



# Spectrophotometric Determination of Carbamazepine Via Oxidative Coupling Reaction with 2,4-dinitrophenyl hydrazine

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## Abstract

An accurate and sensitive spectrophotometric method has been developed for the determination of carbamazepine (CRN.) in pure and dosage forms. The method is based on the oxidation of 2,4-dinitrophenylhydrazine (2,4-DNPHz) by potassium periodate than coupling with carbamazepine (CRN.) in alkaline medium to form a stable yellowish brown colored water-soluble dye with a maximum absorption at 485 nm. The variables that affect the completion of reaction have been carefully optimized. Beer's law is obeyed over the concentration range of (4-50  $\mu\text{g.mL}^{-1}$ ) with molar absorptivity of ( $6.7335 \times 10^3 \text{ L.mol}^{-1}.\text{cm}^{-1}$ ). The limit of detection was ( $0.1052 \mu\text{g.mL}^{-1}$ ) and Sandell's sensitivity value was  $0.0350 \mu\text{g.cm}^{-2}$ .The proposed method has been applied successfully to the determination of carbamazepine in pharmaceutical preparation.

**Keywords:** Spectrophotometric, Carbamazepine, 2,4-dinitrophenyl hydrazine ,Dosage forms, oxidative Coupling.

## Introduction

Carbamazepine (CRN.) has the IUPAC name 5*H*-dibenzo[*b,f*]azepine-5-carboxamid (Scheme 1) of molar mass 236.269 g.mol<sup>-1</sup> and molecular formula C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O. It is a highly lipophilic neutral tricyclic compound with a white to off white color almost odorless crystalline powder. It is slightly soluble in water but soluble in alcohol and acetonitrile.[1,2]

Carbamazepine is an anticonvulsant and mood stabilizing drug used mainly in the treatment of epilepsy and bipolar disorder, it may be used in schizophrenia along with other medications. [3] In 1953 carbamazepine was discovered by Swiss chemist Walter Schindler. It is currently available as a generic medication and is not very expensive.[4]

Several methods have been reported for the determination of carbamazepine in various matrices using spectrophotometry,[5-7] (HPLC) [8,9], flow Injection Analysis,[10,11] electro-analytical method,[12,13] and capillary electrophoresis.[14,15]

The aim of the present work is to suggest a simple and sensitive spectrophotometry procedure for the determination of carbamazepine in pure dosage forms. The method is based on an oxidation of 2,4-dinitrophenylhydrazine by potassium periodate and reaction with carbamazepine in alkaline medium to form a colored product. In addition, the reaction conditions were studied one-factor-at a time to provide an optimized analytical response.

## Experimental

### Instruments

A PG instrument, UV-visible spectrophotometer model T80 (U.K) with 1cm matched quartz cells was used for the absorbance measurements.

Sartorius BL 210 S electronic balance was used for weighing the samples.

### Materials and Methods

All chemicals used were of analytical reagent grade and were obtained from BDH and Panreac. Carbamazepine standard powder was kindly provided by the State Company for Drug Industries and Medical Appliances, Samara-Iraq(SDI).

#### Carbamazepine Stock Solution (1000 µg.mL<sup>-1</sup>)

The stock solution of (CRN.) was prepared by dissolving an accurately weighed 0.1000 g of pure drug in 10ml of ethanol and the volume was made up to the mark in 100mL volumetric flask with ethanol. The stock solution was protected from light and stored at 5°C.

**Carbamazepine working solution (200 µg.mL<sup>-1</sup>):** prepared by diluting 20mL of the stock solution to 100mL in a volumetric flask with ethanol.

**2,4-dinitrophenyl hydrazine solution (2,4-DNPHz) (2×10<sup>-3</sup>M):** prepared by dissolving 0.0396g of 2,4-DNPHz in 3mL of concentrated sulfuric acid and the volume was made up to the mark in 100mL volumetric flask with distilled water.

**Potassium periodate solution (6×10<sup>-3</sup>M):** prepared by dissolving 0.1380g of KIO<sub>4</sub> in a suitable volume of distilled water and the volume was made up to the mark in 100mL volumetric flask.

**Sodium hydroxide solution (~4M):** prepared by dissolving 16.000g of NaOH in a suitable volume of distilled water and the volume was made up to the mark in 100mL volumetric flask.

#### **Carbamazepine tablets solution (1000 $\mu\text{g}\cdot\text{mL}^{-1}$ )**

The content of 10 tablets was accurately weighed and grinded into fine powder then mixed well and an average weight was calculated. An amount of the powder equivalent to 0.0842g and 0.0839g (containing 0.05g of the drug carbamazepine) of Carbasam-200mg and Tegretol-200mg respectively was accurately and separately weighed, dissolved in 10ml ethanol and stirred for 10 min to ensure complete dissolution of the drug, then transferred into 50 mL volumetric flask and diluted to the mark with ethanol to get 1000 $\mu\text{g}\cdot\text{mL}^{-1}$  (CRN.). The solution was filtered by using Whatman filter paper No.41 to avoid any suspended or undissolved material before use.

**Working solution (200 $\mu\text{g}\cdot\text{mL}^{-1}$ )** was freshly prepared and analyzed by the recommended procedure.

#### **General recommended procedure for calibration**

In a series of 10mL volumetric flasks, 1mL of  $2\times 10^{-3}\text{M}$  2,4-DNPHz and 1mL of  $6\times 10^{-3}\text{M}$  potassium periodate were added to each flask. The resulting oxidized product was coupled with (CRN.) by adding 1mL aliquots of the standard solution containing (40 - 500)  $\mu\text{g}$  followed by 1mL of 4M sodium hydroxide to each flask with shaking. After 10min, the solutions were making up to the mark with distilled water, mixed well and left to stand for 10min. The absorbance of yellowish brown colored chromogen was measured at 485nm against the reagent blank.

## **Results and Discussion**

### **Absorption spectra for primary test**

The primary test for the present method involved oxidation of 2,4-dinitrophenylhydrazin with potassium periodate and reaction with (CRN.) in alkaline medium to form a colored product. The test was done by adding 1ml of 200 $\mu\text{g}\cdot\text{mL}^{-1}$  (CRN.), 1mL of  $1\times 10^{-3}\text{M}$  2,4-DNPHz, 1mL of  $5\times 10^{-3}\text{M}$  potassium periodate, and then 1mL of 1M sodium hydroxide in 10mL volumetric flask with shaking. The contents were diluted to the mark with distilled water. The absorbance and  $\lambda_{\text{max}}$  of the colored product was measured against the reagent blank. Figure (1) shows that the maximum absorption was obtained at a wavelength of 485nm.

### **Optimization of reaction variables**

The various parameters related to the colored product formation have been studied by varying the parameters one at a time and controlling all others fixed and optimum conditions have been selected.

#### **1. Effect of 2,4-DNPHz concentration**

The influence of the concentration of 2,4-DNPHz on the absorbance of the colored product was investigated in the range between ( $9\times 10^{-4}$  -  $4\times 10^{-3}$ )M figure (2). It was found that the maximum absorbance of the yellowish brown color was achieved with  $2\times 10^{-3}\text{M}$  of the reagent. Above this value a decrease in absorbance was observed. Therefore, 1mL of  $2\times 10^{-3}\text{M}$  was used during the subsequent work.

#### **2. Effect of potassium periodate concentration**

The study of potassium periodate concentration revealed that the reaction was depending on  $\text{KIO}_4$  as an oxidizing agent. The highest absorbance was attained when the concentration of

KIO<sub>4</sub> was  $6 \times 10^{-3}$ M. Above this value a decrease in the absorbance reading occurred figure (3). Therefore, 1mL of  $6 \times 10^{-3}$ M was used during the subsequent work.

### 3- Effect of different bases

The effect of different alkaline solutions with concentration of 1M on the absorption intensity of the colored dye formed was investigated. Four types of bases namely; sodium hydroxide, potassium hydroxide, sodium carbonate and ammonium hydroxide were tested and the results were listed in table (1).

As can be seen it was found that sodium hydroxide shows the maximum absorption intensity of the colored product, therefore it was selected for subsequent work.

### 4. Effect of sodium hydroxide concentration

The effect of sodium hydroxide concentration on the measured absorbance of the formed colored product was investigated by using 1mL of different concentrations of NaOH solution ranged between (0.5-6.0)M. The results are presented in figure (4), which reveals that the addition of 1mL of 4M NaOH exhibited a better absorbance. Above this concentration the absorbance value decreased. Therefore, 1mL of 4M NaOH was used in all subsequent experiments.

### 5. Effect of coupling reaction time

The optimum time for the reaction between (CRN.) and 2,4-DNPHz was studied at a fixed concentration of (CRN.).  $20 \mu\text{g.mL}^{-1}$  reacted with 2,4-DNPHz and potassium periodate in alkaline medium. Absorbance values were recorded at different intervals ranging from immediate measurement to a waiting period of 25min. The oxidative coupling reaction is completed in 10min as shown in table (2).

### 6. Effect of reagents mixing order

Effect of different orders of components addition on chromogen formation was investigated by changing the order of addition of reactants four times as shown in table (3). From the results shown, it is obvious that mixing order number one was recommended as it resulted in obtaining a maximum absorbance and hence was followed in the subsequent experiments.

### 7. The stability

Stability study of the colored product formed upon reaction of drug solution with 2,4-DNPHz was carried out by measuring its absorbance at different time intervals. 10min was selected as optimum time in the general recommended procedure. The color of the solution was nearly stable for at least 60min as shown in figure (5).

### Final absorption spectra

The absorption spectrum of the yellowish brown product formed from the treatment of (CRN.)  $20 \mu\text{g.mL}^{-1}$  with 2,4-DNPHz in the presence of potassium periodate in alkaline medium under the optimum conditions was recorded and showed a maximum absorption at 485nm against the reagent blank as shown in figure (6).

### Calibration curve and analytical data

Employing the optimum experimental condition, the measured absorbance values at 485 nm versus different standard concentrations of (CRN.) were plotted to construct a calibration curve. The linearity of the obtained plot of the (CRN.) was in the concentration range of (4 - 50)  $\mu\text{g.mL}^{-1}$  as shown in figure (7). The statistical treatments of the analytical data are summarized in table (4).

## Structure of the product

Job's method [16] and mole ratio method [17] have been used in the determination of the stoichiometry of the reaction between (CRN.) and 2,4-DNPHz. The obtained results figures (8) & (9) showed that 1:1 carbamazepine to 2,4-DNPHz ratio is obtained. The proposed mechanism of the reaction between (CRN.) and 2,4- DNPHz can be represented in scheme (2).

## Comparison of the methods

Table (5), shows a comparison between the proposed method and that of another literature spectrophotometric methods throughout some measured analytical parameters.

## Precision and accuracy

The precision and accuracy of the proposed method was tested by analyzing three replicate samples of (CRN.) in three different concentration levels (within Beer's law range). The results listed in table (6) indicate an acceptable accuracy and precision of the method.

## Interference study

The extent of interfering by some excipients which often accompanied pharmaceutical preparations was studied by measuring the absorbance of solution containing  $20 \mu\text{g.mL}^{-1}$  of (CRN.) and  $1000 \mu\text{g.mL}^{-1}$  of excipient. The results in table (7) show that the studied excipients do not interfere in the determination of (CRN.) in its dosage forms.

## Application in pharmaceutical preparation

In order to demonstrate the applicability of the proposed method for the determination of (CRN.), the method was applied to two types of pharmaceutical formulations (tablets) from different manufacturing sources containing (CRN.). The results of the application were satisfactory as shown in table (8).

## Conclusions

The reagents utilized in the proposed method are readily available, cheap and the procedures do not involve any critical reaction conditions. Moreover, the method is free from interference by excipients. The wide applicability of the new procedure for routine quality control was well established by the assay of carbamazepine in pure form and in pharmaceutical preparations.

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**Table (1): The effect of different bases on coupling reaction.**

Base (1M)	Absorbance
NaOH	0.185
KOH	0.162
Na <sub>2</sub> CO <sub>3</sub>	0.064
NH <sub>4</sub> OH	0.103

**Table (2): The effect of coupling reaction time.**

Time (min)	Absorbance
0	0.347
5	0.371
10	0.395
15	0.341
20	0.302
25	0.298

**Table (3): Variation of absorbance with change of reactants addition order in the determination of 20 µg.mL<sup>-1</sup> (CRN.).**

No	Sequence	Absorbance
1.	R+O+D+B	0.421
2.	D+R+O+B	0.395
3.	R+D+O+B	0.116
4.	D+B+R+O	0.048

R: reagent    O: oxidizing agent    D: drug    B: base

**Table (4): Optical characteristics and statistical data for the determination of (CRN.).**

Parameter	Value
$\lambda_{\max}$ (nm)	485
Color	Yellowish brown
Regression equation	$Y = 0.0274[\text{CRN.}] - 0.0756$
Linearity range (µg.mL <sup>-1</sup> )	4 - 50
Calibration sensitivity (mL.µg <sup>-1</sup> )	0.0285
Correlation coefficient (r)	0.9996
Correlation of linearity (R <sup>2</sup> )	0.9995
Molar absorptivity (L.mol <sup>-1</sup> .cm <sup>-1</sup> )	$6.7335 \times 10^3$
Sandell's sensitivity (µg.cm <sup>-2</sup> )	0.0350
L.O.D. (µg.mL <sup>-1</sup> )	0.1052
L.O.Q. (µg.mL <sup>-1</sup> )	0.3508

**Table (5): Analytical parameters for the analysis of carbamazepine by the proposed method comparing to other methods.**

Methods	Linear range $\mu\text{g.mL}^{-1}$	( $\epsilon$ ) $\text{L.mol}^{-1}.\text{cm}^{-1}$	Correlation Coefficient (R)	C.V% range	Ref.
Proposed method	4.0 - 50.0	$6.7335 \times 10^3$	0.9996	0.017-0.462	...
Spectrophotometric	0.2 - 10.0	$2.1450 \times 10^4$	0.9831	0.15-0.25	6
Spectrophotometric	10.0-350.0	$1.90 \times 10^3$	0.9998	0.63-2.17	7
Spectrophotometric	1.0 - 25.0	$1.30 \times 10^9$	0.9996	2.12-0.59	18
RP-HPLC	...	...	0.9902	0.22 0.42	8
HPLC-UV	0.5 - 40.0	...	0.9999	0.53-2.75	9

**Table (6): Evaluation of the accuracy and precision of the proposed method for the determination of (CRN.).**

Conc. of (CRN.) $\mu\text{g.mL}^{-1}$		Er%	C.V%
Taken	Found*		
10.000	9.945	-0.550	0.462
25.000	25.013	0.052	0.031
35.000	35.017	0.048	0.017

\*Average of three measurements.

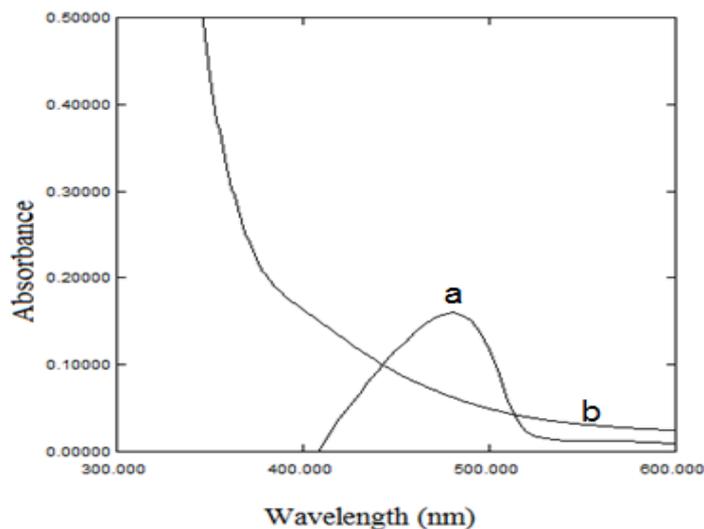
**Table (7): Recovery values for 20  $\mu\text{g.mL}^{-1}$  of (CRN.) in the presence of 1000  $\mu\text{g.mL}^{-1}$  of different excipients.**

Excipients		Carbamazepine Conc.		Recovery (%)
Name	Conc. ( $\mu\text{g.mL}^{-1}$ )	Taken ( $\mu\text{g.mL}^{-1}$ )	Found ( $\mu\text{g.mL}^{-1}$ )	
Lactose	1000	20.000	19.877	99.385
Glucose			19.921	99.605
Sucrose			19.756	98.780
Starch			20.074	100.370
Magnesium Stearate			19.752	98.760

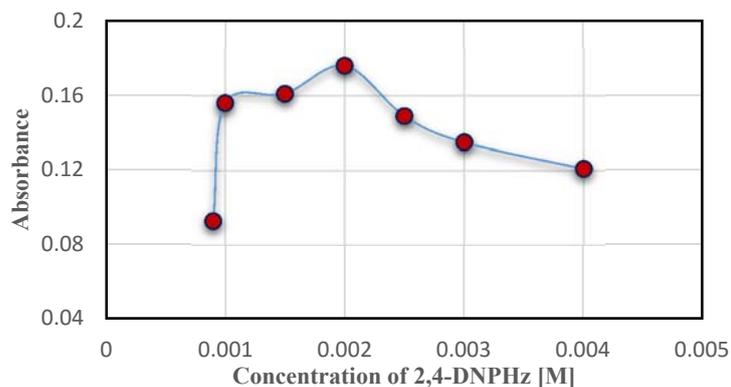
**Table (7): Determination of carbamazepine in pharmaceutical formulations (tablets) by the proposed method.**

Sample	Weight Found* (mg)	Concentration ( $\mu\text{g.mL}^{-1}$ )		Recovery %	C.V %
		Taken	Found*		
Carbasam - 200 mg S.D.I.-Iraq	195.920	10.000	9.796	97.960	1.216
	201.296	25.000	25.162	100.648	0.607
Tegretol - 200mg Novartis-Switzerland	202.020	10.000	10.101	101.140	0.143
	205.344	25.000	25.668	102.675	1.352

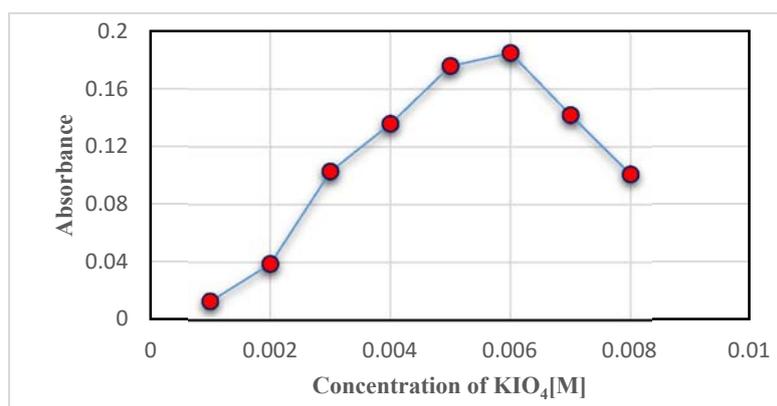
\*Average of three measurements.



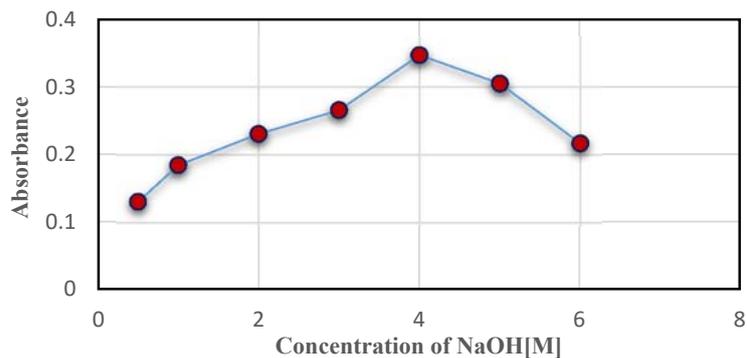
**Figure (1):** Absorption spectra of: (a)  $20 \mu\text{g.mL}^{-1}$  (CRN.) against reagent blank, (b) blank solution against solvent under the primary test conditions.



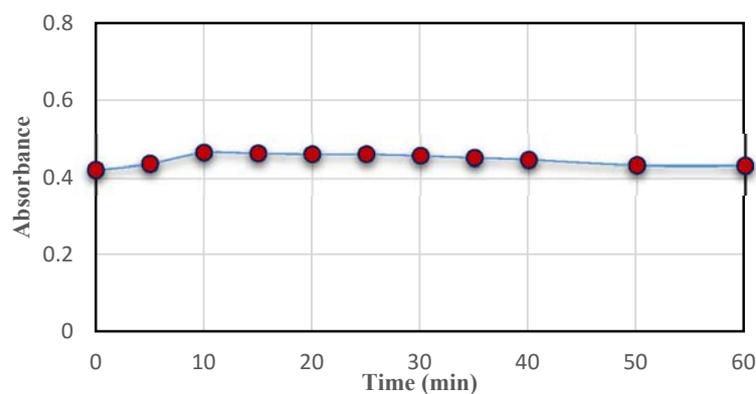
**Figure (2):** The effect of the concentration of 2,4-DNPHz on the color development in the determination of  $20 \mu\text{g.mL}^{-1}$  (CRN.).



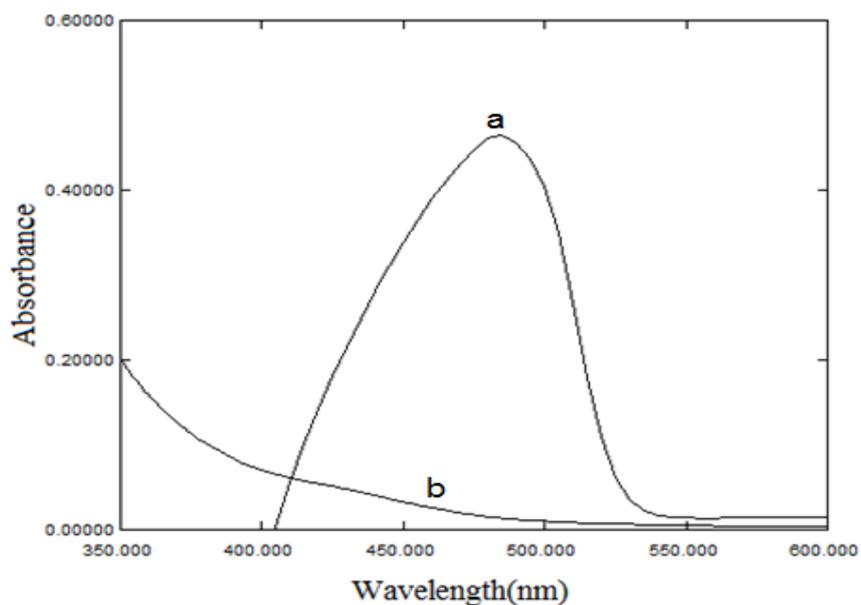
**Figure (3):** The effect of potassium periodate concentration on the color development in the determination of  $20 \mu\text{g.mL}^{-1}$  (CRN.).



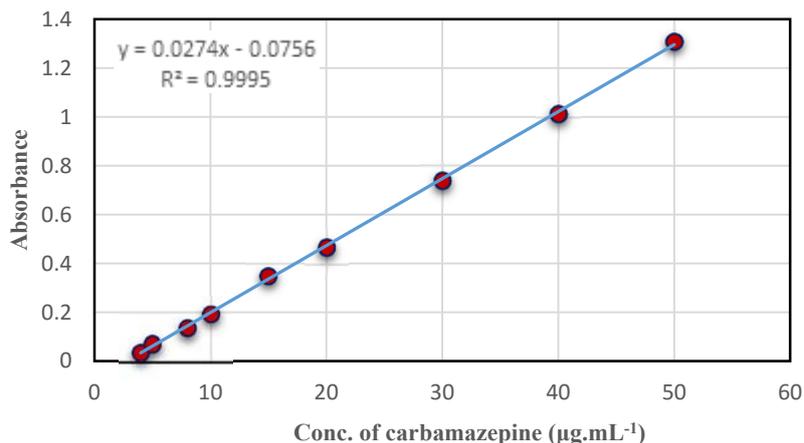
**Figure (4):** The effect of sodium hydroxide concentration on the color development in the determination of  $20 \mu\text{g.mL}^{-1}$ (CRN.).



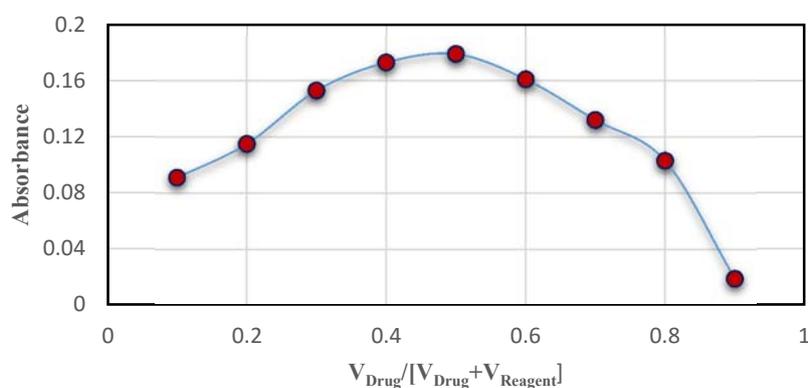
**Figure (5):** The stability of the colored product with time.



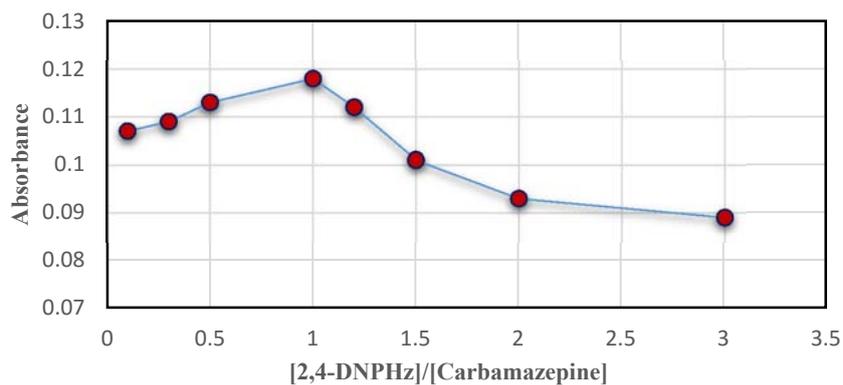
**Figure (6):** Absorption spectra of: (a)  $20 \mu\text{g.mL}^{-1}$ (CRN.) against reagent blank, (b) blank solution against solvent under the optimum conditions.



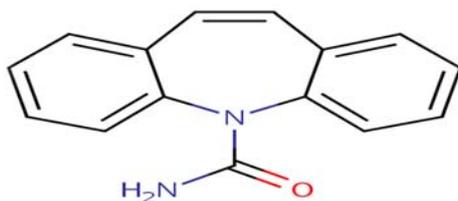
**Figure (7): Calibration curve for the determination of (CRN.) under optimum conditions.**



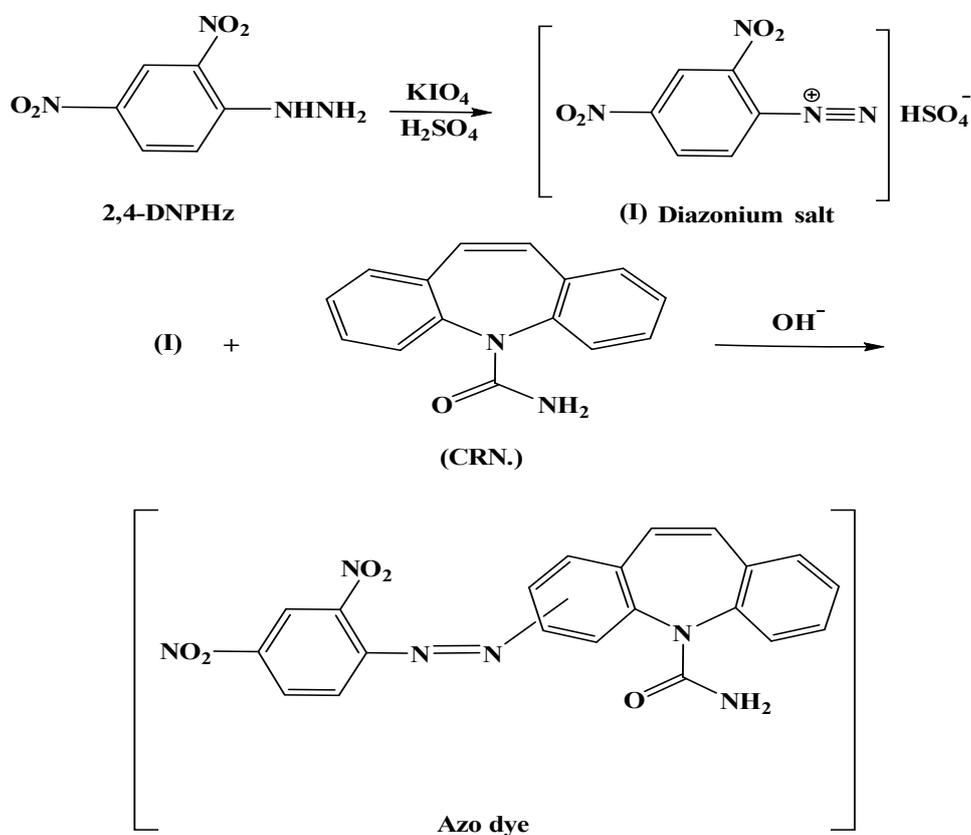
**Figure (8): Continuous variation method for the reaction of (CRN.) with 2,4-DNPHz.**



**Figure (9): Mole ratio method for the reaction of (CRN.) with 2,4-DNPHz.**



Scheme (1): The structural formula of carbamazepine.



Scheme (2): The suggested reaction mechanism between (CRN.) and 2,4-DNPHz.

## التقدير الطيفي للكاربامازيبين بواسطة تفاعل الازدواج التأكسدي مع 4,2-ثنائي نايترو فنيل هيدرازين

سحر ریحان فاضل

قسم الكيمياء/ كلية العلوم/ جامعة ديالى

نجوى اسحاق عبد الله

انتظار داود سليمان

قسم الكيمياء/ كلية التربية للعلوم الصرفة (ابن الهيثم)/ جامعة بغداد

استلم في: 12/كانون الأول/ 2015، قبل في: 7/كانون الأول/ 2016

### الخلاصة

تم تطوير طريقة طيفية دقيقة وحساسة لتقدير الكاربامازيبين بصورته النقية و في المستحضرات الصيدلانية. تعتمد الطريقة على اكسدة الكاشف 4,2-ثنائي نايترو فنيل هيدرازين بوجود بيرايودات البوتاسيوم ثم مفاعله مع الكاربامازيبين في وسط قاعدي لتكوين صبغه مستقرة ذات لون بني مصفر ذائبه في الماء تعطي اعظم امتصاص عند الطول الموجي 485 نانومترا". درست العديد من العوامل التي تؤثر في اكتمال التفاعل بدقة للحصول على الظروف المثلى للتفاعل. قانون بير ينطبق ضمن مدى من التراكيز (4-50)مكغم.مل<sup>-1</sup> والامتصاصيه المولارية  $6.7345 \times 10^3$  لتر.مول<sup>-1</sup>.سم<sup>-1</sup>. و بحد كشف 0.1052 مكغم.مل<sup>-1</sup> و دلالة ساندل تساوي 0.0350 مكغم.سم<sup>-2</sup>. تم تطبيق الطريقة المقترحة بنجاح في تقدير الكاربامازيبين في المستحضرات الصيدلانية.

**الكلمات المفتاحية:** التقدير الطيفي، الكاربامازيبين، 4,2-ثنائي نيتروفنيل هيدرازين، المستحضرات الصيدلانية، الاقتران التأكسدي.