

Spectrophotometric determination of Drug Clonazepam in Pure form and Pharmaceutical Tablets by Oxidative Coupling Reaction with Chlorpromazine hydrochloride

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ABSTRACT

Sensitive, simple and a Precise spectrophotometric methods were development for the estimation reduced clonazepam (CZP) in purity form and in the pharmaceutical tablets from through using potassium bromate as oxidizing reagent for the drug and Chlorpromazine Hydrochloride (CPZ.HCL) as new chromogenic reagent. That led to forms colored products with a maximum absorbance of 530 nm. For the conditions reaction have been studied and optimized. The linearity range for Clonazepam was 0.05–3.5 µg/ ml, while the detections limit 0.0061 and the quantification limit 0.020 µg ml⁻¹. The Molar Absorptivity 1.381×10^4 L/ mol cm with Shandell's sensitivity 0.0228 µg. cm⁻². Finally the proposed methods were successfully Applying for estimation CZP in pure form and commercial formulations.

Keywords: Oxidative coupling reaction, Clonazepam, Spectrophotometric determination.

INTRODUCTION

Benzodiazepines belong to a group of substances known for their muscle, relaxant, sedative; hypnotic, anticonvulsant and anti-depressive properties¹⁻⁵. Clonazepam (CZP) is a medication of benzodiazepine^{6,7}. It used for epilepsy that is one most prevalent serious neurological disorders^{8,9}. Clonazepam (CZP) chemically know [CZP 5-(2-chlorophenyl) 1,3-dihydro-2H -7-nitro-1,4- benzodiazepin-2- one).]¹⁰⁻¹³. It 's freely very soluble in methanol, ethanol¹³. Clonazepam is a little soluble in acetone, acetic anhydride¹⁴. it is a light yellow crystalline powder¹⁵. It is classified as a high potency nitro-benzodiazepine^{16,17}. It affects chemicals in the brain that may be unbalanced¹⁸⁻²⁰. It's chemical formula and molecular weight is C₁₅H₁₀ClN₃O₃ and(315.7) Figure 1^{21,22}.

The literature for using different techniques such as flow injection^{23,27}, HPLC^{28,30}, and spectrophotometric^{31,33}. The aim of this paper development of very simple and highly selective accurate spectrophotometric method to measure the quantity of(CZP) drug depended on oxidative coupling reaction between the drug and Chlorpromazine hydrochloride (CPZ) in addition existence potassium bromate(KBrO₃) as oxidizing reagent .

EXPERIMENTAL

Apparatus

All of these absorbances measure by using : T80 UV-VIS. Spectrophotometer P.G Instrumental; Ltd; U .K. Oven[BS] Size Two; Gallenkamp/ England. With cells quartz 1- cm With temperature range (0-300C). Sartorius; Balance Bp3015 Germany, Heating, Water Bath-Haak Fe.

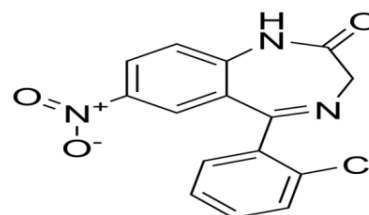


Figure 1: The chemical structure of clonazepam (CZP).

MATERIALS AND METHOD

Each chemical materials utilized in the research have been high grand of purity, They were prepared from the following:

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 2 ml from concentrated HCl 11.64 M and add 0.3 gm from zinc powder, leave the mixture a bout (15 min) then filtered in a volumetric flask, and completed volume with distilled water, as a stock solution which saving away from the light³⁴.

Chlorpromazine hydrochloride (CPZ) company standard material was provided from the stat Company Industries Samarra, Iraq (S.D.I).

The stock solution of CPZ (0.01 M) prepared by dissolving (0.088 g m) of pure material in 25 mL distilled water.

Potassium bromate(KBrO₃)

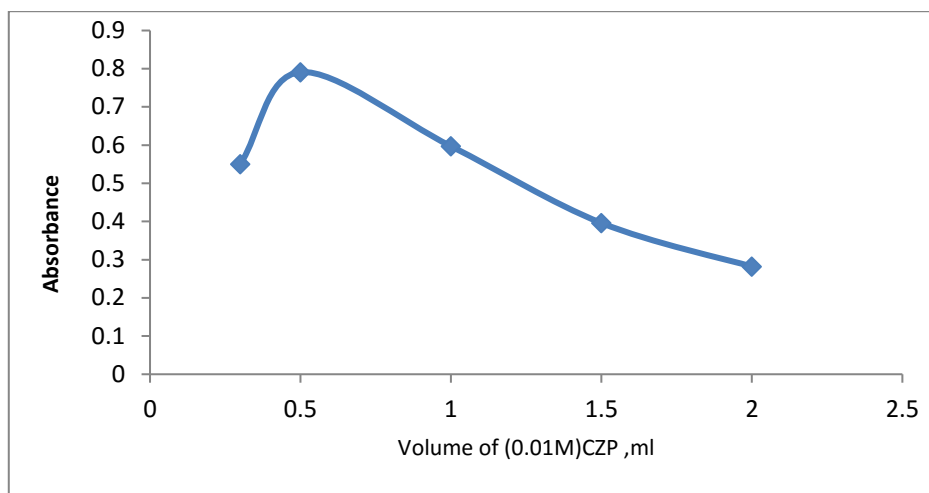


Figure 2: Effect of (0.01M) CPZ volume.

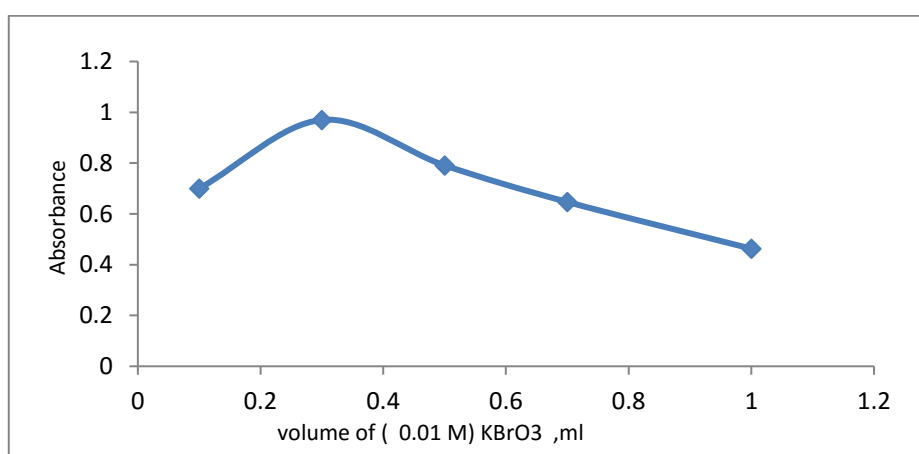


Figure 3: Effect KBrO3(0.01M) volume.

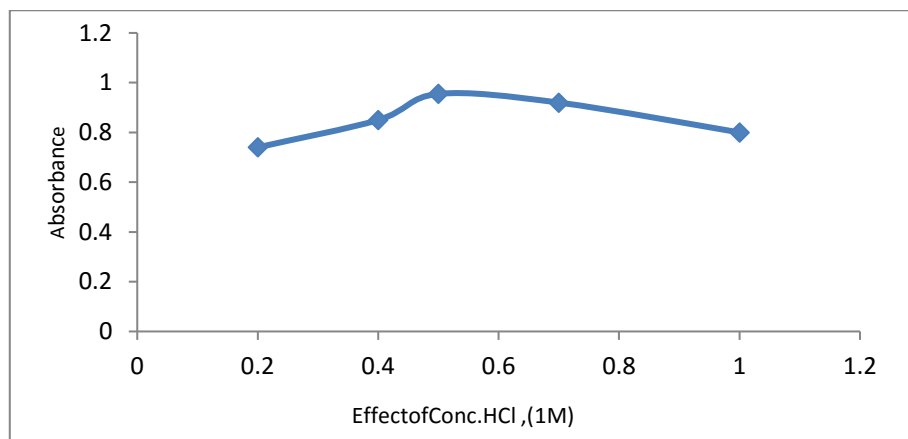


Figure 4: Effect of HCl (1M) volume.

Table 1: Effect of sequence addition.

Subsequent	Absorbance
D+O+A+R	1.03
D+R+O+A	0.410
R+O+A+D	0.408
O+R+D+A	0.385

it was obtainable from [BDH] company that have purity (99 %). This solution prepare by dissolve 0.0417 g

potassium bromate by distilled water in to 25 ml with the in calibrated flask.

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

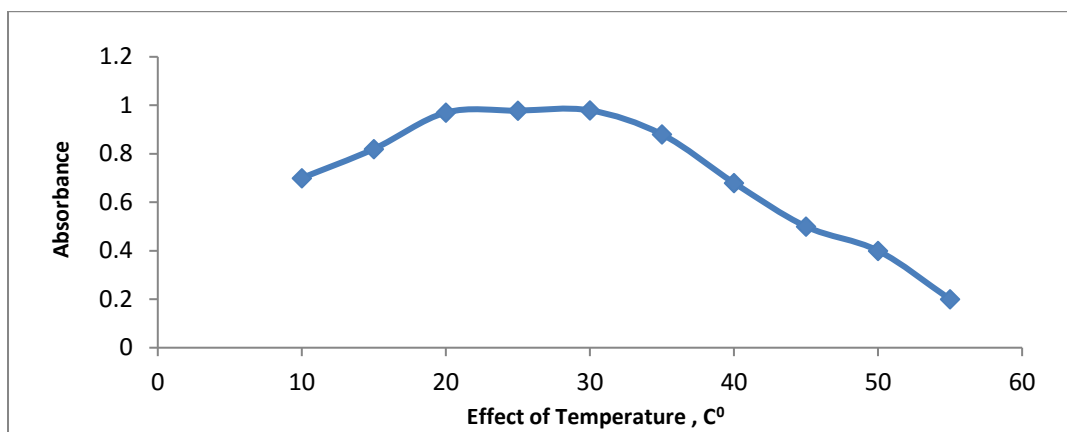


Figure 5: Effect the Temperature.

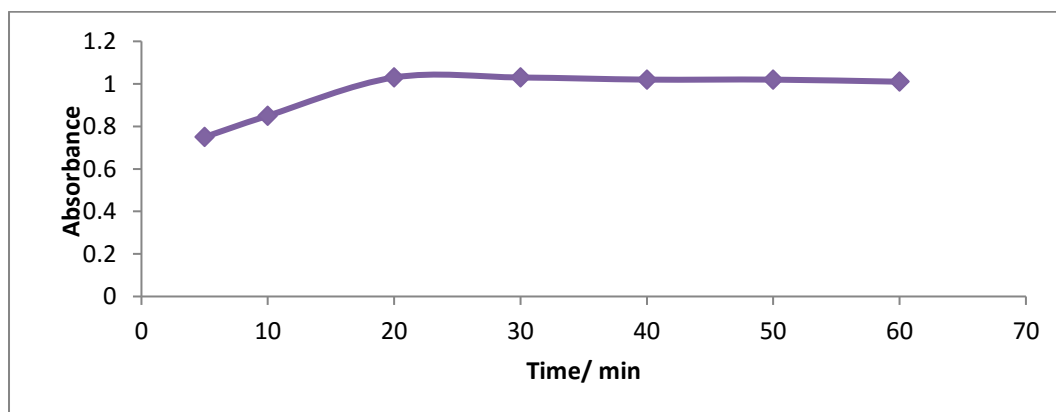


Figure 6: Effect of reaction time

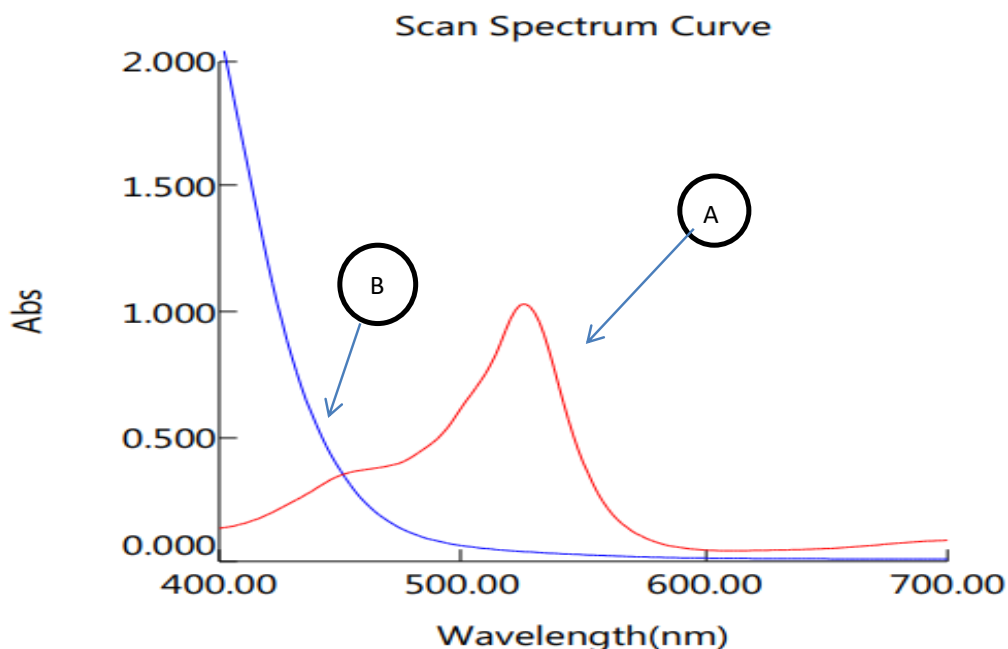


Figure 7: Showed (A) Absorption spectrum of the colored product CZP ($2 \mu\text{g. mL}^{-1}$), and (B) Absorption spectrum of blank solution (CPZ, KBrO₃, HCl) .

powder leave the mixture a bout (15 min) then filtered in a volumetric flask and completed the volume in the final

by using distilled water as a stock solution. Thereafter transferred at 100.0 mL volumetric flask then completed

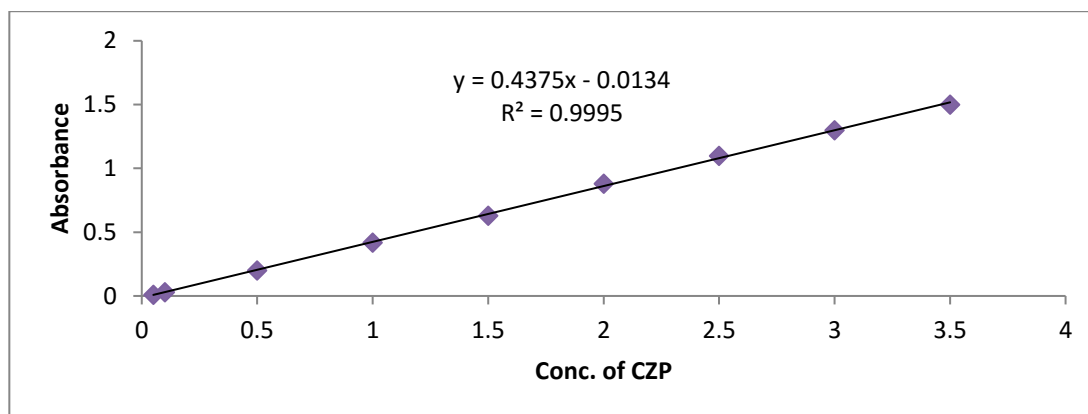


Figure 7: The calibration curve of (CZP).

Table 2: Analytical characteristics of developed procedure to determination CZP.

Parameter	Value
R egression equation	Y=0.4375x-0.0134
slope	0.4375
Correlation coefficient (R ²)	0.9995
Linear range . µg/ ml	0.05-3.5
Molar absorptivity ε L/mol.cm	1.381x10 ⁴
Sandal' sensitivity .µg cm ²	0.0228
Limit of Detection .µg ml.	0.0061
Limit of Quantification .µg ml	0.020

Table 3: Accuracy and precision of the prosed method.

Conce.* of CZP µg ml ⁻¹		E*. %	Rec*%.	RSD*%.
Taken	Found			
0.5	0.495	-1	99.00	0.659
2	2.010	0.5	100.50	1.069
2.5	2.450	2	98.00	0.405

*It's the Average of three Readings.

Table 4: Application of effect the Excipient on the estimation of 2 µg ml⁻¹ (CZP).

Excipient	Conc*. clonazepam found (µg mL ⁻¹)	Error.* (%)	Recovery* (%)
Starch	2.05	2.5	102.5
Mg stearate	2.04	2	102
Lactose	1.95	-2.5	97.5
PVP	2.01	0.5	100.5

Table 5: Examination of clonazepam in bulk drug and dosage forms.

pharmaceutical preparations* .	Average recov* . *%	
	Proposed method*.	Standard method*.
pure CZP(2ppm)	99.166	99.85
Rivotril (Tablets 2 mg)*	100.733	101.98

by using distilled water. Thereafter take the appropriate

volume each of solutions was treated under the conditions that were used in the based way of working was to find concentricity adopted on the calibration curve.

General recommended procedure

In the series of volumetric flasks 10ml , taken from the standard solution 100 µg. ml⁻¹ reduced clonazepam with the concentration 0.05-3.5 µg. ml⁻¹ mixed with (0.3 ml of 0.01 M) from potassium bromate, there after add 0.5 ml (of 0.01 M) chlorpromazine then addition 0.5ml of 1M HCl and completed to the final mark with distilled water .The absorbance of the all color product complex was measured at (530 nm) respectively after(20 min) for all solution versus the blank reagent and Calibration curve was Constructed .

RESULTS AND DISCUSSION

A Study the conditions optimum for reaction between reduced CZP drug with (CPZ) as a new chromogenic reagent that reacted existing (KBrO3) as oxidizing reagent. Various conditions have been studied that affected the absorbance of the product colored formed to until to improve it.

Effect of volume chlorpromazine reagent

A effect of (CPZ) volume reagent on the color intensity product absorption was examined form taken vary volume 0.3-2 ml of (CPZ) (0.01 M) presence (0.5)ml of the potassium bromate and 0.5 ml of 1M HCl and 2ug.mL⁻¹ (CZP). The best volume was (0.5) ml that mean optimum volume in the experiments and gives that the maximum intensity absorption and it was upon in the following of experiments Fig(2)

Effect of volume of potassium bromate(0.01M) as oxidizing agent

Various volume of KBrO3 (0.3_1.0) ml at concentration (0.01M) and adding (0.5)ml of (0.01M) CPZ reagent and (0.5 ml) of(1M) HCl obtained higher color intensity, which was used in the following experiments.

Effect of volume HCl(1M)

A Based on an presence of HCl acid that give increasing the intence of the colourd products, Consequently , the different volumes of (1M) HCl were examined, and it was found 0.5 mL from this acid gave the highest absorbance , which chosen in subsequence experiment. (Figure 4).

Effect of addition order

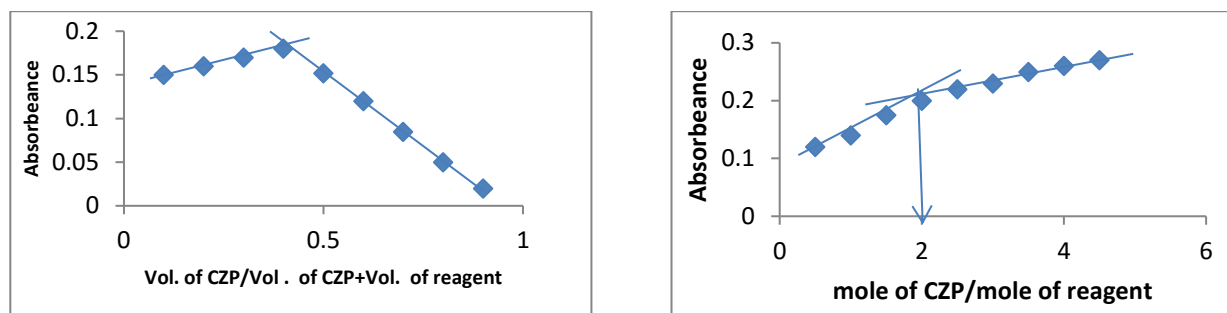
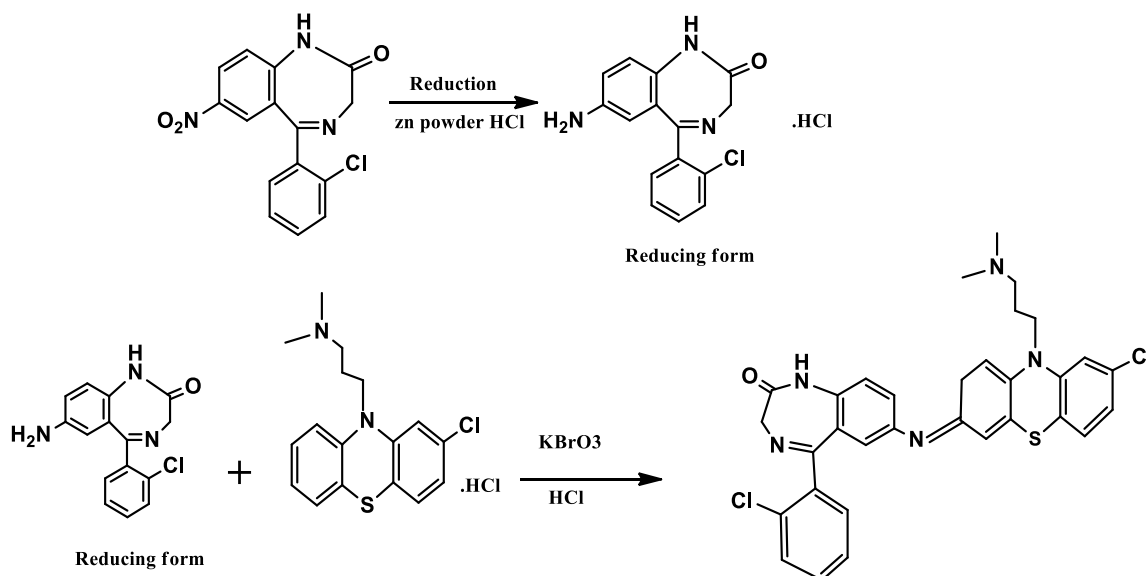


Figure 8: Continuous Variation (Job's method) and Mole Ratio for reaction (CZP) with (CPZ) reagent .



Scheme 1: The Proposed mechanism reaction between reduced (CZP) with (CPZ) in the existing of (KBrO3) as oxidant reagent and HCl.

The best result of order addition effect that (D +O +A +R) where (D = drug; O = oxidizing; R = reagent) because it give maximum absorption which selected in subsequent experiment Table (1).

Effect of the Temperature

Temperature effected on the colourd intensity of the products were studied between clonazepam (CZP) and chlorpromazine (CPZ) in the presence KBrO3 and acidic solution at different temperature (0-60°C) . The colourd products were more stable in the temperature (20-30°C) which gave highest absorbance was obtained, whereas higher temperature give low absorbance may be cause to the partial dissociation of complex.(Fig.5)

Effect of reaction of Time

Optimum coupling reaction was estimated at different time periods (5_60 min) for development absorption of clonazepam (CZP) with the reagent (CPZ) in the existing of oxidative agent of KBrO3 in the acidic medium . A colour products for (CZP) obtained after (20 min) which remain constant at least (60 min). Fig(5)

Absorption Spectra

The spectral scan was accomplished to obtain the product coupling between (CZP)and (CPZ) in the of presence oxidizing agent KBrO3 and acidic solution HCl against the blank solution ,Fig(6) shows the absorption maximum at (530 nm) where [A] spectrum Absorption

describe colored product and [B] the spectrum of the blank reagent .

Calibration curve

After the conditions optimum, the spectrum colored product for reaction clonazepam with chlorpromazine in presence potassium bromate as a oxidant reagent .shows in Fig(7). A Linear calibration graph obeyed the concentration of (0.05-3.5) Ppm . The slope curve(0.4316), molar absorptivity 1.381×10^4 L/mol.cm . Sandell sensitivity $0.0228 \mu\text{g cm}^{-2}$ and Detection limit $0.0061 \mu\text{g ml}^{-1}$. The are shows [LOQ] $0.020 \mu\text{g .ml}^{-1}$. Each the Analytical values for determination (CZP) drug illustrated in the Table (2).

Accuracy and Precision

The Accuracy and Precision developed analytical method was used to estimation of CZP using CPZ reagent by taking three different concentrations of CPZ and calculation each of Relative Error value (%E) and Recovery value (%Rec.) and Standard Deviation value (R.S.D). The results shows in table (3) indicated the analytical method are good Accuracy with precision.

Stoichiometry Reaction Ratio

The stoichiometry ratio between clonazepam (CZP) drug and chlorpromazine (CPZ) from through using Job's method and mole ratio method under the analytical optimum conditions. The obtained result was (1:1) (Drug:

Table 6: Comparison between The proposed and Standard methods.

Pharmaceutical preparations	Proposed method		Standard method	
	Recovery.*% $\bar{x}_{i(1)}$	$(X_{i(1)} - X_1)^2$	Recovery *.*% $\bar{x}_{i(2)}$	$(X_{i(2)} - X_2)^2$
pure CZP	99.166	0.1638	99.85	1.1342
Rivotril (2 mg)	100.733	0.1638	101.98	1.1342
	$X_1 = 99.9495$	$\Sigma = 0.3276$	$X_2 = 100.915$	$\Sigma = 2.2684$

T-(Value) exp. = -0.8475, Critical T- Value =4.303, F-(Value) exp. = 0.1444, Critical F- Value =19.000

Reagent complex) was formation at wavelength 530 nm. show Fig. (8) while the stability constant can be calculated by used (five times) of reagent for each concentration of (CZP)with (CPZ) equal $2.16 \times 10^5 \text{L}^2 \cdot \text{mol}^{-2}$.

Depending on the reaction between clonazepam drug and chlorpromazine is explained in Scheme (1). Chlorpromazine. HCl have a strong electrons donating that make to coupling with the reduced clonazepam after attainment the oxidation by (KBrO₃) to form oxidative coupled reaction³⁵.

Interference

The effect different additives studied were: Starch, PVP, Mg Stearate, Lactose that containing in clonazepam pharmaceutical preparations by addition separately excess quantities ten: one from each excipient to 20 µg /ml (CZP) solution by using conditions optimum reaction and following in the calibration curve. (Table-4) the final results explain no effect interfere in the proposed analytical method.

APPLICATIONS

A applicability for the method for examination of the pharmaceutical formulations was examined. A results of the examination obtainable formulations of clonazepam drug and pharmaceutical is summarized in table (5). When the average of three estimation while the standard method taken from the British Pharmacopoeia. The results were replicated and the examination formulations was express checked by Standard method³⁶.

Evaluation the Results of the Proposed Method

To exam the competence and success for the methods proposed analytical have to compare between results that maintained applied in the standard method that taken by the British Pharmacopoeia. By through utilize several statistical tests (T and F) tests. To explain the confidence of the proposed method with observed there is no considerable variation between the methods. Table (6): Applying (T and F) test to make compare accuracy with precision of the proposed method with the standard method for the oxidative coupling product (CZP) and (CPZ).

CONCLUSION

A precise, sensitive and simple spectrophotometric method for the purpose of estimation of very little amounts of clonazepam in the aqueous solution depended its coupling oxidative reaction with CPZ and KBrO₃ presence HCl (1M). The method applying, more successfully for the estimation of small an amounts commercial (CZP) drug and pharmaceutical depended on

the oxidative coupling product between the clonazepam with chlorpromazine as a new chromogenic agent in the existing of KBrO₃ as oxidative reagent.

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