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Development of the new spectrophotometric method for determination of Chlordiazepoxide by ion-pair complex formation reaction

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Abstract

The study focuses on the development of a spectrophotometric method for quantifying chlordiazepoxide in its pure form and pharmaceutical formulation. The developed method is based on the formation of a colored ion-association complex through the reaction of chlordiazepoxide with Bengal rose B dye, facilitated by KH-phthalate buffer at pH 4.0. The effects of various factors on the formation of the complex were studied at 568 nm., in the concentration range of 3 to 30 µg. mL^{-1}. The calibration curve exhibited linearity following Beer's law, demonstrating molar absorptivity and Sandell's sensitivity were $1.2771x10^4$ $L.mol^{-1}.cm^{-1}$and 0.0234 $\mu g \cdot cm^{-2}$ respectively. The detection limit was determined to be 0.0190 $\mu g \cdot ml^{-1}$, while the quantitation limit reached 0.0633 $\mu g \cdot ml^{-1}$. Investigation of the reaction stoichiometry revealed a 1:1 molar ratio between chlordiazepoxide and the dye. The standard addition method has been used to determine chlordiazepoxide drug quantity and ensure there were no interferences with pharmaceutical additives.

Keywords: Bengal rose B dye, Chlordiazepoxide , Ion-pair complex, Spectrophotometric method.

التقدير الطيفي للكلورديازيبوكسيد باستخدام تفاعل تكوين معقد التجمع الايوني

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الخلاصة

تركز الدراسة على تطوير طريقة طيفية لتقدير الكلورديازيبوكسيد بشكله النقي وفي مستحضره الصيدلاني(أقراص).تعتمد الطريقة المطورة على تكوين معقد تجمع ايوني ملون بين الدواء قيد الدراسة وصبغة وردة البنكال ب وباستخدام محلول منظم من فثالات البوتاسيوم الهيدروجينية عند pH4.0 .تم دراسة عدد من العوامل المؤثرة على تفاعل تكوين المعقد عند الطول الموجي 568 نانوميتر.في مدى التراكيز من 3الى 30 مايكروغرام .مل$^{-1}$.اظهر منحنى المعايرة خطية وفقا لقانون بير ،مبينا ان معامل الامتصاص المولاري و حساسية ساندل كانتا $1.2771X10^4$لتر.مول$^{-1}$.سم$^{-1}$ و0.0234 مايكروغرام .سم$^{-2}$ على التوالي .كان حد الكشف 0.0190 ميكروغرام. مل$^{-1}$ بينما بلغ الحد الكمي 0.0633 ميكروغرام. مل$^{-1}$.كشفت دراسة نسبة التفاعل عن نسبة مولية 1:1 بين الكلورديازيبوكسيد والصبغة واستُخدمت طريقة الإضافة القياسية لتحديد كمية دواء الكلورديازيبوكسيد والتأكد من عدم وجود تداخلات مع المضافات الصيدلانية.

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1. Introduction

Chlordiazepoxide, chemically known as 7-chloro-2-methylamino-5-phenyl-3H-1, 4-benzodiazepine-4- oxide(Fig.1) [1], is a member of the benzodiazepine class that is broadly used as a sedative and anxiolytic, and enhance effectively the neurotransmitter gamma-aminobutyric acid (GABA) complex formation, which strengthens pharmacological activity of benzodiazepine derivatives. These effects include not only anxiolytic activity but also sedative and hypnotic effects, skeletal muscle relaxant and anticonvulsant activity[2,3].

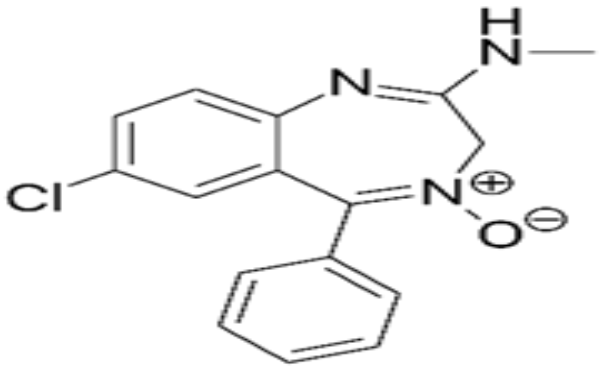


Figure 1: Chemical structure of Chlordiazepoxide

Numerous techniques have been employed to quantify chlordiazepoxide in its pure form, pharmaceutical combinations, and biological samples, including high-performance liquid chromatography (HPLC) [4], UHPLC-MS/MS [5], HPTLC[6], LC-MS/MS[7,8], GC-MS[9], electrochemical methods [10-12], and spectrophotometric methods[13-18].
Ionic association complexes are formed through Coulombic attraction between oppositely charged ions without covalent bonding, a mechanism widely applied in analytical chemistry to determine a variety of pharmaceutical and organic compounds. Organic dyes have been employed in the formation of ionic pair complexes to determine pharmaceutical compounds [19,20].

This study introduces a sensitive, precise, and rapid spectrophotometric technique using Bengal rose B dye to quantify chlordiazepoxide in pharmaceutical preparations. The method eliminates the need for extraction steps or temperature control, streamlining analysis while maintaining accuracy.

Experimental

Apparatus

A SHIMADZU UV-VIS spectrophotometer (UV-1900i) with a double beam was employed, while the BEL-Sensitive balance was used for precise weighting, and solution pH was measured using a BP3001 pH meter.

Chemicals used

Chlordiazepoxide solution ,(100 µg·mL^{-1})was prepared by dissolving 0.0100 g (S.D.I-Iraq) in 5 mL absolute ethanol, followed by dilution to 100 ml with distilled water in a volumetric flask.

Bengal rose B solution, (1x10^{-3} M) was prepared by dissolving 0.5088 g of pure reagent (Fluka) in distilled water and completed the volume in 50 mL volumetric flask with the same solvent to obtain (1x10^{-2} M) then transferred 10 mL from this solution into a volumetric flask (100 mL) and finally marked up with distilled water [21].

KH-Phthalate buffer solution,(pH 4.0) was prepared by mixing suitable volumes of 0.1 M KH-phthalate and 0.1 M of NaOH and adjusting pH 4.0 using a pH meter [22].

Pharmaceutical preparation, (100 µg·mL^{-1}): twenty Libroxide® tablets containing either 5 mg or 10 mg of chlordiazepoxide were accurately weighed and finely ground. Portions weighing 0.3842 g (from 5 mg tablets) and 0.1833 g (from 10 mg tablets), each equivalent to 0.0100 g of pure chlordiazepoxide were dissolved in 5 mL ethanol absolute and diluted to 100 mL with distilled water in a volumetric flask, then shaken, filtered, and treated as the general procedure.

General procedure

Using a spectrophotometric method, a set of 10 mL volumetric flasks were filled with standard chlordiazepoxide solution in increasing amounts (3–30 µg. mL^{-1}). Next, 1 ml of KH-phthalate buffer (pH 4.0) and 1.5 mL of 1×10^{-3} M Bengal rose B solution were added to each flask. The volume was then filled with distilled water, and the absorbance at 568 nm was measured.

Results and Discussion

This study utilizes a reaction between chlordiazepoxide and Bengal rose B at pH 4.0, driven by electrostatic interaction between the secondary amino group of the drug and Bengal rose B's anionic functional group in an acidic medium. The resulting ion pair association exhibits a reddish-orange with maximum absorbance at 568 nm (Fig. 2).

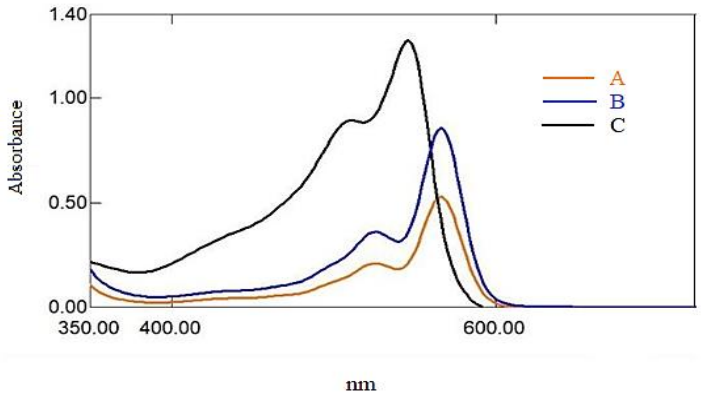


Figure 2: Absorption spectra of: (A)reaction product of chlordiazepoxide (10 µg. mL $^{-1}$), (B) reaction product of chlordiazepoxide (20 µg. mL $^{-1}$) against blank of Bengal rose B, (C)absorption of blank.

Optimization conditions

The method achieved high sensitivity by studying the influence of many parameters, including reagent concentration, pH, temperature, and development time.

Effect of pH

The study evaluated 1 mL of citrate, acetate, and KH-phthalate buffers across pH ranges of 3.8–5.0. Results indicated that KH- phthalate buffer produced the highest absorbance of the complex at pH 4.0, leading to its selection in further experiments, as shown in Fig.3.

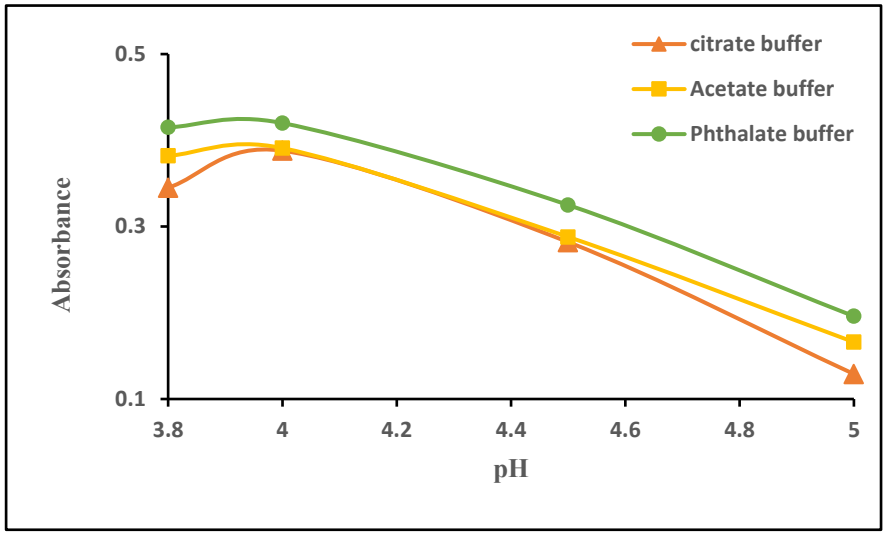


Figure 3: Effect of type buffer solution.

Effect of KH- phthalate buffer solution amount

The effect of KH-phthalate buffer (pH 4.0) volume on absorbance was tested using 0.5–3 mL (Figure 4). 1 mL yielded the highest absorbance, therefore, it was used as an optimum volume in further experiments.

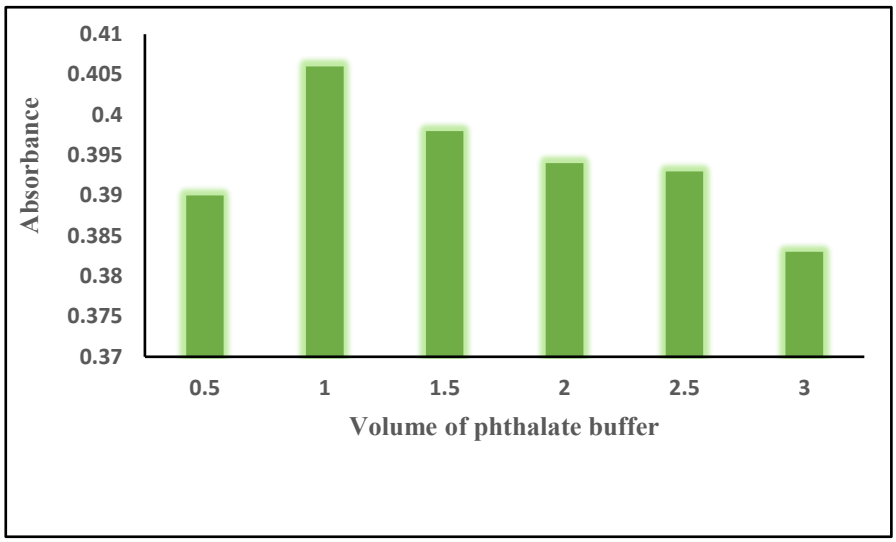


Figure 4: Effect of KH-phthalate buffer volume on the intensity of coloured complex.

Effect of Bengal rose B amount:

To choose the optimum volume of Bengal rose B, different volumes of 0.5- 2.5 mL of the dye concentration ($1x10^{-3}$M) were introduced into a set of volumetric flasks (10 ml). A 1.5ml of reagent produced the highest absorbance and was chosen in all subsequent experiments, as shown in Fig.5.

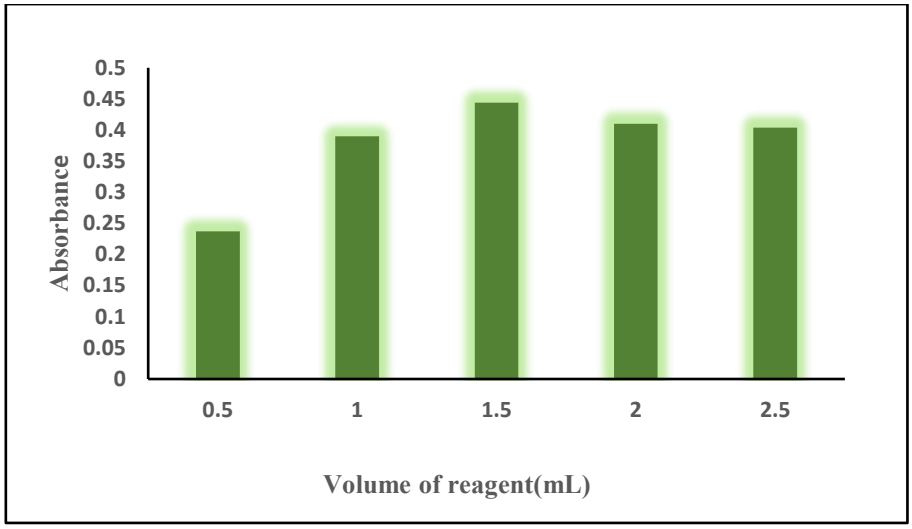


Figure 5. Effect of Bengal rose B volume on the intensity of coloured complex.

Effect of Temperature and time on the ion pair complex.

The study investigated ion-pair complex formation at 0 º C, RT=22±2 ºC, and 40 °to determine the optimal temperature of the reaction as shown in (Figure 6).

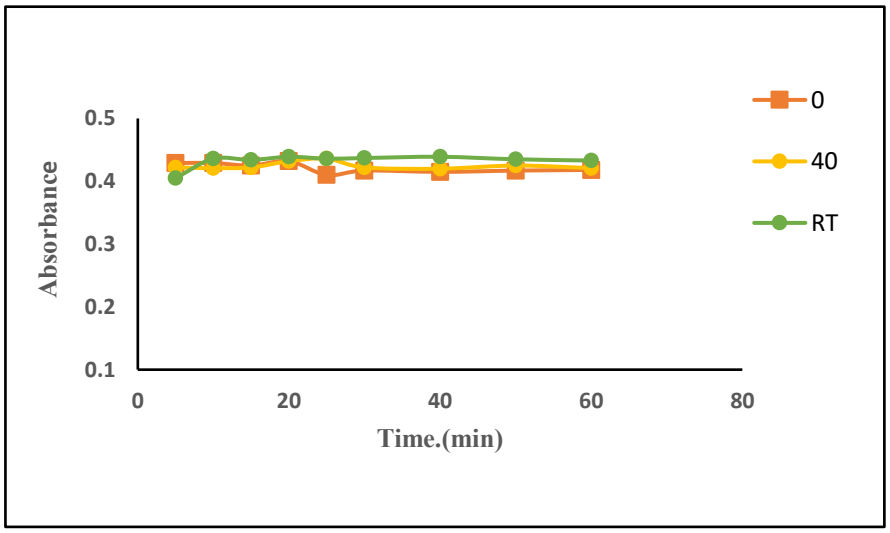


Figure 6: Effect of temperature and time on ion-pair complex formation

The results in Fig.6 showed that the absorbance of ion-pair complex is not greatly affected by temperature changes and the highest absorbance of the complex was obtained after 10 minutes of addition of Bengal rose B to Chlordiazepoxide in KH-phthalate buffer at room temperature and remained stable for another 50 minutes.

Table 1 presents the optimum parameters of the proposed method.

Table1: Optimum experimental conditions of developed colorimetric method

Parameter	Optimal
Wavelength, (nm)	568
pH	4.0(KH-Phthalate buffer)
Buffer volume, (mL)	1.0
Bengal rose B(Ix10^{-3}) M, (mL)	1.5
Standing time,(min.)	10
Time of stability, (min)	60

Calibration curve

The absorbance demonstrated linear proportionality to chlordiazepoxide concentrations within the range of 3-30 µg.ml^{-1}, with a deviation of the linearity at a higher concentration than 30µg.mL^{-1}. The molar absorptivity was 1.27x10^{4} l.mol^{-1} .cm^{-1} and Sandell's index value was 0.0234 µg.cm^{-2}(Fig. 7).

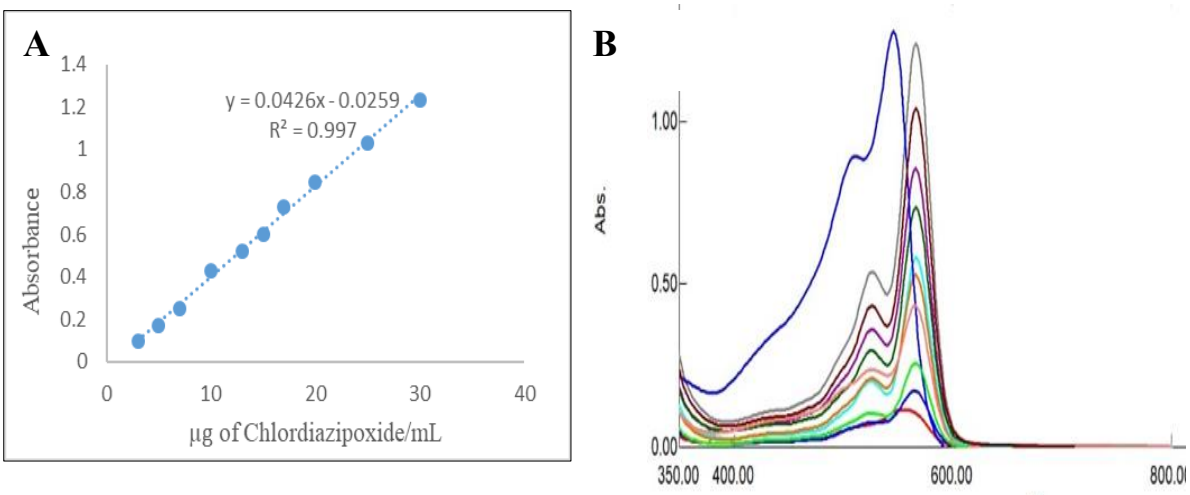


Figure 7: (A)Calibration graph for gradient concentration of Chlordiazepoxide , (B) gradient spectrums according to calibration curve verses blank.

The limit of detection (LOD=0.0190 µg·ml^{-1}) was calculated using the following equation:
LOD= 3σβ/ S
σβ: is the standard deviation of n blank signals
S: is the slope of the calibration curve
10 volumetric flasks that contain blank solution were prepared and each was measured spectrophotometrically at 568 nm against distilled water. And σ was found using the following equation:
$\boldsymbol{\sigma}=\sqrt{\Sigma(\boldsymbol{Xi}-\bar{\boldsymbol{X}})^2/\boldsymbol{n}-1}$
Xi: is absorbance for each blank measurement
n: is the number of blank measurements
$\bar{X}$: is the mean of n blank signals

While the limit of quantification (LOQ=0.0633µg·ml^{-1}) was calculated using the following equation: $\boldsymbol{LOQ}=\boldsymbol{10\sigma\beta}\ /\boldsymbol{slope}$ [23] .

Stoichiometric study

Job's method (continuous variation) [24] and slop ratio method [25] were used to study the composition of the ion-pair complex between Chlordiazepoxide and Bengal Rose B dye using equimolar solutions (1×10^{-3} M). In job's method, to a series of 10 ml volumetric flasks, different volumes of the drug from 0.25 to 2.8 mL,1mL of KH-phthalate buffer solution, and different volumes of dye from 2.75 to 0.2 mL were added so that the final volume of the two components (drug and dye) in all volumetric flasks are equal, the volume was completed with distilled water, and the absorbance was measured at 568nm. The results obtained in (Fig.8) referred to that the ion pair association was formed by1:1 concerning the drug to dye.

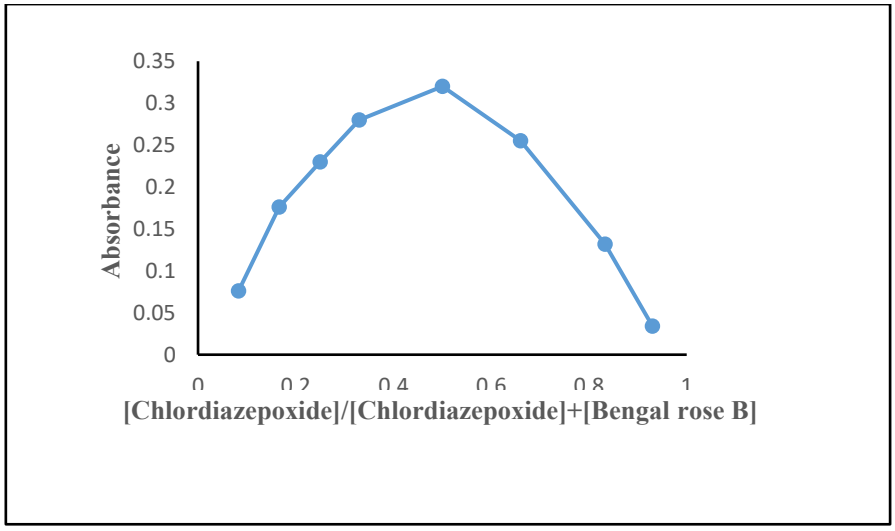


Figure 8: Job's method for ion-pair complex

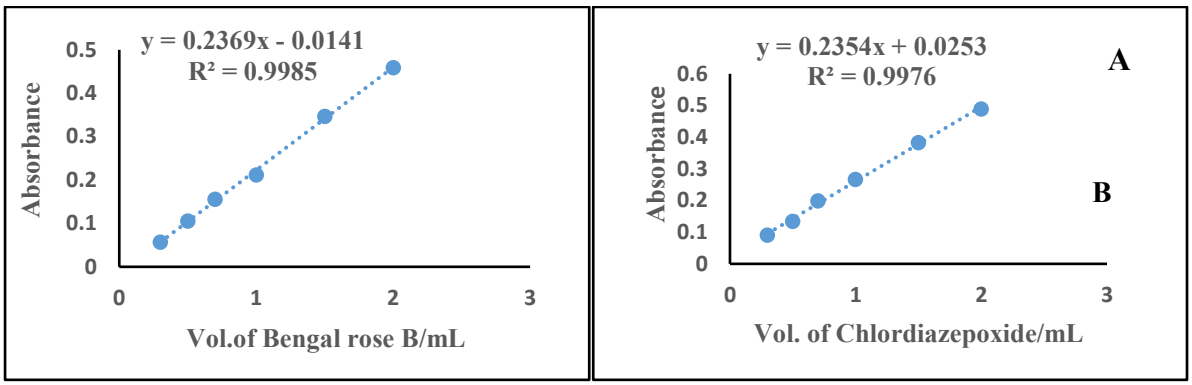
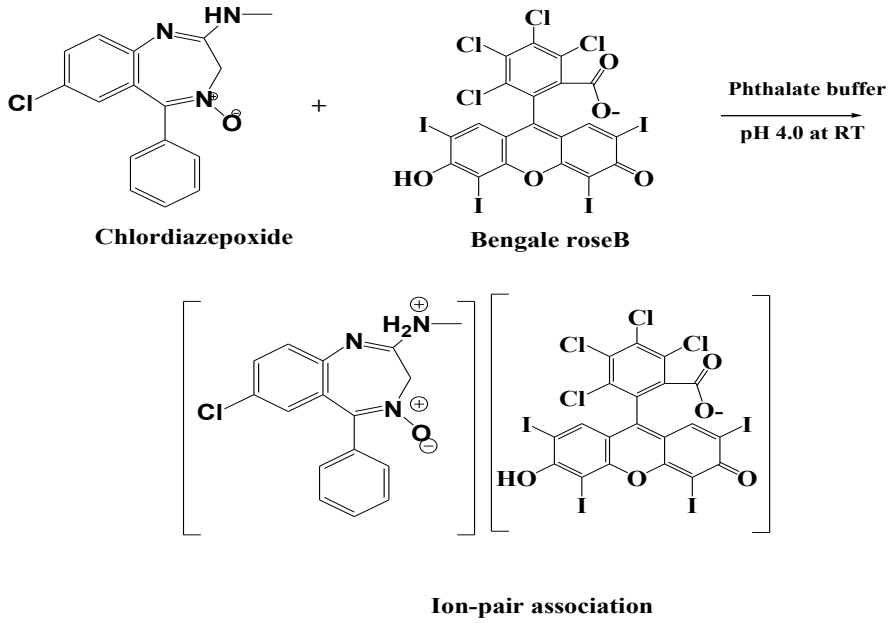


Figure 9: Slope ratio method, (A) volume of Chlordiazepoxide against absorbance, (B) volume of Bengal rose B against absorbance

The slope ratio method also has been studied. First, it was done by fixing the volume of the drug and changing the volume of the dye, then the volume of the dye was fixed and changing the volume of the drug to obtain two linea r graphs as shown in Fig 9:

Based on the results presented in Figures 8 and 9, the proposed reaction mechanism of formed product is illustrated in Scheme 1 [21]:



Scheme 1: The suggested reaction for ion pair association.

Analytical applications

The present method was studied by determining the amount of the drug in pharmaceutical preparation (Tablets). Additionally, the results of the proposed method were statistically examined by applying a t-test for accuracy, using the following equation [26]: $t_{exp}=(\mu-\bar{X})\sqrt{N}/S$

Where:

μ: Certified amount of drug

$\bar{X}$: Average of recovered amount

S: Standard deviation

n: Number of replicates

The results are cited in Table 2.

Table 2: Analytical application for estimation of Chlordiazepoxide in dosage form (tablets).

Pharmaceutical preparation	Amount taken ($\mu g.mL^{-1}$)	Amount measured ($\mu g.mL^{-1}$)	Recovery*, %	Relative error,%	RSD,%	Drug contains (mg)	t-test
Libroxide® Tablets (5 mg-SDI)	5	4.90	98.00	-2.00	1.64	4.90	0.3098
	10	10.23	102.30	+2.30	1.25	5.11	
	15	15.21	101.40	+1.40	1.30	5.07	
Libroxide® Tablets (10 mg-SDI)	5	5.21	104.20	+4.20	1.91	10.42	1.4733
	10	10.28	102.80	+2.80	0.93	10.28	
	15	14.91	99.40	-0.60	0.86	9.94 10.04	

*Average of four determinations

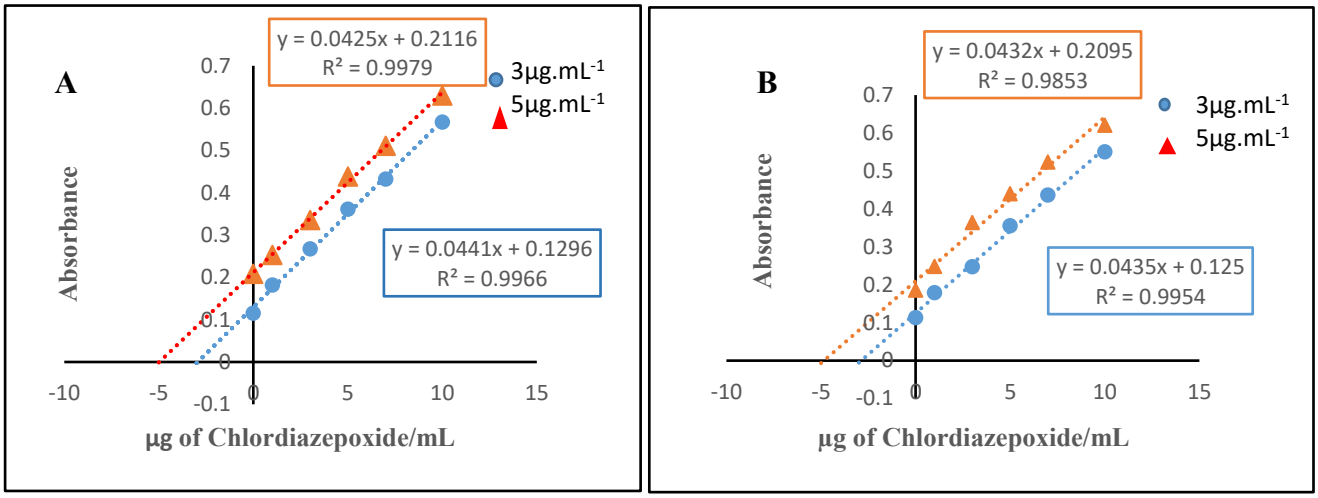


Figure 10: Standard addition method curve for determining Libroxide ® Tablets in two doses (A) 5 mg, (B) 10mg

The experimental t-test value fell below the theoretical value (t=3.18), at the 95% confidence level for 4 replicates, confirming no appreciable difference is observed between the certified value and the determined amount.

Standard additions method

A standard addition method was applied to validate the method's efficacy, demonstrating its accuracy in quantifying chlordiazepoxide in tablets, as supported by (Fig.10 and Table 3).

Table 3: Standard addition method for determining Chlordiazepoxide.

Pharmaceutical Preparation	µg Chlordizepoxide present/mL	µg Chlordizepoxide measured /mL	Recovery, %	Drug content(mg)
Libroxide 5 mg/Tablet (S.D.I Iraq)	3	2.93	97.66	4.88
	5	4.97	99.40	4.97
Libroxide 10 mg/Tablet (S.D.I Iraq)	3	2.87	95.66	9.56
	5	4.84	96.99	9.68

Conclusion

The study presents a simple, accurate, and sensitive spectrophotometric method for determining Chlordiazepoxide through an ion-pair association reaction with Bengal rose B dye at pH 4.0. This approach eliminates the need for solvent extraction or heating, enabling efficient analysis of pharmaceutical preparation (tablets) successfully.

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