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DETERMINATION OF CHLORDIAZEPOXIDE BY INDIRECT SPECTROPHOTOMETRIC METHOD VIA DIAZOTISATION AND COUPLING REACTION

Safaa A. Zakaria*, Nabeel S. Othman

Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq

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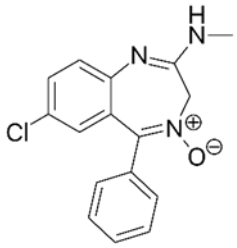
*Corresponding author
safaa-ahmed2017@uomosul.edu.iq

Graphical abstract



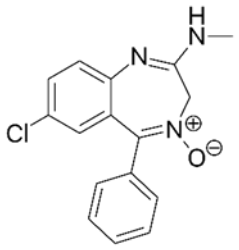
Abstract

The investigation research involves the development of a new spectrophotometric method for determining chlordiazepoxide indirectly in both pure and pharmaceutical forms by either measuring the absorbance or area under the peak. The main reaction is based on acidic hydrolysis of chlordiazepoxide after that converted the resulting compound to its corresponding diazotized form then coupling with 2,4,6-trihydroxyacetophenone to produce a yellow azo dye in a basic medium. The effects of different factors on intensity of resulting azo dye were investigated. In concentration ranges of 0.5 to 15 µg. mL^{-1} the plots were linear by monitoring the colored product's absorption at 432 nm as well estimating the area under the peak within range of 425-440 nm. Sandell sensitivity were 0.0156 and 0.07142 µg·cm^{-2}, the determination coefficient were found to be 0.9967and 0.9944, the molar absorptivity were$1.4781x10^4$ and $0.3243x10^4$ $L.mol^{-1}.cm^{-1}$, respectively. The reaction ratio between hydrolysed drug and the reagent was studied and found to be 1:1. Measuring absorbance or area under the peak provide a promising tool for the sensitive and accurate determination of chlordiazepoxide indirectly in its pure form and commercial dosage forms (Tablets).

Keywords: Spectrophotometric method, diazotization, chlordiazepoxide, 2,4,6-trihydroxyacetophenone

1.0 INTRODUCTION

The IUPAC name for the generic name chlordiazepoxide is 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide (Figure 1) was the first benzodiazepine drug to be introduced in 1960. It is a crystalline powder, nearly white or pale yellow in color, insoluble in water, and slightly soluble in ethanol. It has sedative, hypnotic, and anxiolytic effects that are dose dependent. It is a central nervous system depressant. In addition, It has anticonvulsant and muscle-relaxing properties. It might also be helpful in treating the withdrawal symptoms from ethanol. Similar to other medications in the benzodiazepine class, chlordiazepoxide exhibits a strong affinity for the benzodiazepine binding site found in the gamma-aminobutyric acid-A (GABAA) receptor complex. Consequently, it strengthens GABA's effects and promotes inhibitory synaptic transmission [1, 2].



M.wt. 299.80 g/mol.

Figure 1 Chemical structure of chlordiazepoxide

Various techniques were used for determining either Chlordiazepoxide alone or in the presence of other compounds in both different dosage forms and biological samples, enlist electrochemical analysis by different electrodes [3-5], GC-M [6], high-performance liquid chromatography (HPLC) [7], LC-MS/MS[8,9], UHPLC-MS/MS[10],HPTLC[11]and spectrophotometric methods[12,13,14,15,16].

The idea of this research involves using the diazotization coupling reaction for determining relied Chlordiazepoxide on the formation of corresponding aminobenzophenone after acidic decomposition followed by converting the free amino group in the compound to diazonium salt using equimolar concentration of NaNO2 to become ready to react with coupling agent 2,4,6-trihydroxyacetophenone. A yellow azo dye is formed and studied by monitoring absorbance at 432 nm and area under the peak within range of 425-440 nm.

2.0 METHODOLOGY

Instruments

Measurements of absorbance and spectral were obtained with great care using a double beam SHIMADZU UV-VIS spectrophotometer (UV-1900i) with two glass cells have 1 cm light path. A BEL-Sensitive balance was used to accurate and precise weight, also a BP3001 pH meter was used to measure the pH of solutions under investigation.

Reagents and Solutions

The reagents utilized in this research were of a high degree of purity and the medication in its pure form and dosage form will be obtained from The State Company for Drug Industry and Medical Appliances - Samarra" (SDI).

Hydrolysed chlordiazepoxide standard solution(HCLD), 50µg/mL: This solution was prepared by dissolving 0.0250g of standard chlordiazipoxide in 25 mL of 6 M hydrochloric acid and then heating the solution for 1 hour in a boiling water bath. The hydrolysed drug was transferred into a 50 mL volumetric flask and completed the volume to the mark with distilled water [17], then from this solution 10 mL was transferred into a 100 mL volumetric flask and completed the volume using the same solvent. This solution was transferred to a darkened bottle and remained stable for one month if it was kept at room temperature.

Sodium Nitrite, 2.15 x 10^{-4} M

This solution was prepared by dissolving 0.0148 g (BDH) in a few ml distilled water then the mixture was transferred to a 100 ml in a volumetric flask and completed the volume with the same solvent then 10 ml of this solution was transferred to a 100 ml volumetric flask and diluted with the same solvent, finally, it was transferred to a darkened bottle and remained stable for one month at room temperature.

2,4,6-Trihydroxyacetophenone Solution,0.05%

A 0.0500 g (Sigma) of 2,4,6-trihydroxyacetophenone was dissolved in 5ml ethanol absolute with good mixing then the volume was completed with distilled water in a 100ml volumetric flask. Finally, the reagent was transferred to a darkened bottle and remained stable for one week.

Sodium hydroxide Solution,1M

Using a volumetric flask and distilled water, the concentrated ampoule solution(BDH) was diluted to make 1000 ml of this solution, which was then stored in a plastic container.

Pharmaceutical Preparation,50 µg/mL

20 tablets (Libroxide ® 5 and 10 mg chlordiazepoxide) were weighed accurately and powdered then an equivalent to 0.0500 g of chlordiazepoxide of the powder was taken and dissolved in 50 mL of 6M HCl and after shaking and filtering the solution 25 mL was transferred into a beaker and the hydrolysis process and preparation solution were performed as described previously.

3.0 RESULTS AND DISCUSSION

Primary Experiment

To a 10 ml volumetric flask ,1 ml of HCLD, 1ml of $NaNO_2$ (2.15 x 10^{-4}), 1mL of 2,4,6-trihydroxy acetophenone and 1 ml of sodium hydroxide (1M) were added respectively then completed the volume to the mark with distilled water. The maximum absorbance was recommended at 432 nm in the next experiments.

Optimal Conditions

The following tests evaluated the optimum condition which affect the azo dye's absorption. Among these conditions were:

Effect of Acid

The effect of different acids on the reaction process has been studied and the highest absorption of the product formed was obtained when no acid was used and depended only on the acidity resulting from hydrolysis of the sample to produce diazonium salt, the details are shown in Table 1.

Table1 Effect of acid on the intensity of yellow azo dye

1mLofacid, (1M)	Absorbance	λ_{max},nm	$\Delta\lambda^*$,nm	Final pH
HCl	0.080	448	122	2.18
H_2SO_4	0.051	458	132	1.93
HNO_3	0.168	429	103	3.08
CH_3COOH	0.126	484	158	5.36
Without	**0.312**	**432**	**106**	**12.64**

Effect of Reagent Amount

To find the optimum volume of the reagent, different concentrations of HCLD with different volumes of reagent concentration (0.05%) were introduced into a set of 10 ml volumetric flasks. A 2ml of reagent which gave the highest value for correlation coefficient was chosen and fixed in the subsequent experiments (Table 2).

Table 2 Effect of 2,4,6-trihydroxyacetophenone on the intensity of yellow azo dye

Reagent, (0.05%)	Absorbance of µg HCLD/mL					R^2
	0.5	1.5	2.5	5	7.5	
0.5	0.035	0.089	0.153	0.290	0.412	0.9982
1.0	0.043	0.098	0.159	0.306	0.422	0.9972
1.5	0.053	0.101	0.179	0.314	0.472	0.9980
2.0	0.062	0.129	0.189	0.326	0.493	0.9984
2.5	0.088	0.136	0.194	0.318	0.480	0.9968

Effect of Type and Amount of Different Bases

A 1mL of (1M) for different bases have been employed as a reaction medium before dilution of the components of the reaction. The highest absorbance was obtained when NaOH was used, therefore it was selected and fixed in the subsequent experiments, as seen in Figure 2.

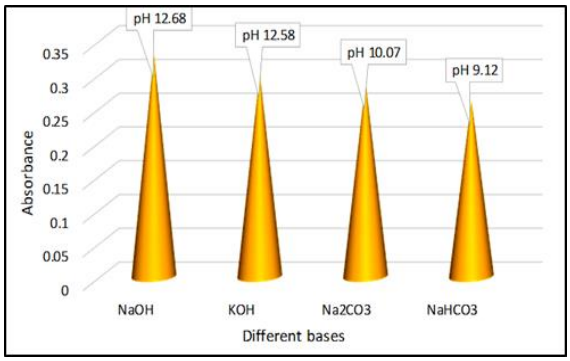


Figure 2 Effect of different types of bases on absorbance

Also, different volumes of the selected base were studied with gradient concentrations to know their effect on the intensity of the yellow azo dye. A high correlation coefficient with high absorbance was obtained when 1.5 mL of NaOH was added, as seen in Table 3.

Table 3 Effect of different amount of bae the intensity of yellow azo dye

ML of NaOH,1M	Absorbance of µg HCLD/mL				R^2
	1.5	2.5	5	7.5	
0.5	0.126	0.168	0.321	0.148	0.0612
1.0	0.116	0.168	0.335	0.480	0.9987
1.5	0.119	0.176	0.343	0.506	0.9996
2.0	0.118	0.160	0.340	0.491	0.9965

Effect of Addition Sequences

Different sequences were studied for high stability and intensity for yellow azo dye. The experiments ensured that the best sequence was HCLD, sodium nitrite, reagent and base which utilized in the initial investigation. Alternatively, caused a loss in absorbance, so it was fixed and dependent.

Absorption Spectrum

The absorption spectrum was taken for azo dye product which resulted from the reaction of 50 µg of HCLD with 1mL of $2.15x10^{-4}$ $NaNO_2$ followed by adding 2mL of (0.05%) 2,4,6-trihydroxyacetophenone in the presence of 1.5 mL of 1M sodium hydroxide. The formation of a colored azo dye gives at the wavelength of 432 nm the highest absorption (Figure 3), hence for all subsequent measurements, 432 nm was fixed.

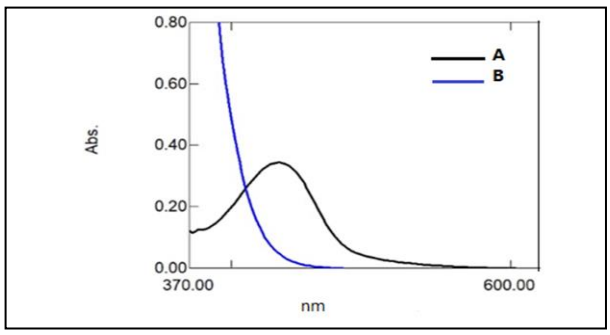


Figure 3 Absorption spectra (A)50 µg HCLD versus reagent blank and (B)reagent blank versus Distilled water

Calibration Graph

Using the optimal conditions outlined in the preceding experiments, a linear calibration graph for the HCLD assay via the recommended method is created, showing that Beer's law is followed over the concentration range of 0.5–15 µg/ml with a determination coefficient of 0.9967. The molar absorptivity of the yellow azo dye product was 1.4781×10^4 $l.mol^{-1}$. Cm^{-1} and Sandell index was 0.0156 µg /cm^2, Figure 4.

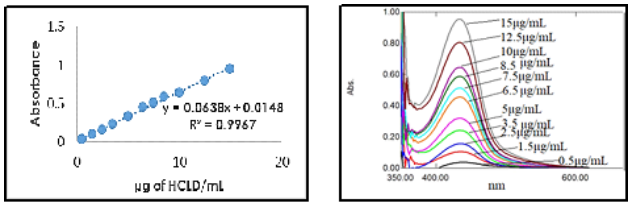


Figure 4 Calibration graph with their spectrum for determination of HCLD

The Stoichiometry of the Colored Azo Dye

The stoichiometry reaction between HCLD and 2,4,6-trihydroxyacetophenone was investigated by applying the Job's and Mole ratio methods using equimolar solutions (2.15×10^{-4} M) of HCLD and 2,4,6-trihydroxyacetophenone [THAP], as seen in Figure 5.

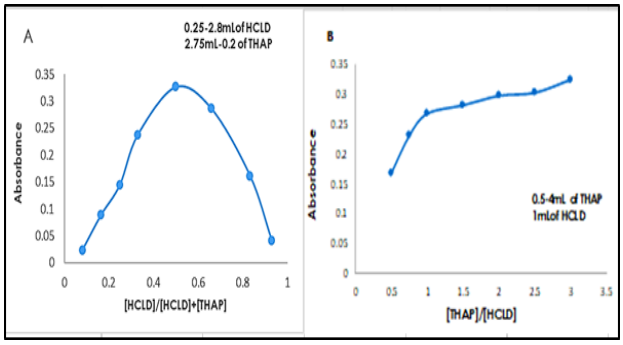
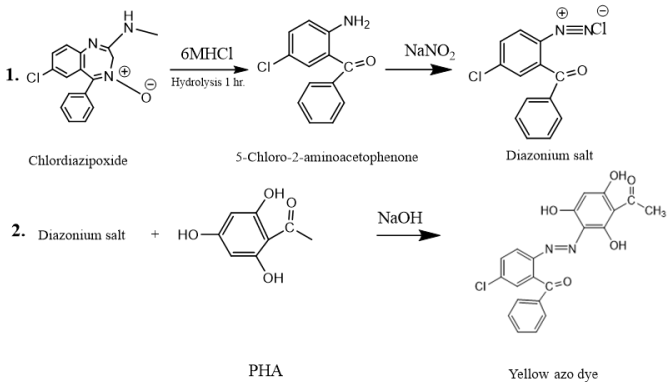


Figure 5A Job's method plot, B: Molar ratio method plot

Figure 5 indicates that the ratio of formed azo dye is 1:1 HCLD to THAP. Thus, the mechanism of forming the yellow azo dye which product under diazotisation and coupling reaction may be recommended in accordance with the Scheme that follows:



Scheme 1 Suggested reaction in two steps:1. forming diazonium salt ,2.coupling reaction

Analytical Application

HCLD was determined in its pharmaceutical preparations (tablets) and the results are shown in Table 4.

Table 4 Analytical application for pharmaceutical in dosage form (tablets)

Pharmaceutical preparation	Libroxide® Tablets (10mg-SDI)			Libroxide® Tablets (5mg-SDI)		
Amount taken (µg/ml)	7.5	5	2.5	7.5	5	2.5
Amount measured(µg /ml)	7.53	4.96	2.42	7.48	4.97	2.43
Recovery* %	100.4	99.20	96.80	99.73	99.40	97.20
Relative error, %	+0.40	-0.80	-3.20	-0.27	-0.60	-2.80
RSD, %	0.99	0.49	2.96	0.27	1.33	1.83
Drug contains (mg)	10.0	9.92	9.68	4.98	4.97	4.86
t-test	0.54	1.91	1.70	0.56	0.63	2.66

*Average of three determinations

Standard Addition Method

The standard addition method was also used to determine the drug content in the tablets to prove that there was no interference with additives used in pharmaceutical preparations. Two concentrations were taken from two doses of Libroxide ® Tablets (5

and 10 mg) after hydrolysis, followed by adding different concentrations of HCLD under study in its pure form, the total concentration of it must be within the calibration curve then the solutions were treated according to the optimal condition for suggested method. Figure 6 and Table 5 show the results were obtained.

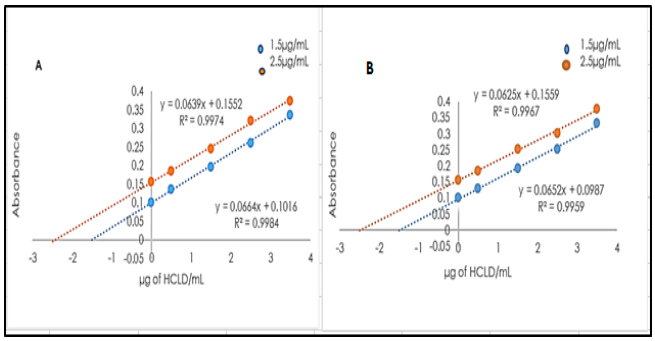


Figure 6 Standard addition method curve for determination two doses of Libroxide ® Tablets tablet (A) 5 mg, (B) 10mg

Table 5 Standard addition method for the determination of HCLD

Pharmaceuti-cal Preparation	µg HCLD present/ mL	µg HCLD measured /mL	Reco-very,%	Drug content (mg)
Libroxide 5mg/ Tablet (S.D.I Iraq)	1.5	1.53	102.00	5.10
	2.5	2.42	96.80	4.84
Libroxide 10mg/ Tablet(S.D.I Iraq)	1.5	1.51	100.66	10.06
	2.5	2.49	99.60	9.96

The second spectrophotometric method was used for determining HCLD in its pure and dosage form depending on measuring peak area instead of absorbance using optimum conditions that were obtained previously in the first method. This method involved detecting a fixed range of wavelengths from 425-440nm including the maximum absorption which depended formerly on measuring a yellow azo dye as shown in Figure 8

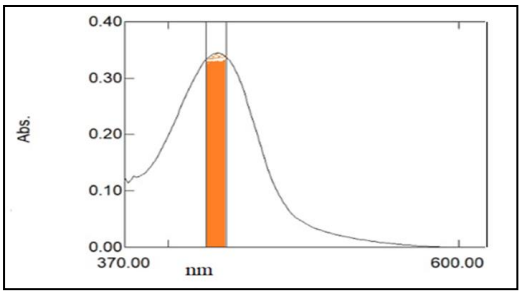


Figure 7 Spectrum of area under the peak for optimum conditions

The peak area of the yellow azo dye solutions was measured at 425-440 nm against the blank solution. A linear calibration graph was obtained over a concentration of 0.5-15 µg HCLD /ml), as shown in Figure 8:

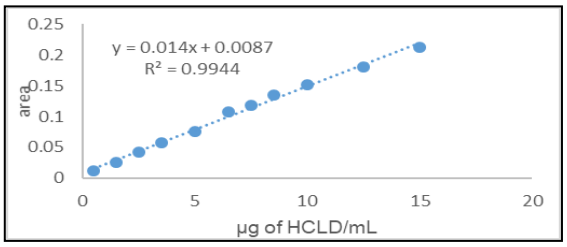


Figure 8 Calibration curve according to peak area method

The suggested area under the peak method has been employed for the quantification of HCLD in tablet dosage form. This method has yielded better results of recovery, accuracy, and precision than the spectrophotometric method depends on measuring absorbance as illustrated in Table 6.

Table 6 Analytical application by peak area method for tablet dosage form

Pharmaceutical preparation	Libroxide® Tablets (5mg-SDI)		
Amount taken µg/ml	2.5	5	7.5
Amount measured µg/ml	2.56	5.00	7.43
Recovery*, %	102.40	100.00	99.06
Relative error, %	+2.40	0.00	-0.94
RSD, %	2.70	0.93	1.08
Drug contains mg	5.12	5.00	4.95

4.0 CONCLUSIONS

For determing trace quantities of chlordiazepoxide in pure and dosage forms, a simple, fast, exact, and practical spectrophotometric methods have been established using two ways for measuring which is based on acidic hydrolysis of chlordiazepoxide to produce a compound with a free amino group then diazotizing it by adding equimolar of sodium nitrite followed by coupling with the reagent 2,4,6-trihydroxyacetophenone in a basic medium. The spectrophotometric method depended on measuring either absorbance or area under the peak for yellow azo dye formed. A high sensitivity was obtained when measuring the absorbance while more recovery, accuracy, and precision were obtained when the area under the peak was measured.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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