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Spectrophotometric Determination of Chlordiazepoxide in Pharmaceutical Formulations via Oxidative Coupling Reaction with Phenothiazine

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Abstract

A sensitive, precise and reliable indirect spectrophotometric method for the determination of chlordiazepoxide (CDE) in pure and pharmaceutical dosage forms is described. The method is based on oxidative coupling reaction between amino group resulting from acidic decomposition of CDE with phenothiazine in the presence of sodium periodate to produce an intense green soluble dye that is stable and shows a maximum absorption at 602 nm. The calibration plot indicates that Beer's law is obeyed over the concentration range of 0.1−50 µg/mL, with a molar absorptivity of 1×10^4 L/mol cm and correlation coefficient of 0.9994.All the conditions that affecting on the stability and sensitivity of the formed product were studied and optimized and the suggested method was effectively applied for the determination of CDE in commercial dosage forms.

Keywords: Chlordiazepoxide, phenothiazine, oxidative coupling reaction.

التقدير الطيفي للكلورديازيبوكسيد في الأشكال الصيدلانية بواسطة تفاعل الازدواج التاكسدي مع الفينوثيازين

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الخلاصة

يتضمن البحث استخدام طريقة طيفية غير مباشرة ذات حساسية ، دقة ، و مصداقية لتقدير دواء الكلورديازيبوكسيد في شكله النقي ومستحضراته الصيدلانية. تعتمد الطريقة المقترحة على تفاعل الازدواج التاكسدي بين مجموعة الامين الناتجة من التحلل الحامضي للدواء مع كاشف الفينوثيازين بوجود بيريودات الصوديوم لتكوين صبغة خضراء ذائبة بالماء و مستقرة تعطي أعلى امتصاص عند الطول الموجي ٦٠٢ نانومتر. منحني المعايرة يطاوع قانون بير وبمدى التركيز (٠,١- ٥٠) مايكروغرام\مل, وكانت قيمة الامتصاصية المولارية مساوية إلى ١×١٠[٤] لتر\مول .سم, وبمعامل ارتباط لا يقل عن ٠,٩٩٩٤ . و قد تم دراسة جميع الظروف المؤثرة على استقرارية وحساسية الناتج المتكون و تثبيتها, وتبين أن الطريقة المقترحة طبقت بنجاح لتقدير الكلورديازيبوكسيد في أشكال مختلفة من الأشكال الصيدلانية التجارية .

الكلمات المفتاحية: الكلورديازيبوكسيد ، الفينوثيازين ، تفاعل الازدواج التاكسدي.

Introduction

Chlordiazepoxide (CDE) chemically known as 7-chloro-2-methylamino-5-phenyl-3H-l,4-benzodiazepine-4-oxide (Figure 1), is a benzodiazepine showing powerful antianxiety effects in humans and effectively used to enhance the effect of the neurotransmitter gamma-amino butyric acid resulting in sedative, hypnotic, anxiolytic, anticonvulsant and muscle relaxant properties [1-3].

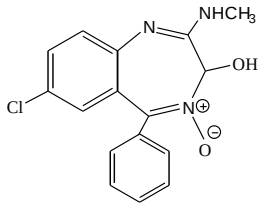

Figure 1. Chlordiazepoxide

The literatures reported different methods for simultaneous determination of CDE both in pharmaceutical formulations and biological samples, including spectrophotometry [4-6], flourimetry [7], dispersive nanomaterial-ultrasound assisted microextraction [8], voltammetry [9,10], polarography [11], capillary electrophoresis [12], gas chromatography [13, 14], and high performance liquid chromatography [15,16].Although the sensitive visible spectrophotometric methods are very little, the literature contained a simple colorimetric methods for the determination of benzodiazepine drugs included CDE using

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diazotization reaction, depends upon the formation of their corresponding amino benzophenones after acidic hydrolysis and then conversion the amino group to diazonium salt using $HCl/NaNO_2$ [17]. This simple reaction was depended on the development of simple and very sensitive method based on oxidative coupling reaction between hydrolysis product (decomposed CDE) and phenothiazine (PHT) as a coupling reagent in the presence of sodium periodate. Absorbance measurements were established by recording the absorbance of the green colored product at 602 nm versus reagent blank which has a minimum absorbance at the same wavelength.

Experimental

Apparatus

Double beam Shimadzu UV–VIS 260 spectrophotometer (Kyoto-Japan) was used in all measurements (spectral and absorbance). The absorbance measurements were carried out using matched 1cm quarts cells.

Materials and solutions

The reagents grade materials were used throughout this work. Chlordiazepoxide (CDE) standard 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 25 mg amount of standard CDE was accurately weighted and dissolved in a 25 mL of 6 M hydrochloric acid then heating the solution in a boiling water-bath for 1 hour. The decomposed drug transferred into 50 mL volumetric flask and complete the volume to the mark with distilled water to obtain 500 µg/mL of CDE hydrolyzed solution [17]. This solution is stable for more than one week if kept in room temperature. Simple dilution using the same concentration of acid was used to prepare more diluted solutions.

Phenothiazine solution (0.01 M, BDH)

The solution of this reagent was prepared by dissolving 0.0996 g of the reagent in 50 mL ethanol and kept in dark bottle.

Sodiumperiodate solution (0.05 M, BDH)

This solution was prepared in 50 mL volumetric flask via dissolving 0.5347 g of $NaIO_4$ in distilled water.

Hydrochloric acid solution (6 M, BDH)

This 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 Na_2CO_3 solution.

Preparation the solutions of pharmaceutical tablets

A 30 tablets (Libroxide ® 5 and 10 mg chlordiazepoxide) were accurately weighed and powdered then an equivalent to 50 mg of CDE of the powder was taken and dissolved in about 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 the decomposition procedure was accomplished the decomposed solution transfer into 50 mL volumetric flask and complete the volume to the mark with distilled water to obtain a 500 µg/mL of CDE solution.

General procedure

With using a volumetric flasks (10 mL) an increasing amounts of sample containing 1–500 µg of hydrolyzed standard CDE (100 µg/mL) was transferred (cover the range 0.1-50 µg/mL of CDE) then added 0.2 mL of 10 mM PHT solution and about 0.1 mL of 50 mM of sodium periodate solution. The reactants then diluted with distilled water and mixed. The absorbance of resulting solutions was measured after 10 min at 602 nm at room temperature (25°C) against reagent blank (prepared under the same procedure but without CDE). Alternatively the corresponding calibration curve and regression equation were constructed. For the optimization of conditions and in all later experiments, a solution of 250µg of CDE was used in a final volume of 10 mL (i.e. 25 ppm).

Stoichiometric relationship

The stoichiometry of the suggested reaction was investigated. An equimolar of CDE and PHT (1mM for both) under optimum concentration of sodium periodate were prepared using Job's method [18] (Fig. 1).In Job's method of continuous variation a sequence solutions (total volume of the CDE and PHT was 5 mL) have been prepared. Hydrolyzed drug and reagent indifferent 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 602 nm. Figure 2 showed that a 1:1 ratio product (CDE:PHT) is formed.

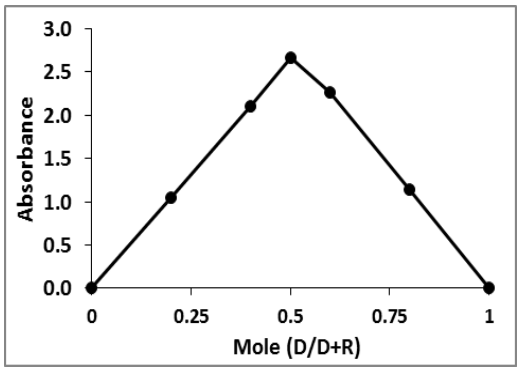
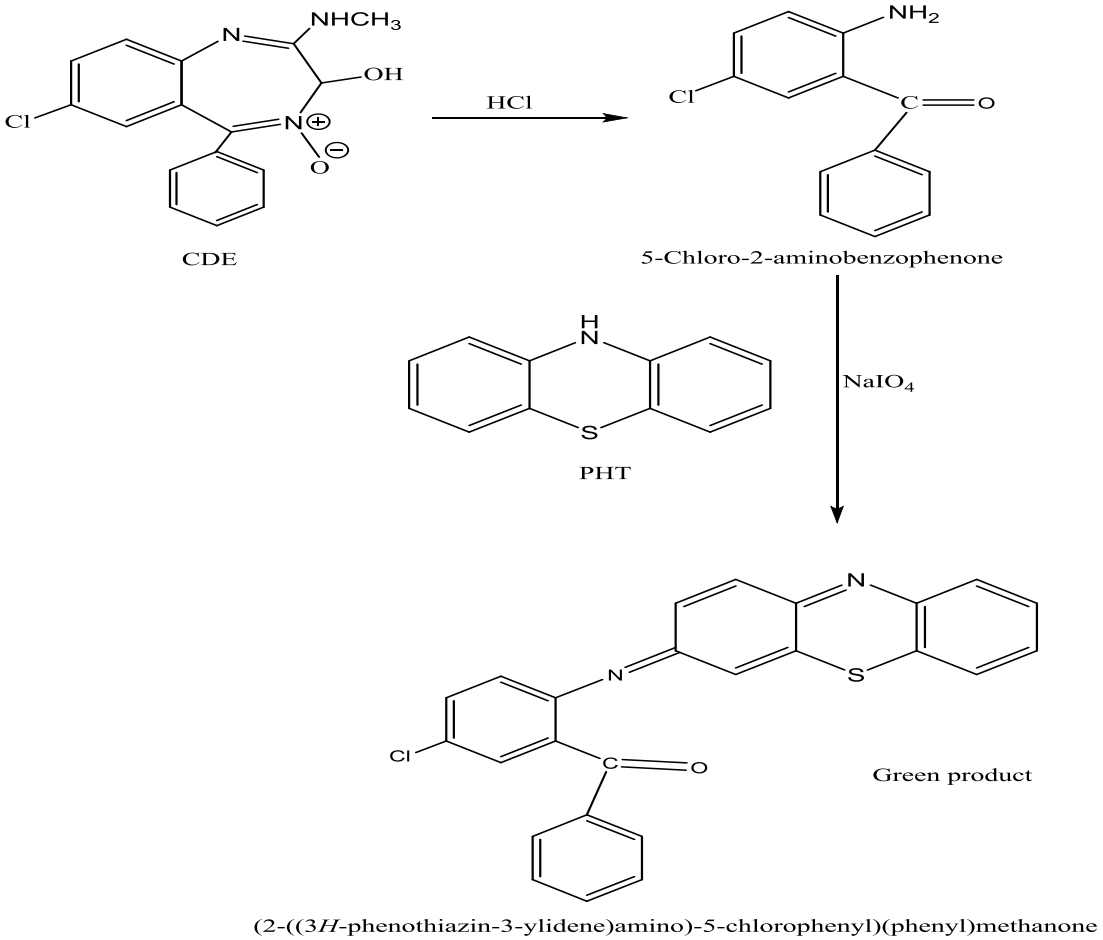


Figure 2. Job's method

CDE is completely hydrolyzed to 5-chloro-2-aminobenzophenone when heated with hydrochloric acid in a boiling water-bath. The strong electron donating ability of amino group in hydrolyzed CDE make it coupled with oxidized PHT easily and formation the oxidative coupled product. Depending on the result obtained from job's method, the mechanism of formation of the product (2 -((3H-phenothiazin-3-ylidene) amino) – 5 – chloropheny l) (phenyl) methanone) under oxidative coupling product may be suggested according to the following Scheme [19-21]:



Scheme 1. Proposed mechanism of reaction product (2-((3H-phenothiazin-3-ylidene) amino)-5-chlorophenyl)(phenyl)methanone)

Results and Discussion

Absorption spectra

According to the previous procedure, the absorption spectrum of the green product formed by the reaction between the hydrolyzed product of the CDE and PHT in acidic medium has been recorded (Fig. 3). After 10 minutes from the beginning of reaction between hydrolyzed CDE with PHT reagent, the spectra were obtained. The maximum wavelength of the green colored product was appeared at 602 nm.

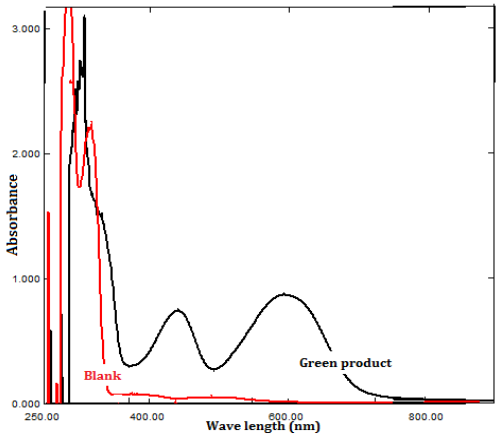


Figure 3. Absorption spectra of 25 µg/mL of decomposed CDE treated as described procedure and measured against blank, and the blank measured against water.

Study of the method variables

The experimental factors such as the reagents concentrations, order of addition, temperature, medium of reaction and stability time that may affecting mainly the sensitivity of the colored product were studied by variable one parameter with the time, and kept the rest fixed. As we mentioned previously all experiments were done using 25 µg/mL of CDE and the absorbance measurements were carried out after 10 min from the beginning of the reaction at λ_{max}=602 nm and laboratory ambient temperature (25 ± 2C°).

Effect of reaction time

Effect of reaction time on the steadiness of absorbance readings after dilution was studied (Table 1). The absorbance of colored product started to be stable after ° min and remains stable more than 60 min. The large stability time offer an advantage of measuring large number of samples comfortably at any time within the period without changing in the values of readings. This experiment was repeated after optimization all variables and the result was the same.

Table 1. The effect time of reaction

Time, min	2	5	10	15	20	25	30	40	50	60	70
Absorbance	0.508	0.538	0.538	0.537	0.538	0.537	0.537	0.538	0.538	0.534	0.528

Effect of temperature and order of addition

Three different temperatures were used for study the effect of temperature on the suggested reaction (5, 25, and 65°C) and the experiment indicated that maximum absorbance was obtained at room temperature and at 65°C, more than at 5°C, which that may be due to increase the coupling affinity between the reactants at high temperature (Fig. 4a). Ambient temperature was chosen in all the following experiments because the product is more stable than at 65°C.Also variable addition orders of reagents were studied and it was found the order of (CDE+PHT+$NaIO_4$) was gave the best results and was used in all the following experiments (Figure 4b).

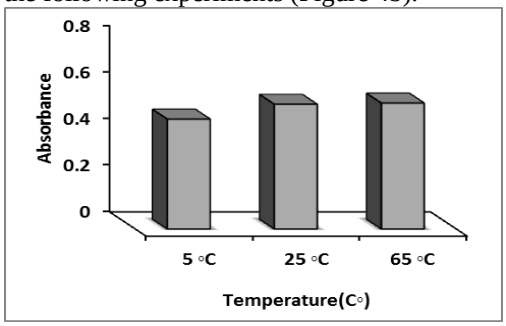


Figure 4 (a). Effect of temperature

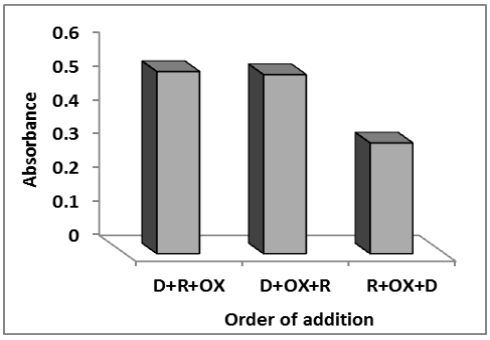


Figure 4 (b). Effect of order of addition (D; Drug, OX; Oxidant, R; Reagent

Effect of reagents concentration ($NaIO_4$and PHT)

$NaIO_4$ was found to be a useful oxidizing agent for the proposed reaction, other oxidizing agents (potassium per sulphate, potassium dichromate, potassium hexacyanoferrate, and ferric chloride) have also been tested, but none give product when tried instead of $NaIO_4$. Effects of the variable volumes of PHT 10mM (from 0.1–0.5 mL) and $NaIO_4$ 50 mM (from 0.05–1 mL) were examined. Results indicated that 0.2 mL of

PHT and 0.1 mL of oxidant gave the higher absorbance of product (Fig. 5) and were considered to be optimum for the next experiments. The absorbance was decreased with increase of the volume of $NaIO_4$ that added, this may be due to the increase in the absorbance of the blank with each addition of oxidant.

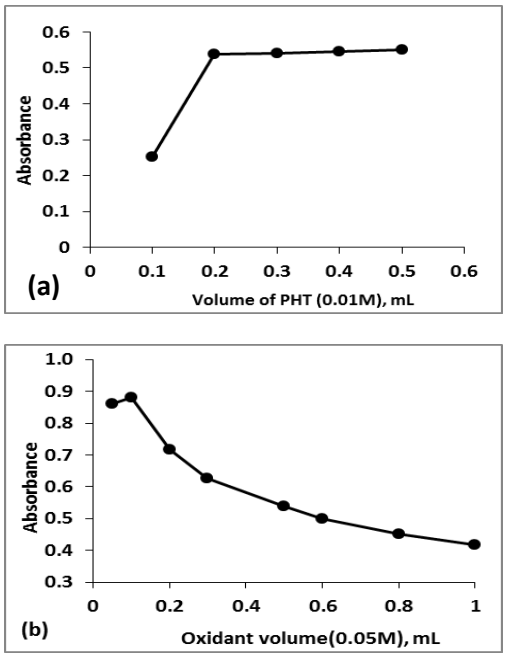


Figure 5. (a) Effect of volume of PHT, (b) Effect of volume of oxidant.

Effect of reaction medium

The green colored product appeared directly during coupling reaction between reactants; and the acidic of the reaction medium (used for decomposition process of CDE) was enough for development of the color. More acidic solution was not change the absorbance, while the basic medium caused a shift in maximum wave length value to 542 nm accompanied with decrease in sensitivity; therefore, there is no need to add an acidic or basic solution.

Method of validation

Linearity

Under the optimized experimental conditions for CDE determination, standard calibration curve was constructed (Fig. 6). Into (10 mL) of sequences standard flasks increasing amounts of sample containing 1–500 µg of CDE was transferred (cover the range 0.1-50 µg/mL) then a volume of 0.2 mL of 10 mM PHT and 0.1 mL of 50 mM of oxidant ($NaIO_4$) were added. The reactants into the flasks were mixed and diluted with distilled water, and finally left for 10 min to reach to the stability. The absorbance later measured at 602 nm at 25°C (room temperature). The regression equation was derived using the least-squares method. The intercept, slope, correlation coefficient, and molar absorptivity values in addition to statistical analytical values are calculated and listed in Table 2. Results listed indicate a good sensitivity with linearity over the range of 0.1-50 µg/mL.

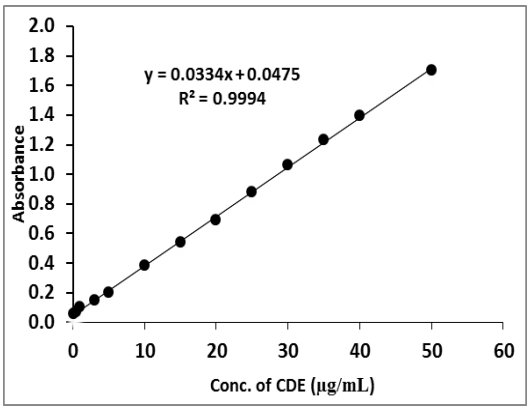


Figure 6. Calibration curve.

Table 2. Summary of characteristics data of suggested method

Parameter	Value
Regression equation	Y= 0.034X + 0.0475
Linear range (µg/mL)	0.1-50
Correlation coefficient(R^2)	0.9994
LOD (µg/mL)	0.11
LOQ (µg/mL)	0.36
Reproducibility (%)	0.2-1.5
Average of recovery (%)	100.43
Molar absorptivity(L/mole cm)	1.00×10^4
Sandell's sensitivity (µg/cm^2)	2.99×10^{-2}

Accuracy and repeatability

To estimate the accuracy of the proposed method, and the repeatability of readings, two altered concentrations solutions of CDE were prepared. The assay process was applied in three replicates, and the RSD% (percentage relative standard deviation) 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 the suggested method were obtained.

Table 3. Accuracy and precision of the proposed method

Conc. of CDE, µg/mL		Error *%	Rec.*%	RSD *%
Present	Found			
25	24.92	-0.32	99.68	0.17
30	29.96	-0.13	99.87	1.49
35	35.62	1.77	101.77	0.31

*Average of three determinations.

Method specificity (Study of pharmaceutical additives)

Examination of the specificity and selectivity of the suggested method was done by analysis of target drug in the existence of 10-fold of common additives (talc, starch, polyvinyl pyrrolidone and magnesium stearate) which often accompany CDE in its dosage forms. The satisfactory obtained recovery values (99.8-101.7%) indicating no interfering with these additives were observed which representing the selectivity of the method (Table 4).

Table 4.Effect of common additives

Additives (250 µg/mL)	Conc. of CDE, µg/mL		Rec.%	RSD%
	Present	Found		
Poly vinyl pyrrolidone	25	25.27	101.08	0.31
Talc		25.32	101.28	0.99
Starch		24.94	99.76	0.83
Magnesium stearate		25.03	100.12	0.43
All the above additives		25.44	101.76	0.98

Analytical applications

The current procedure was applied profitably to the estimation of CDE in tablets. 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 mentioned previously, and the results obtained in Table 5 and 6 (good precision <2.6 and high recoveries best than 98%) were in agreement with those of common method (UV spectroscopy method) [22], using two common tests (Student t-test and F-test) at 95% of confidence level [23]. The obtained results tabulated in Table 6 (calculated values <<tabulated values) showed that there was no significant differences in accuracy or precision between the two methods.

Table 5. Application of the proposed method to the determination of CDE in different dosage forms using standard addition method

Dosage form	Taken conc. (µg/mL)	Proposed method		
		Pure drug added conc. (µg/mL)	Total found conc. (µg/mL)	(%Rec.± SD) n=4
Libroxide® Tablets (5mg-SDI)	25	5	29.59	98.63±0.61
		10	34.54	98.69±0.50
	30	5	34.71	99.17±0.58
		10	39.71	99.28±0.85
Libroxide® Tablets (10mg-SDI)	25	5	29.74	99.13±0.26
		10	35.39	101.11±0.32
	30	5	34.80	99.43±0.60
		10	39.72	99.30±0.64

Table 6. Comparison and application of the current and UV methods.

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)	25	24.79	99.16	98.88	0.20	20	19.66	98.30	98.56	0.58
	30	29.58	98.60		0.29	30	29.61	98.70		0.15
						40	39.47	98.68		0.33
Libroxide® Tablets (10mg)	25	24.98	99.92	99.49	0.45	20	20.23	101.15	100.77	0.37
	30	29.72	99.07		0.49	30	30.17	100.57		0.65
						40	40.24	100.60		0.12
Pure CDE				100.44					100.89	
t (2.776)[b] F (19.000)[b]	0.307 2.785	$(n_1-1)=2$, $(n_2-1)=2$, $(n_1+n_2-2)=4$								

a, (n=5); b Theoretical value.

Conclusion

Oxidative coupling reaction is considered as one of the most significant reaction used for drug analysis. A few researches were proposed for determination this important drug, but most of them were not sensitive or requiring difficult steps and expensive techniques. The suggested method is simple, specific and in addition, it does not require an extraction or any expensive solvents or technique. This method applied successfully to the analysis of CDE in tablets with good accuracy and precision. The proposed method was compared with UV method (classical method) of CDE determination and by using the statistical methods (t- and F test) and the suggested method proved accuracy and precision and can be used routinely in quality control studies.

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