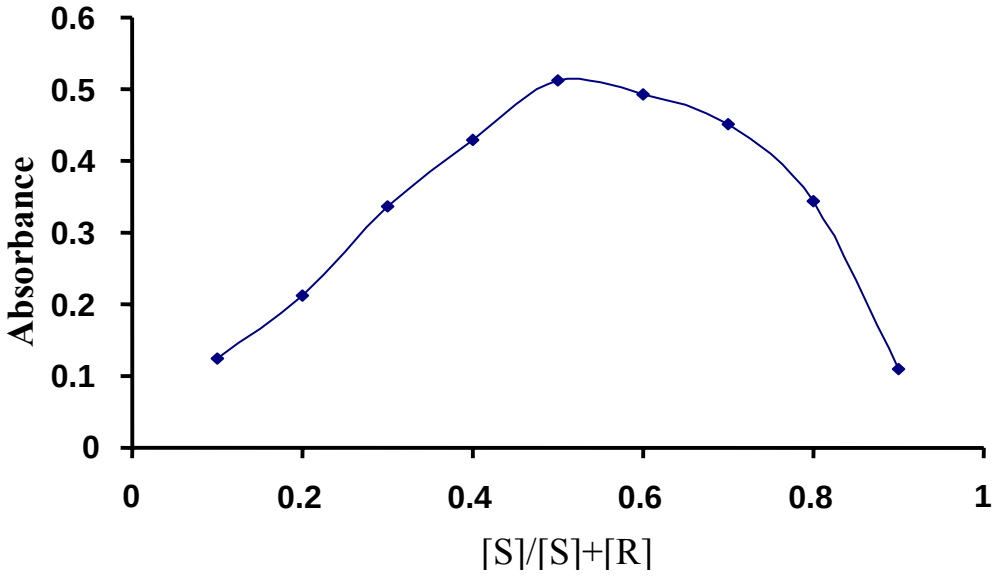


Fig. 3: Job's plot for nitrazepam (NEDA) reagent.

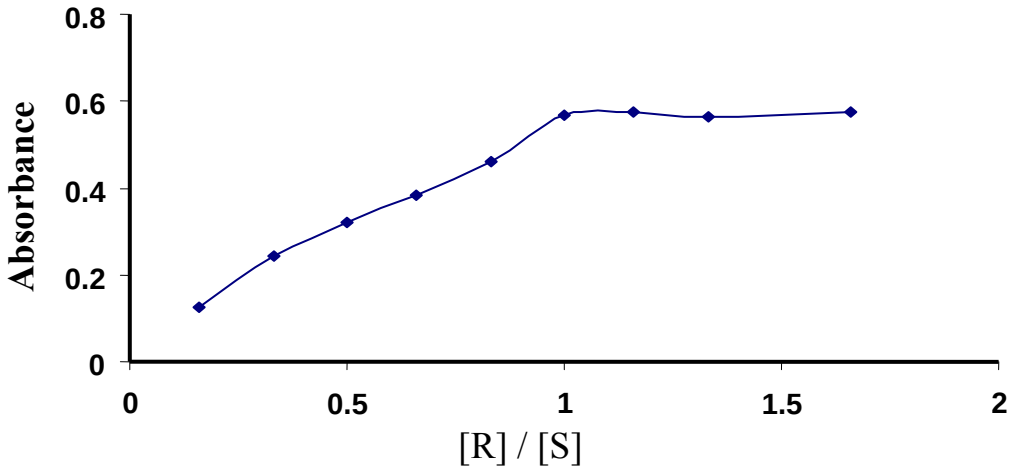
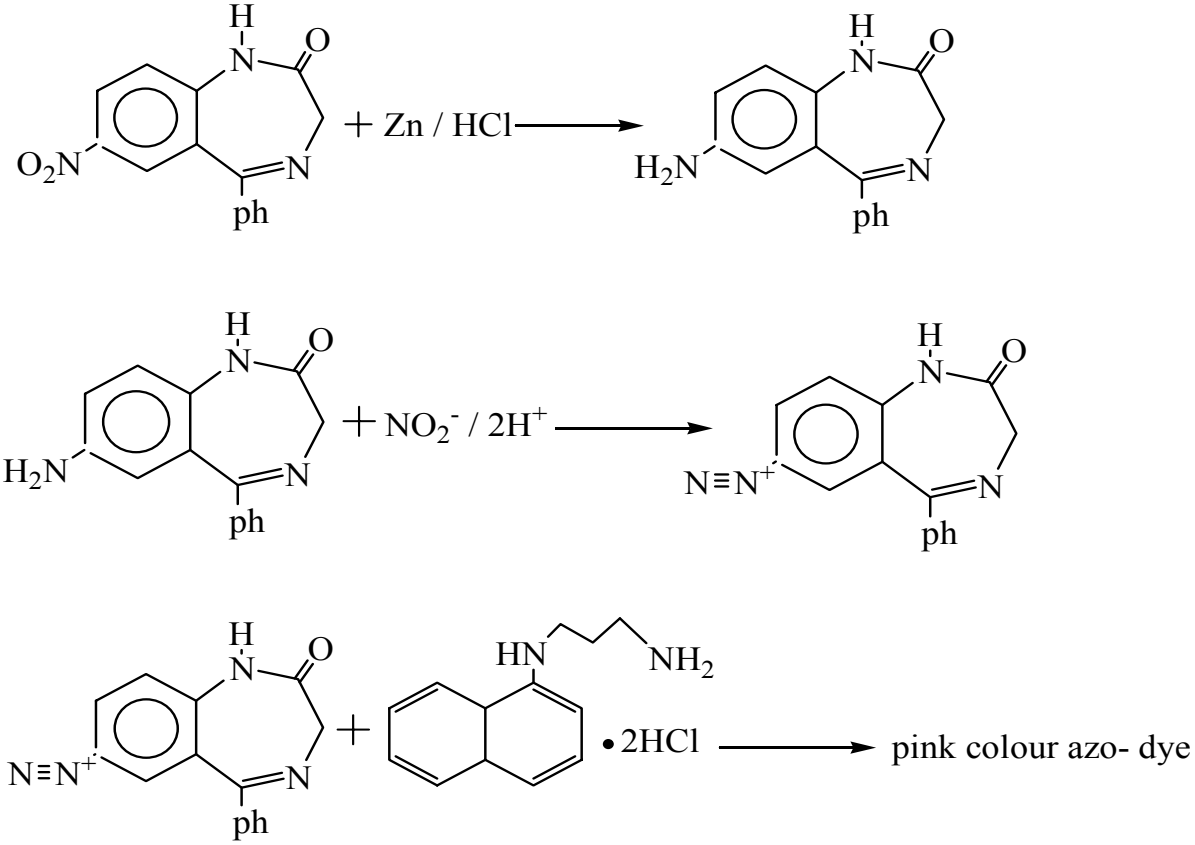


Fig. 4: Mole ratio's plot for nitrazepam-(NEDA) reagent complex.

From the above resulting data the probable azo dye formation is shown as follow:



Stability constant of formed dye (Haris, 1998)

Stability constant of formed dye is calculated with ratio 1:1.The results are shown in which Table 7, indicate that the average of stability constant is 4.348×10^3 $l.mol^{-1}$, indicating that the coloured complex is stable.

Table 7: Stability constant of formed dye.

ml of nitrazepam 3.555×10^{-4} M	**Absorbance**			**$K.M^{-1}$**
	As	**Am**	**α**	
1	0.099	0.219	0.548	4.233×10^3
2.5	0.433	0.939	0.539	4.463×10^3

Interference of foreign species

The effect of various species on the determination of nitrazepam is investigated. The results are shown in Table 8.

Table 8: Effect of interferences on the determination of nitrazepam.

Interference	Recovery(%) of 100 µg nitrazepam / µg of foreign compound added			
	50	100	300	500
Starch	101.04	101.73	101.21	101.56
Glucose	101.91	98.78	99.65	99.82
Acacia	100.69	100.52	98.78	101.56
Glycerol	101.04	97.39	100.69	100.86
Dextrose	101.04	99.30	98.26	100.34
Lactose	100.69	98.78	98.26	100.86
Sucrose	97.04	101.04	100.86	100.21

Application of the method

The proposed method was successfully applied to the determination of nitrazepam in its pharmaceutical preparations. The results which are shown in Table 9 indicate that a good recovery was obtained.

Table 9: Analytical applications.

Nitrazepam amount, µg	Absorbance of standard solution	Morgan (mg/ tablet) -syria	
		Absorbance	Recovery(%)
25	0.121	0.115	95.04
50	0.224	0.219	97.76
100	0.452	0.461	101.99
200	0.829	0.833	100.48

Comparison of the methods

Table 10 shows the comparison between some of analytical variables obtained from the present method with that of the recent spectrophotometric method.

Table 10: Comparison of methods.

Analytical parameters	Present method	Literature method (Al-Gabsha.et al,2008)
Medium reaction	Acidic	Neutral
Temperature (C°)	Room temperature	Room temperature
Development time (minutes)	Direct measuring	Direct measuring
λmax (nm)	564	485
Reagent coupling	N-(1-naphthyl) ethylene-diaminehydrochloric acid	Resorcinol
Beer's law range (ppm)	0.04-8.0	0.4-8.0
Molar absorbtivity($l.mol^{-1}.cm^{-1}$)	1.633×10^{3}	14900
RSD (%)	1.07	>%±3.47
Color of the dye	Pink	Orang-yellowish
Application of method	Determination of nitrazepam in tublets	Determination of nitrazepam in tablets

CONCLUSION

A simple, rapid, accurate and precise spectrophotometric method is evaluated for the determination of nitrazepam in pure form. The method is based on diazotization-coupling reaction between reduced product of nitrazepam and N-(1-naphthyl) ethylenediaminedihydrochloride in acidic medium to produce a pink colour which is stable, water soluble and has a maximum absorption at 546 nm with a molar absorpitivity of 1.633×10^3 $l.mol^{-1}.cm^{-1}$. Beer's law is obeyed in the concentration range from 0.4-20 µg/ml of reduced form of nitrazepam. The proposed method has been applied successfully to the assay of nitrazepam with, out interferences.

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