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Spectrophotometric Determination of Clonazepam Drug in Pure form and Pharmaceutical Preparation by Diazotization Reaction

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Abstract--- *By diazotization coupling reaction as accurate, selective and rapid spectrophotometric method for determination drug of clonazepam by reliant on azo-coupling reaction between CZP and 1,2-phenylenediamine for To achieve the purpose of obtaining the colored product with a maximum absorbance 480nm. For the conditions reaction have been studied and optimized. The linearity range for clonazepam was (0.1_6µg\ml) ,while the detections limit 0.04327and quantification limit 0.14425µg\ml The Molar Absorptivity 8.505×104 L/mol.cm with Sandal's sensitivity 0.00370 µg\cm2. Eventually this methods were successfully completed to determination of CZP in pure from and commercial form.*

Keywords--- *Spectrophotometric Determination, Clonazepam, Azo-coupling Reaction.*

I. INTRODUCTION

Pharmaceutical drugs are chemicals that are designed to prevent, diagnose, treat, or cure a disorder. In laymen terms, we simply call them medicines. Pharmaceutical chemistry is the study of drugs, and it involves drug development. This includes drug discovery, delivery, absorption, metabolism, and more. There are elements of biomedical analysis, pharmacology, pharmacokinetics, and pharmacodynamics. Pharmaceutical chemistry work is usually done in a lab setting. Clonazepam (CZP.) is a medication of benzodiazepine[1-3]

Clonazepam using multi-component mode of analysis [4, 5] is available. An isocratic chiral sensitive HPLC method was developed for the separation of Escitalopram oxalate drug substance [6, 7].

Clonazepam [8] [5-(o-chlorphenyl)-7-nitro-1H-1,4-benzodiazepin-2(3H)-one] is mainly used as anticonvulsant, muscle relaxant and anxiolytic agent as shown in figure 1. [9]

Clonazepam is slightly soluble in acetone, chloroform, acetic anhydride, hardly soluble in methanol, isopropanol, ether, almost insoluble in water. It has a molecular weight of 315.72 and the following structural It is a light yellow crystal inform[5].

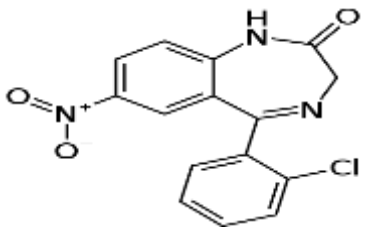

Fig. 1: The Chemical Structure of Clonazepam

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several and various methods have been reported in literature for determination clonazepam drug in either pharmaceutical forms or biological fluids .Several methods have been reported in the literature for the estimation of drugs of clonazepam in either pharmaceutical forms or biological fluids, including HPLC[10-13]liquid and gas chromatography[14-16] clonazepamin blood using LC-MS/MS chemiluminescence and electrochemiluminescence, electrical methods[17-19]flow injection[20-23] colorimetric and various spectrophotometric method[17, 24, 25] The aim of this search is development a rapid, sensitive and simple spectral methods to determination clonazepam drug in Pure form and in Pharmaceutical Preparation by using Azo-coupling reaction also - Applying the spectral methods used in estimating of clonazepam drug .

Experimental Apparatus

- Each the absorption are measurement T-8 .UV-Visible Spectrophotometric (P.G) Instrumental (I.T.D) and used (1.0) cm quartz cells.
- Heating -cooling water bath (Hakk ,F)
- Sartourious B.P-3015-Germany
- Oven(B.S) size two

II. MATERIALS

Each chemical materials are used in this research highly purity from They were prepared as following method:

- Clonazepam reduced (CZP): It obtainable from State the company of the Drug Industry with the Medical Apparatus; (S.D.I) /(Iraq) . The stock solution of reduced clonazepam (CZP) at 100 µg ml^{-1}) prepared from taken 0.005 gm of (CZP)and then dissolved it 25 ml of ethanol with 2ml from the distilled water and 2ml from concentrated HCl 11.64 M and add 0.3 gm from zinc powder, leave the mixture a bout (15 min) after that filtered by filter paper in a volumetric flask, and completed volume with distilled water, as a stock solution which taking away from the light[26]
- 1,2-phenylenediamine[0.01M]:Prepare by dissolve (0.054)gm of 1,2phenylenediamine in 50ml of ethyl alcohol
- Sodium nitrite ($NaNO_2$) [0.1M]: It supplied from (BDH-Chemical I.T.D) by dissolve (0.1725gm) as a pure material in (25 ml) Distilled water . this solution prepared daily.
- Sulfamic acid 0.2[M]: Prepared by dissolving (0.485gm) from the substance in 25 ml distilled water .
- Hydrochloric acid1 [M] it was provided by from (GCC) at 98% prepared by diluting appropriate concentration hydrochloric acid at 25ml distilled water.

Procedure

In the series of calibrated flask of 10ml _aliquots of stock solution from (CZP) to obtain the final concentration in the range 0.05 - 6 ppm, then take out 0.6ml of hydrochloric acid (1M) with 0.8ml of (0.1M) Sodium Nitrite . Wait for (10min) in order to complete the (azo-coupling) reactionsuccessivelyadd0.4ml from Sulfamic acid (0.2M) and stand for (2min) to remove the excess of nitronuim ion then add 1m from the reagent 1,2-phenylenediamine (0.01M) and complete by distilled water to the mark of calibrated flask. The obtained solutions can be sit stable for (60 min) at room temperature and absorbance 480 nm was measured versus blank reagent.

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Procedure for Pharmaceutical Preparations

Tablet Rivotril: Solution 100 µg. m-1 prepare by grinded according the procedure stock solution of reduced CZP weighed the adequate amounts. Three tablet were weighed and finely crushed. An accurately weighted amount of the powder equivalent (0.425gm) from (CZP) that adopted on type of Tablets which can be used. It was dissolved in 25 mL ethanol with 2ml distilled water and also 2 mL (HCl ~11.64 N) and add 0.3 gm from zincpowder.

III. RESULTS AND DISCUSSION

In the procedure, effects of different parameters on color characteristics for azo dye by gain optimum conditions reaction.

Effect of Acid Volume

To be sure through experiment of existence effect acid on the absorbance product, using hydrochloric1[M]with series different volumes (0.1-0.7ml).0.6ml from acid give the perfect absorbance. Figure (2)

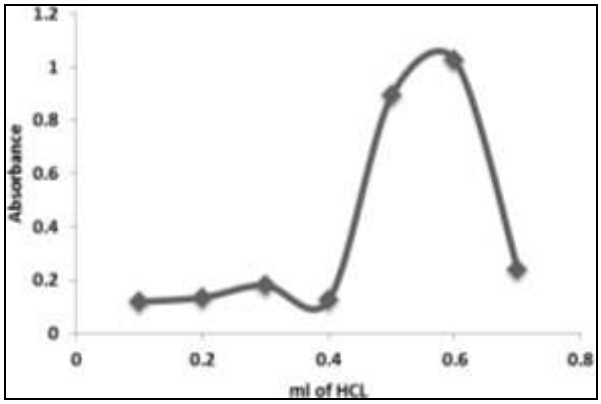

Fig. 2: Effect of Volume of Hydrochloric Acid0. [1M]

Effect of Sodium Nitrite Volume

Series of different volume (0.2-1 ml) from Sodium Nitrite (0.1M) and found that the perfect absorbance obtain at 0.8ml of Sodium Nitrite while the best time (5min) that enough the result colored intenseness have been for complete diazotization of CZP. Figr.(3)

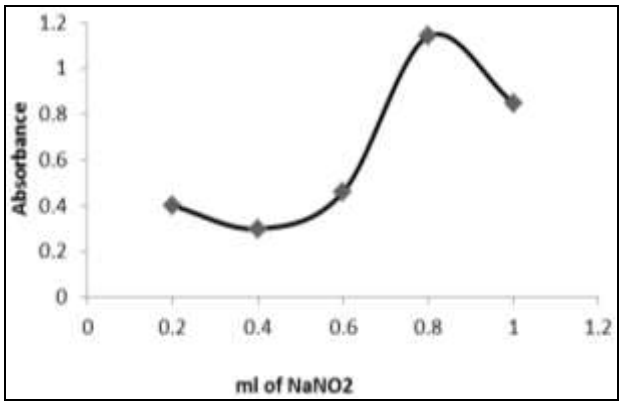

Fig. 3: Effect of Sodium Nitrite [0.1M]

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Effect of Sulfamic Acid Volume

The impact of volume of sulfamic acid is necessary to remove the excess of nitrite , the purpose from this addition is to avoid reaction with reagent. The result show that (0.4ml) and time (2min) is suitable to give highly intense reaction with reagent 1,2-phenylenediamine. Fig(4)

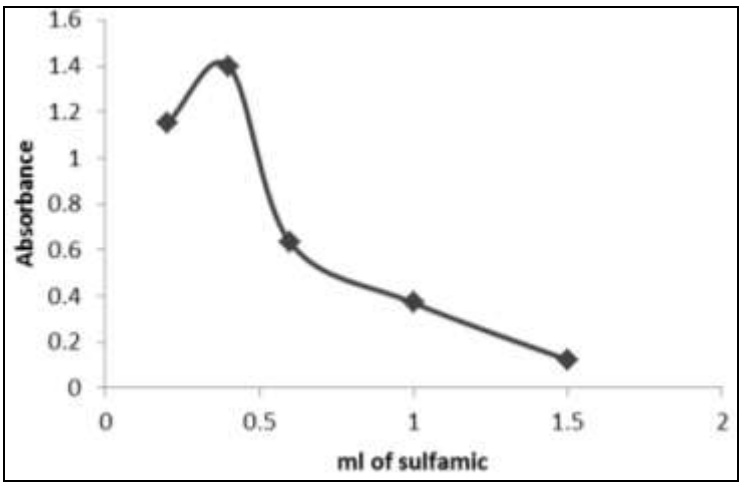

Fig. 4: Effect of Sulfamic Acid 0.2M

Effect of Reagent Volume

Series of different concentration from reagent (0.2-2ml) effect on color intense and the absorbance measured and found (1ml) was the optimum volume as shown in Fig(5)

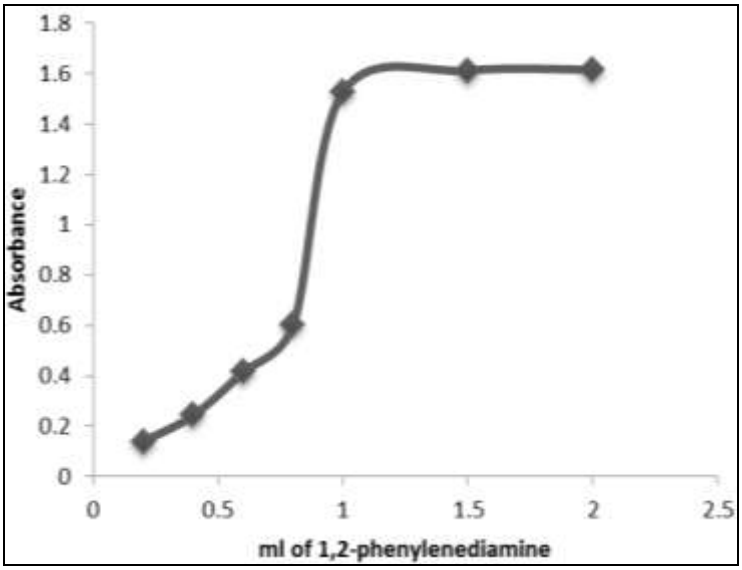

Fig. 5: Effect of Reagent Volume[0.01M]

Effect of Reaction Time

To be sure effect on the stable intense colored prepared CZP with 1,2-phenylenediamine under the perfect conditions and measured at different intervals time approachat 60 min the result obtain refer to that the color

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intensity improve and stay constant at least 60 min.as shown in Fig.(6)

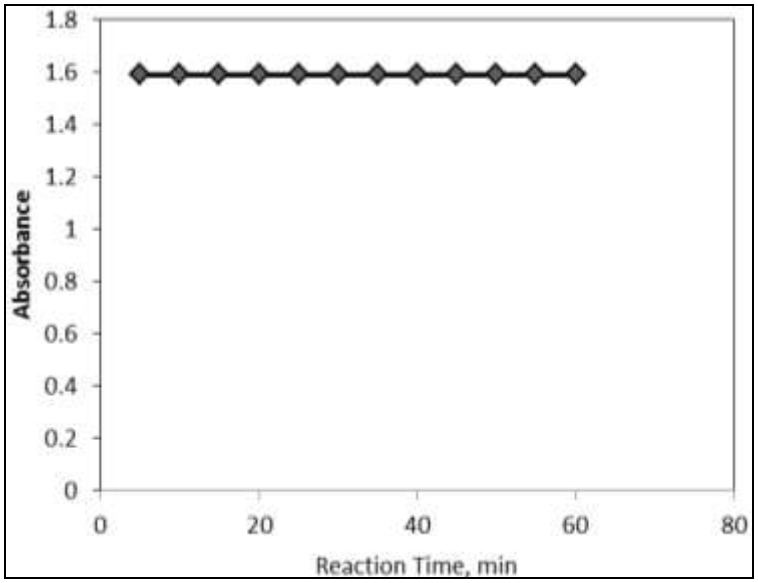

Fig. 6: Effect of Reaction Time

Effect of Temperature

To examined weather conditions effect on the result of the test**.** Take the range (5-45C^{O}) and according to the result conclusion on the (25 C^{O}**)** giving higher color intense, While higher temperature lead to decomposition and attributed the product appear in Fig(7)

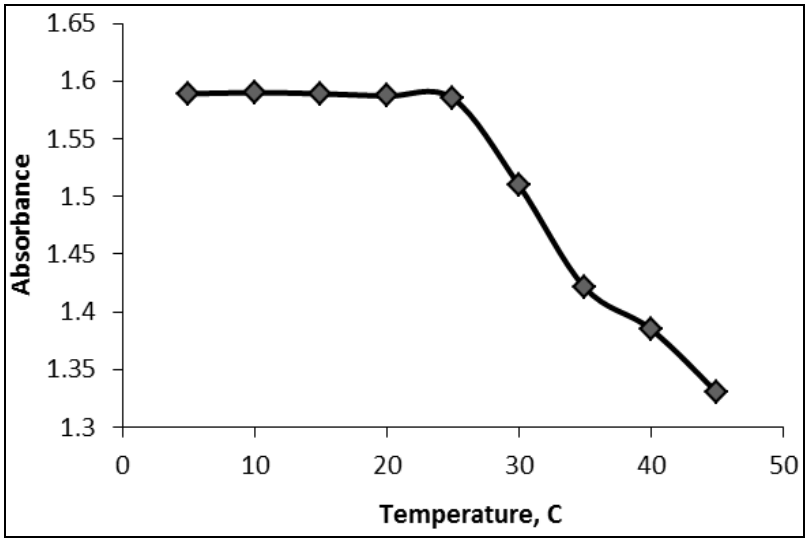

Fig. 7: Effect of Temperature

Absorption Spectra

The reaction of 1,2-phenylenediamine under optimum conditions give orange intense color as product at maximum wave length 480nm while solution blank colorless absorption as shown in Fig.(8)

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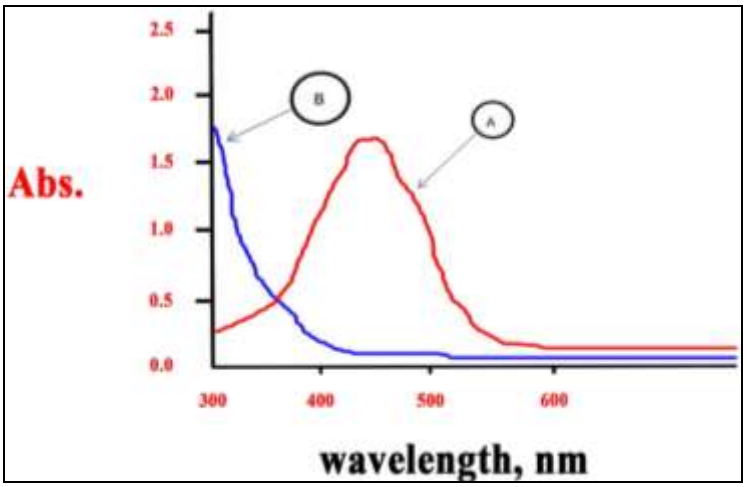

Fig. 8: A: Clonazepam (10 µg/ml) with 1,2-pheneylenediamine(0.01M)Product versus solution blank. B:Soloution blank versus distilled water

Calibration Curve

By benefit from the optimum conditions. The linearity range for clonazepam was on the range (0.1_6µg\ml) Fig. (8) with slope 0.294 L.mg^{-1}and Correlation Coefficient 0.996 an intercept was 0.002 . The Molar Absorptive 9.2818×10^{4} L/mol.cm^{-1}with Sandal's sensitivity (0.00340µg\cm^{2}), LOD 0.03973and LOQ 0.13245µg\ml. The characteristics of the spectrophotometric method improved are as shown in Table(2)

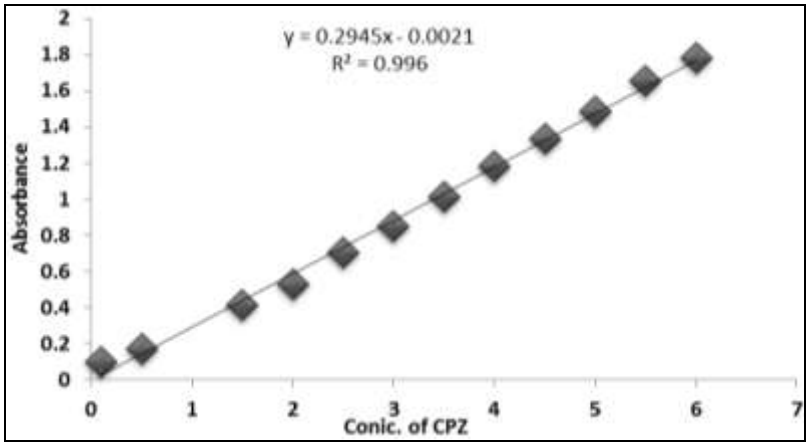

Fig. 9: Calibration Curve of Clonazepam

Table 1: Characteristics of the Method Developed for Determination CZP

Parameters	***Value***
Regression equation	y=0.294x-0.002
Slope	0.294
Correlation Coefficient	0.996
linearity range mg.ml^{-1}	0.05-6
Molar Absorptivity L/mol.cm^{-1}	9.2818×10^{4}
Sandal's sensitivity µg\cm^{2}	0.00340µg\cm^{2}
L.O.D µg\ml	0.03973
L.O.Q µg\ml	0.13245

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Precision and Accuracy

To determinate the precision and accuracy that checked by measuring three solution of CZP and repeated test three times. The result values give a good satisfactory accuracy and precision show in Table (2).

Table 2: Precision and Accuracy

Concentration of CZP µg\ml		***%E***	***%Rec****	***%RSD****
Taken	***Found***			
2	1.993	-1.269	98.73	0.818
4	4.00	-0.819	99.180	2.78
5	5.072	-1.440	98.56	0.435

*Average of three determinations

Stoichiometry of Reaction

For determination the nature accuracy of the product complex so as to give an intense colored complex that available by applying (Job s and mole ratio method). The results demonstrate existence of (2:1) [CPZ: Reagent] at 458nm complex was product . The Stability constant (K_{stab}) of color formed under the optimum conditions was 4.081×10^{6}L.mole^{-1}. [4, 27, 28]

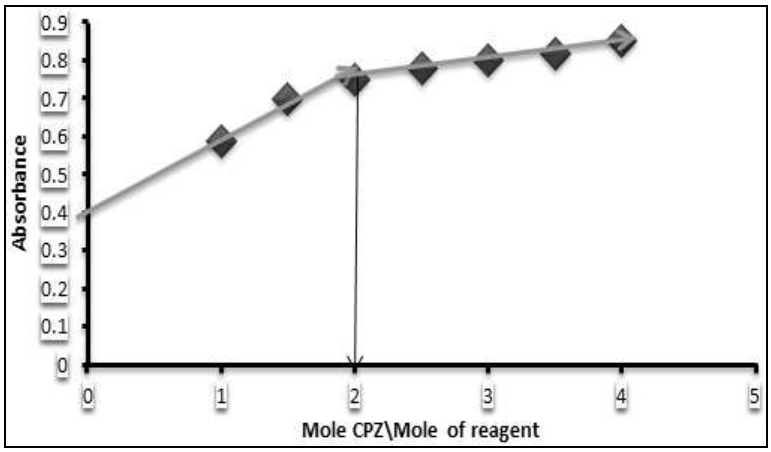

Fig. 10: Mole- Ratio Method

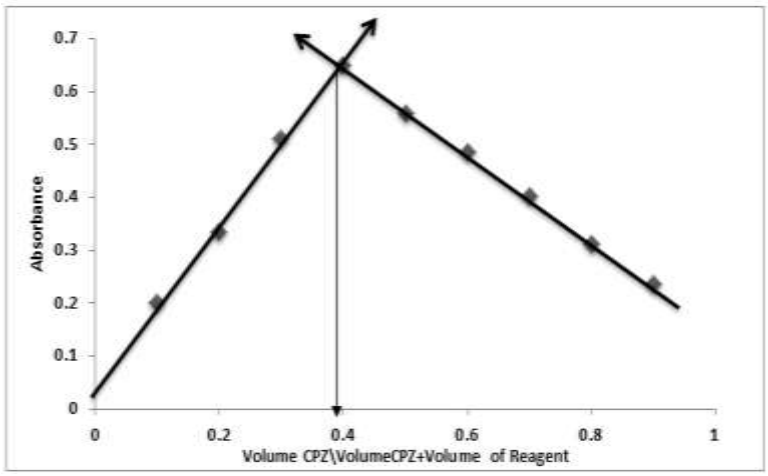

Fig. 11: Continuous Variation Method

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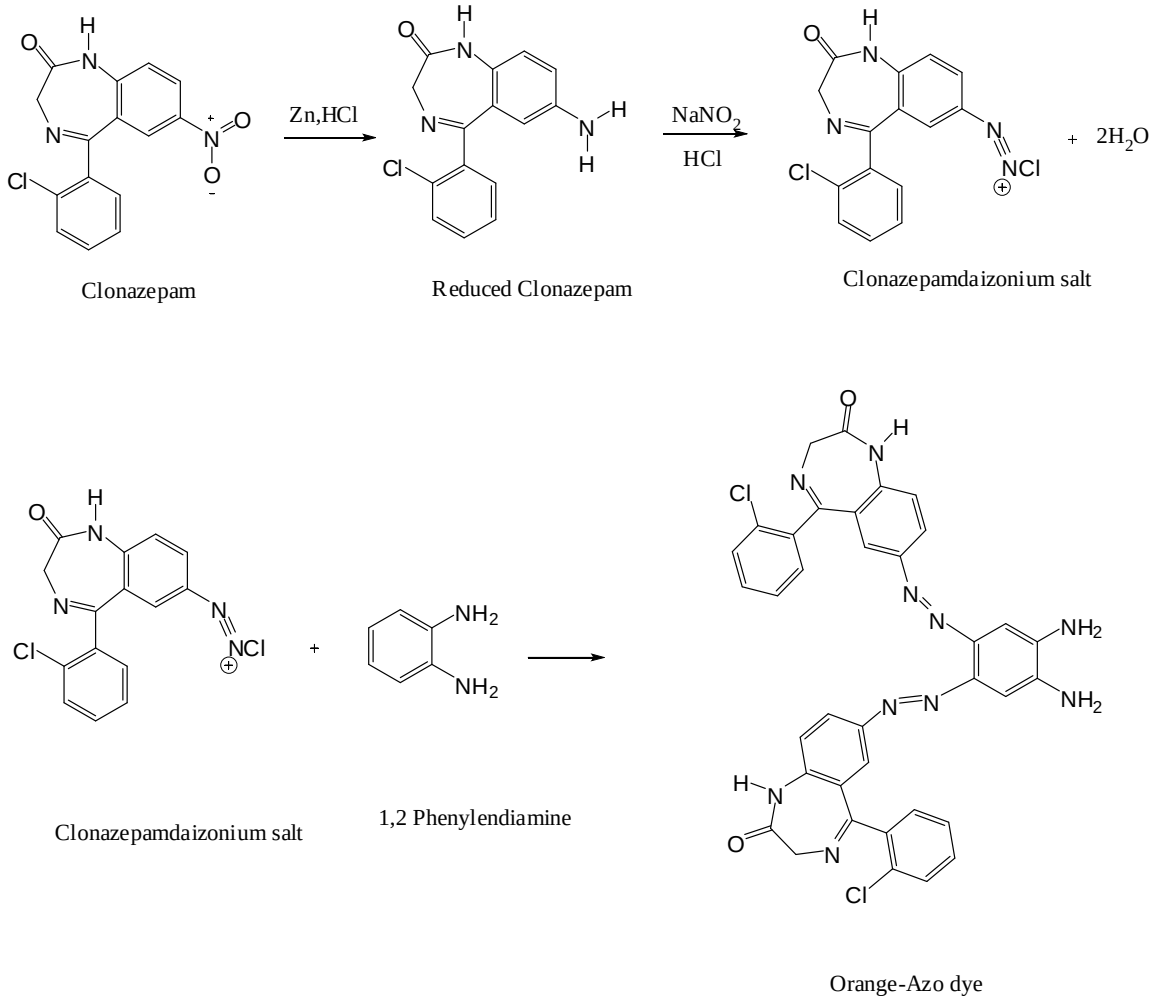

Fig. 12: Show the proposed Reaction Mechansium between CPZ and Reagent in Presence $NaNO_2$and HCl

Application The suggested method is applying for determination Clonazepam in Revotril (tablets) the results explain in Table (1) show values of a good recovery obtainable , The proposed method scored more successfully and effectively for the standard method as appear in Table(3).

Table 3: Results Show the Comparison between Standard and Proposed Methods

Ph. Preparation Containing CPZ	***Proposed Method***		***Standard Method***	
	Recovery $(Xi)_1$	$(Xi_1 - X_2)_2$	***Recovery***	$(Xi_2 - X_2)$
Pure CPZ	**98.82**	**0.1521**	**99.85**	**1.1342**
Rivoteril (Tablets)	**98.04**	**0.1524**	**101.98**	**1.1342**
	X_{eva}**= 98.43**	**∑= 0.3042**	X_2 **= 100.915**	**∑=2.2684**

T $Value_{(exp)}$= -1.731 **, Critical** Value=4.303**, F** Value=0.1444

IV. CONCLUSION

A precise ,simple and accurate spectrophotometric method have been improve investigation of trace an amount of CPZ adopted on diazotization _coupling reaction with 1,2-phenylenediamine with present of sodium nitrate (0.01M) and (1M) HCl addition with sulfamic acid in pure and doses forms.

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