Spectrophotometric -Indirect Method for Chlordiazepoxide Estimation in Dosage Forms via Charge-Transfer Complex Formation with Metol

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ABSTRACT

Aims: An accurate, simple, and reliable spectrophotometric indirect method for chlordiazepoxide (CDE) determination in its pure and pharmaceutical dosage forms is demonstrated.

Methods: The method is based on the formation of charge- transfer complex between decomposed CDE and Metol (N-methyl-p-aminophenol sulfate) in the presence of potassium dichromate to produce an intense red complex that is stable and have a maximum absorbance at 516 nm.

Results: The calibration curve shows that Beer's law is obeyed in the concentration range of 3−60 µg/mL, with a correlation coefficient of 0.9994, and a molar absorptivity of 3.24×10^3 L/mol cm. All the variables that affected on the sensitivity and stability of the formed product were studied and optimized

Conclusion: The suggested method was applied successfully for CDE determination in pharmaceutical dosage forms.

Keywords: Chlordiazepoxide, Metol, Charge transfer complex.

INTRODUCTION

Chlordiazepoxide (CDE) is 7-chloro-2-methylamino-5-phenyl-3H-l,4-benzodiazepine-4-oxide [1] (Fig. 1), it is a benzodiazepine class sedative and hypnotic medication used to enhance the effect of the neurotransmitter gamma-amino butyric acid (GABA) resulting in hypnotic, anxiolytic, sedative, anticonvulsant, and muscle relaxant properties [2,3].

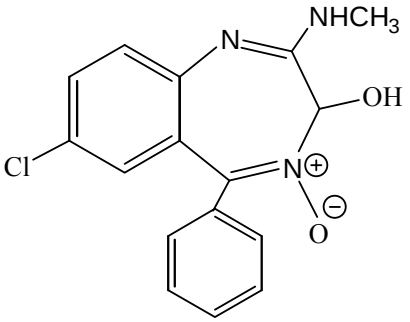

Fig. 1: structure of Chlordiazepoxide

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There are various methods in the literature for simultaneous estimation of CDE in biological samples and dosage forms are reported, these methods including dispersive nanomaterial-ultrasound assisted microextraction[4], LC–MS[5], voltammetry[6,7], flourimetry [8], GC–MS [9], polarography[10], and HPLC methods [11-14]. Although there are very little sensitive visible spectrophotometric methods, the literature contained a simple colorimetric methods for benzodiazepine drugs (included chlordiazepoxide) estimation using diazotization reaction, depends upon the formation of their corresponding amino benzophenones after acidic hydrolysis [15]. This reaction was based on development of simple and selective method based on a charge- transfer reaction between decomposed CDE as n-donor and Metol (N-methyl-p-aminophenol sulfate) as a π- acceptor using potassium dichromate as an oxidant. The measurement of absorbance were established by recording the absorbance of charge- transfer red colored complex at 516 nm against the blank which have a minimum absorbance at the same wavelength.

EXPERIMENTAL

Apparatus

Spectrophotometer (Kyoto-Japan) has a double beam Shimadzu UV–VIS 260 was used in all spectral and absorbance measurements. A 1cm matched quarts cells were used to carry out the absorbance measurements.

Materials and solutions

Throughout this work, the analytical grade reagents were used. Standard Chlordiazepoxide (CDE) was supplied by the State Company for Drug (SDI), Samarra-Iraq. Libroxide ® 5 and 10 mg chlordiazepoxide (SDI, Samarra-Iraq) tablets were obtained from local markets.

Preparation of hydrolyzed chlordiazepoxide standard solution

A 0.025 g amount of standard CDE was accurately weighted and dissolved in a 25mL of 6M hydrochloric acid and heating this solution in a boiling water-bath for 1 h. Then transfer the decomposed drug into 50 mL volumetric flask and completes the volume to the mark with distilled water to gain 500 µg/mL of hydrolyzed CDE solution [15]. The stability of this solution is more than one week if kept in room temperature. Simple dilution was used to prepare more diluted solutions by using the same concentration of acid.

Metol (p-Methyl aminophenol sulfate) (0.025M, Sigma-Aldrich)

This reagent was prepared daily by dissolving 0.4305 g in 50 mL distilled water and keep in dark flask.

Potassium dichromate ($K_2Cr_2O_7$) (0.02M, Sigma-Aldrich)

A 0.2942 g of $K_2Cr_2O_7$ dissolved in distilled water in volumetric flask (50 mL).

Hydrochloric acid solution (6 M, BDH)

This solution was prepared by appropriate dilution of 250.6 mL of concentrated solution (11.97 M) with 500 mL distilled water in volumetric flask then standardized with Sodium carbonate solution.

Preparation the solutions of pharmaceutical tablet

A 30 tablets were accurately weighed and powdered then an amount of powder equal to 0.05 g of CDE were taken and dissolved in 25 mL of ethanol and shaken then filtered (this solution is 2000 µg/mL of CDE). After transferring 12.5 mL of the result solution into a beaker, A 12.5 mL of concentrated hydrochloric acid was added then the hydrolysis process was performed as described previously. After accomplished the decomposition procedure, the decomposed solution was transferred into 50 mL volumetric flask and completed the volume to the mark with distilled water to obtain a 500 µg/mL of CDE solution.

General procedure

A 30-600 µg of CDE was transferred into sequences of 10 mL flasks (cover the range 3-60 µg/mL) then a volume of 1 mL of 0.025 M metol and 0.7 mL of 0.02 M of $K_2Cr_2O_7$ were added. The reactants into the flasks were mixed and diluted with distilled water. The absorbance later measured at 516 nm after 20 min at room temperature (25°C) against reagent blank that containing all materials except CDE. The corresponding calibration curve and regression equation were constructed. For optimization all later experiments, a solution of 25 µg/mL of CDE was used.

Stoichiometric relationship

The stoichiometry of the suggested reaction was accomplished, An equimolar of CDE and metol (1mM for both drug and reagent) under optimum concentration of $K_2Cr_2O_7$ were prepared using Job's method [16]. In Job's method of continuous variation a sequence solutions (total volume of the chlordiazepoxide and metol was 5 mL) have been prepared. Decomposed drug and reagent in different complementary ratio (0:5, 1:4, 2:3, 3:2…..5:0) were mixed, diluted and directed under the suggested procedure in 10 mL volumetric flask. And the absorbance was measured at 516 nm. Fig. 2 showed that a 1:1 ratio product (CDE: metol) is formed**.**

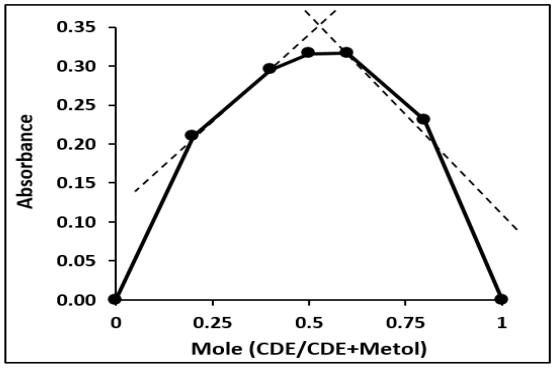
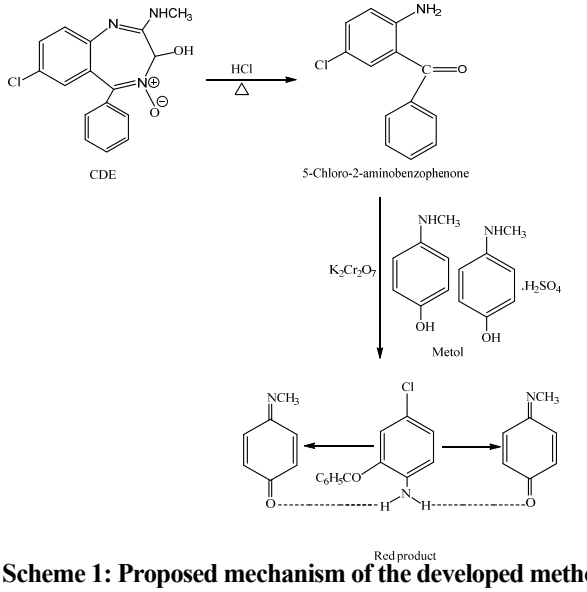


Fig. 2: Job's method

Metol is p-methylaminophenol sulfate, $C_{14}H_{20}N_2O_6S$, is widely used in charge transfer reaction as π-accepter after its oxidation with oxidizing agent [17]. The red product (charge transfer complex) can be formed by electron transfer from the highest π molecular orbital of hydrolyzed CDE to the lowest empty orbital (π*) of two adjacent p-N-methyl benzoquinoneimine molecules (formed under oxidizing metol with potassium dichromate). The two p-N-methyl-benzoquinoneimine molecules are being closer by primary arylamine through hydrogen bonding thus facilitating the simultaneous overlap of the π molecular orbital of CDE with the π* orbitals of the two p-N-methylbenzoquinoneimine molecules as shown in Scheme 1 [18].



Scheme 1: Proposed mechanism of the developed method

RESULTS AND DISCUSSION

Absorption spectra

As the previous procedure, the reaction between the decomposed CDE and metol in the presence of $K_2Cr_2O_7$ produced an intense red colored charge-transfer complex has the absorption spectrum that have been recorded in Figure 3. The spectrum was obtained after 20 minutes from the beginning of the reaction and the maximum wavelength was appeared at 516 nm.

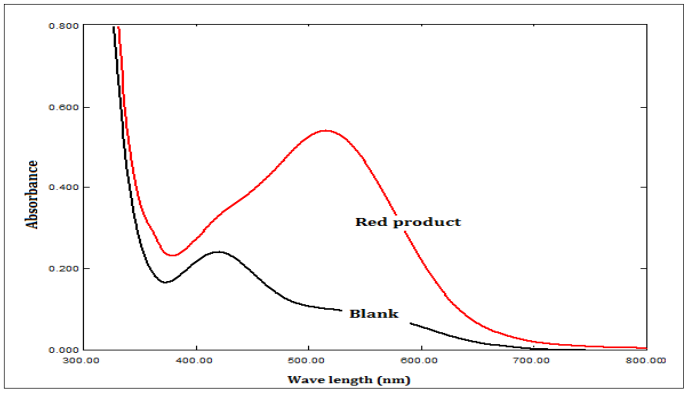


Fig. 3: Absorption spectra of 25 µg/mL of decomposed CDE treated with metol/$K_2Cr_2O_7$ and measured against blank and the blank against distilled water.

Optimization of reaction variables

The experimental factors such as the concentrations of reagents, order of addition, reaction medium, stability time and temperature, that affecting mainly on the sensitivity of the colored product were studied by variable one parameter with the time, while keeping the other constant. All experiments were done using 25 µg/mL of CDE and the measurements of absorbance were carried out after 20 min from the beginning of the reaction at laboratory ambient temperature (25 ± 2°C) and 516 nm.

Effect of reaction time

Effect of reaction time

On the value of absorbance after dilution was considered (Table 1). Absorbance of product started to be stable after 20 min and remains stable more than 55 min.

The large stability time offer many advantages one of them is measuring large number of samples at any time within the period without changing in the values of readings. After optimization all variables, this experiment was repeated and the results were the same.

Table 1. Effect of the time on the reaction

Time, minute	2	5	10	15	20	25	30	40	50	60	70
Absorbance	0.183	0.246	0.295	0.321	0.338	0.338	0.339	0.344	0.345	0.345	0.342

Effect of temperature and order of addition

Three different temperatures were used for examine this parameter on suggested reaction (5, 25, and 65°C) and the experiment indicated that maximum absorbance was attained at 25°C, more than at 5 or 65°C, which that due to increase the coupling affinity between the reactants at room temperature (Fig.4). As a result a 25°C was chosen in all experiments.

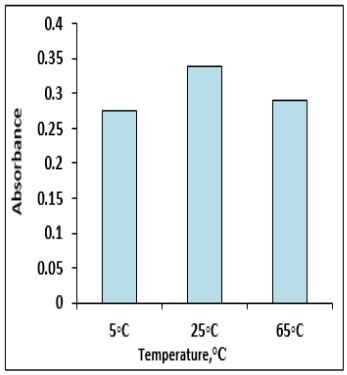


Fig. 4: Effect of temperature

Also different order additions of reagents were studied and between them the order (CDE+ metol +$K_2Cr_2O_7$) was gave the highest response for both suggested methods and accordingly was chosen (Fig. 5).

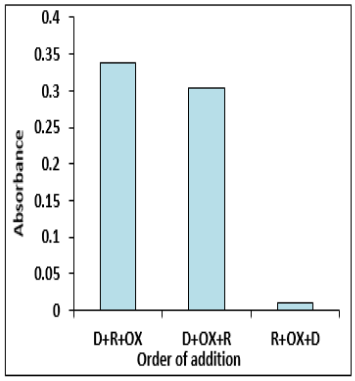


Fig. 5: Effect of order of addition (D; CDE, OX; $K_2Cr_2O_7$, R; metol)

Effect of reagents concentration (metol and $K_2Cr_2O_7$)

Effect of variable volumes of metol 0.025M (from 0.2–1.5 mL), were examined. The results indicated that (1 mL) of metol appeared the best absorbance of products with minimum blank value (Fig.6a), so 1mL was selected for the next experiments. $K_2Cr_2O_7$ was found to be a useful oxidizing agent for the proposed reaction, other oxidizing agents like ($K_2S_2O_8$, $FeCl_3$, $K_3Fe(CN)_6$) have also examined but none of them give product except $K_2Cr_2O_7$. Variable volumes of $K_2Cr_2O_7$ 0.02 M (from 0.1-1 mL) were studied and optimized (Fig.6b). A 0.7 mL of $K_2Cr_2O_7$ gave the higher absorbance of products, so it was used in all the following experiments.

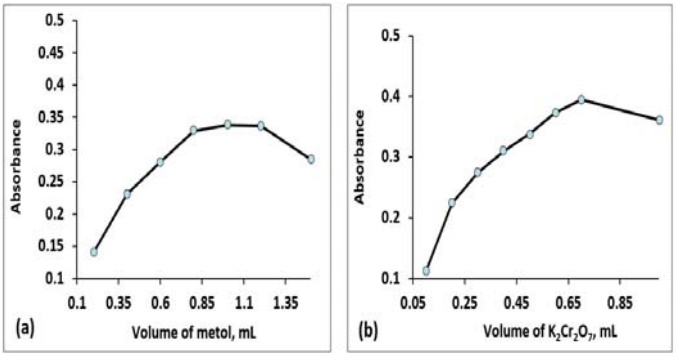


Fig. 6: Selected best volume of (a) metol (b) $K_2Cr_2O_7$.

Effect of the medium of reaction

Under experiments the products was appeared at the moments of addition and reacted all reactants. It was noticed that the amount of acid used for hydrolyzed of CDE was sufficient for progress of reactions, while additional acidity would not change the absorbance. The

basic medium lead to disappear the red color of complex; therefore, there is no need to add an acidic or basic solution for the proposed reaction.

Method of validation

Linearity

After study and optimization all the experimental variables, a standard curve was prepared (Fig. 7). Into a sequences of 10 mL flasks 30-600 µg of CDE was transferred (cover the range 3-60 µg/mL) then a volume of 1 mL of 0.025 M metol and 0.7 mL of 0.02 M of $K_2Cr_2O_7$ were added. The reactants into the flasks were mixed and diluted with distilled water. The absorbance later measured at 516 nm (at ambient temperature) after 20 min. The regression equation, slope, correlation coefficient and molar absorptivity values besides statistical values were considered and recommended in Table 2, which referred to good linearity and high sensitivity in estimation of CDE.

Table 2. Summary of characteristics data for suggested method

Parameter	Value
λmax (nm)	516
Regression equation	Y= 0.0108x + 0.1125
Linearity range (µg/mL)	3-60
Correlation coefficient(r)	0.9994
Linearity percentage, % r^2	99.87
Slope, b (mL/µg)	1.08×10^{-2}
Intercept, a	1.13×10^{-1}
$S_{y/x}$	8.01×10^{-3}
S_b	1.39×10^{-4}
S_a	4.38×10^{-3}
LOD (µg/mL)	1.16
LOQ (µg/mL)	3.85
RSD (%)	0.3-0.6
Average of recovery (%)	101.13
Molar absorptivity (L/mole cm)	3.24×10^{3}

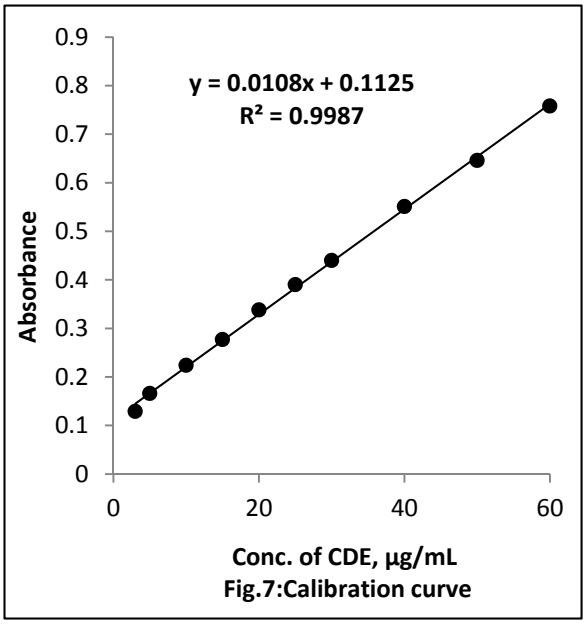


Fig.7:Calibration curve

Accuracy and repeatability

To estimate the accuracy of suggested methods, and repeatability of readings, three altered concentrations solutions of CDE were prepared. The assay process was applied in five replicates and the RSD% was obtained. The satisfactory results in Table 3 showed that a low values for the RSD (good precision) and values of relative error (accuracy) of methods were attained.

Table 3. Accuracy and precision of the suggested method

Sample	Conc. of CDE, (µg/mL)		Error*	Rec. %	RSD%
	Present	Found			
1	20	20.01	0.05	100.05	0.27
2	25	25.34	1.36	101.36	0.52
3	30	30.59	1.97	101.97	0.57

Study of method specificity (pharmaceutical additives)

Examination of the specificity and selectivity of the suggested methods were done by analysis of target drug in the existence of 10-fold of common additives which often accompany CDE in its dosage forms. The satisfactory obtained recovery values demonstrating no interfering

with these additives were observed which representing the selectivity of the proposed methods (Table 4).

Table 4. Effect of common additives

Additives (250 µg/mL)	Conc. of CDE, µg/mL		Rec.%	RSD%
	Present	Found		
PVP	25	25.19	100.76	0.89
Talc		25.18	100.72	0.34
Starch		25.46	101.84	0.60
Mg-stearate		25.37	101.48	1.00
All the above		25.66	102.64	0.88

Analytical applications

The estimation of CDE in tablets was applied successfully in current procedure. Two doses of tablets containing 5 and 10 mg of CDE have been analyzed by applying direct and standard addition methods. The solutions of tablets were prepared as we mentioned previously, and the results obtained in Table 5 and Table 6 (good precision and high recoveries best than 97%) were agreement with those of common method (UV method) [19], using t-and F-tests (95% confidence level) [20]. The obtained results tabulated in Table 6 (calculated values <<tabulated values) showed that there was no significant differences in accuracy or precision between the standard and the two proposed methods.

Table 5. Estimation of CDE in tablets using standard addition method

Dosage form	Taken conc. (µg/mL)	Pure drug added conc. (µg/mL)	Total found conc. (µg/mL)	(%Rec.± SD) n=4
Libroxide® Tablets (5mg-SDI)	20	5	24.38	97.52±0.21
		10	29.20	97.33±0.15
	30	5	34.35	98.14±0.40
		10	39.00	97.50±0.12
Libroxide® Tablets (10mg-SDI)	20	5	25.02	100.08±0.23
		10	30.17	100.57±0.31
	30	5	35.45	101.29±0.17
		10	40.3	100.75±0.44

Table 6. Comparison and application of the current and UV methods directly a, average of 5 readings; b Theoretical value.

Pharmaceutical form	Proposed method					UV method				
	Taken conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%)[a]	Mean Rec. (%)	RSD (%)[a]	Taken conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%)[a]	Mean Rec. (%)	RSD (%)[a]
Libroxide® Tablets (5mg)	30	29.48	98.27	97.65	0.97	20	19.66	98.30	98.56	0.58
	40	38.81	97.03		0.81	30	29.61	98.70		0.15
						40	39.47	98.68		0.33
Libroxide® Tablets (10mg)	30	31.13	103.77	102.02	0.23	20	20.23	101.15	100.77	0.37
	40	40.11	100.27		0.99	30	30.17	100.57		0.65
						40	40.24	100.60		0.12
Pure CDE				101.13					100.89	
t (2.776)[b] F (19.000)[b]	0.073 3.098	$(n_1-1)=2$, $(n_2-1)=2$, $(n_1+n_2-2)=4$								

a, average of 5 readings; b Theoretical value.

CONCLUSION

Charge-transfer reaction is one of the common significant reactions used for the analysis of the drugs. There are few papers that proposed for estimation of CDE; but most of them requiring a complicated steps and high cost techniques in addition they are not sensitive. The suggested method is simple, specific, and did not require an extraction or solvents consumption or any expensive technique. The current study proved that batch system is uncomplicated and cost effective method for estimation of CDE in tablets. The suggested method was applied successfully for analysis of CDE in tablets with good accuracy and precision, and proved that they can be used routinely in quality control studies.

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الطريقة الطيفية غير المباشرة لتقدير الكلورديازيبوكسيد في أشكال الجرع عن طريق تكوين معقدات انتقال الشحنة مع الميتول

هند هادي* ومريم جمال*

***قسم الكيمياء، كلية العلوم، جامعة بغداد، الجادرية، بغداد، العراق.**

ملخص

الأهداف: تم توضيح طريقة طيفية غير مباشرة دقيقة وبسيطة وموثوقة لتقدير الكلورديازيبوكسيد (CDE) في أشكال الجرع النقية والدوائية.

الطريقة: تعتمد الطريقة على تكوين معقد انتقال الشحنة بين CDE المتفكك والميتول (كبريتات N–الميثيل–بارا–أمينوفينول) بوجود ثنائي كرومات البوتاسيوم لإنتاج مركب أحمر مستقر ولديه أقصى امتصاص عند 516 نانومتر.

النتائج: يوضح منحنى المعايرة أن قانون بير يطاع في مدى التراكيز 3–60 مكغم/مل ، ومعامل ارتباط قدره 0.9994 ، وكانت الامتصاصية المولارية 3.24 × 103 لتر/مول.سم.

الاستنتاجات: تم دراسة جميع المتغيرات التي تؤثر على حساسية واستقرار الناتج المتكون وتحسينها. وتم تطبيق الطريقة المقترحة بنجاح لتقدير دواء CDE في أشكاله الصيدلانية.

الكلمات الدالة: الكلورديازيبوكسيد ، ميتول ، معقد انتقال الشحنة.

تاريخ استلام البحث 2018/9/26 وتاريخ قبوله للنشر 2019/3/25.