RESEARCH ARTICLE

Eco-friendly Analytical Method for Estimation for Benzodiazepine Drug in Pure and Pharmaceuticals Formulations by Oxidative Coupling Reaction with Phenylephrine Hydrochloride

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ABSTRACT

A simple, fast, sensitive and selective spectrophotometric method has been developed based on the oxidative coupling reaction process, which depends on the determination of clonazepam (CZP) drug using an oxidizing agent sodium periodate ($NaIO_4$) in the presence of a reagent, phenylephrine hydrochloride (PH-HCL), that uses the pink color of CZP at a wavelength of 495 nm. Where several factors affecting the color intensity and absorbance were studied, including the effect of color stability time, effect of the volume of the reagent, the effect of the volume of the oxidizing agent, order of addition and temperature. The calibration curve found to obey Lambert beer at range concentration (2–20 mg/L), while the limit of detection (LoD) ($8.9*10^{-2}$), limit of quantitation (LoQ) ($2.6*10^{-2}$) and molar absorbance is 1.4×10^2 liters $mol^{-1}cm^{-1}$. The method was applied to pharmaceutical preparations (Tablet) and was found to characterize the best precision and accuracy. The standard methods not need any control of temperature and also not affected by interferences and henceforth successfully utilized to determine CZP in pharmaceutical preparations.

Keywords: Benzodiazepine, Clonazepam, Oxidative coupling, Phenylephrine hydrochloride, Spectrophotometric method.

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INTRODUCTION

Benzodiazepines, called "benzos", are considered to be medicines that treat insomnia, anxiety, and psychological effects and reduce depression. One of the most important species is chloroziban. Clonazepam (CZP) its Known; 5-(o-chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzo-diazepin 2-one) is a benzodiazepine. It is used to treat muscle spasms tooth extraction operations, and treat muscle imbalance, and it is also used to treat cases of insomnia, anxiety and epilepsy.[1-4] Different ways have been reported in the literature to determination of CZP drug in pharmaceutical forms or fluids biological, having electrical ways spectrophotometry,[5-8] liquid and gas chromatography voltammetry (LC-MS/MS), high performance liquid chromatography (HPLC), flow injection,[9] Ag nanoparticles/MWCNT modified electrode, capillary electrophoresis, chemiluminescence and electro-chemiluminescence, paleography.[10,11] The literature reported several way for estimated of benzodiazepines in biological samples and pharmaceutical formulations, counting spectrophotometry, polarography, capillary electrophoresis, fluorimeter, GC-MS, HPLC, electro-chemiluminescence, dispersive nanomaterial ultrasound assisted microextraction, chemiluminescence, flow injection, and electrical methods. Despite the sensitive visible spectrophotometric ways being so little, the literature contained easy colorimetric ways for the estimation of benzo-diazepine drugs.[12-15] In this study, the colorimetric method for spectrophotometric determination was used to identify chloroziban drug, based on a reaction known as oxidative coupling reaction, The method was sensitive, simple, fast and inexpensive based on the oxidative coupling reaction of chloroziban and phenylephrine hydrochloride conjugation reagent in the presence of sodium periodide oxidizing agent.

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REAGENTS AND MATERIALS

All chemicals and reagents used in this research that high degree of purity and were obtained from Sigma Aldridge Company. Chloroziban and Ph-HCl were obtained from factory Samarra-Iraq. Pharmaceutical preparations like (tablet) containing chloroziban were purchased from local commercial suppliers.

Preparation of CZP Stock Solutions (100 mg/L)

The stander solution were prepared *via* dissolving 0.1 g of CZP in 100 mL conical flask by using 25 mL of ethanol and complete the volume by distilled water.

Preparation of Ph-HCl and NaIO4 Solution (0.05 N)

The solution of Ph-HCl was prepared daily *via* dissolving 0.52 g in 50 mL conical flask in DW and the solution of $NaIO_4$ was prepared *via* dissolving 0.54 gm in 50 mL conical flask in distilled water.

General Procedure of Estimation of Clonazepam

In 10 mL of several series conical flask, 2 mL of reduced CZP with $NaIO_4$ (0.05N) 2 mL and mixing about 2 minute after that added 3 mL Ph–HCl. After 5 minutes at 25ºC the solutions were complot the volume to mark by distilled water and give the maximum absorbance at 495 nm against the blank solution. A linear calibration curve was constructed and appears in Figure 1. All the values are analytical for the way utilized to determine CZP drug shown in Table 1.

RESULT AND DISCUSSION

Effect of Volume of a Coupling Reagent

several volume (0.1–4 mL) of reagent Ph-HCl on colored products absorption of CZP as appear in Figure 2, that found when the volume of reagent Ph-HCl increase lead to an increase of intensity of color and give higher absorbance.[16-18] Thus selected 3 mL as optimum volume of the drug.

Effect Volume of $NaIO_4$

Several volumes (0.1–4 mL) of oxidation agent $NaIO_4$ (0.05N) that were found when the volume of oxidation agent $NaIO_4$ increase lead to an increase of the intensity of color and give higher absorbance and higher sensitivity.[19-21] Thus selected 2 mL as the optimum volume of the oxidation agent $NaIO_4$ for the CZP drug as appears in Figure 3.

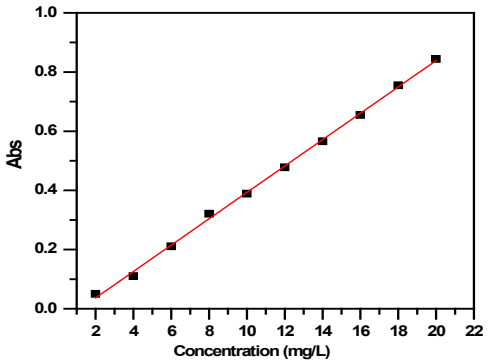

Figure 1: Calibration curve of CZP.

Effect of Addition Order

Several additional order were examined as appeared in Table 2. The optimum number of addition orders. One for solutions of drug CZP because it give the best absorbance and higher intensity color.[22]

Effect of solution temperature

Effect of several temperatures (10–40ºC) that found the higher stability of color product and give best absorption at 25ºC temperatures. Thus when using high temperature gives low stability of color product and absorption because of partial dissociation of colored products[23,24] as appeared in (Figure 4).

Effect of Time Stability of Color

Through the results that were reached, the optimal conditions for the reaction were determined by the effect of the reagent's

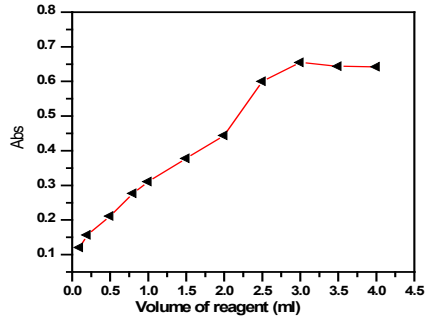

Figure 2: Effect of several volume of coupling reagent

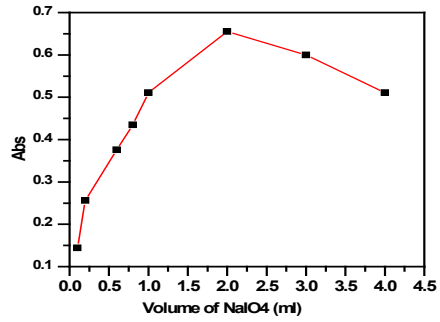

Figure 3: Effect volume of oxidation agent $NaIO_4$

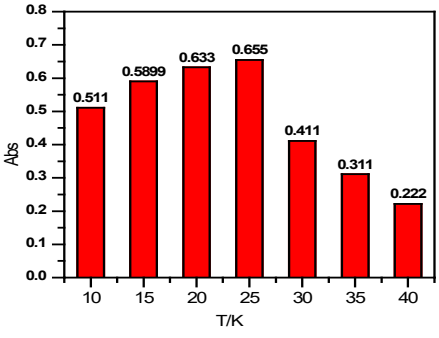

Figure 4: Effect of solution temperature of the stability of color product.

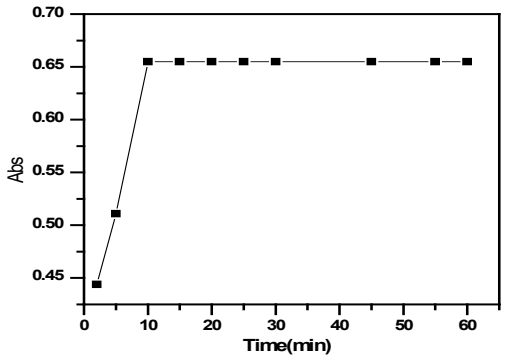

Figure 5: Effect of time stability of color.

Table 1: The values analytical for the way utilized to determine CZP drug.

Parameters	*Proposed method*
λ_{max} (nm)	495
Beer's law limit (μg mL^{-1})	2–20
Regression equation	(Y = m X + C)
Slope (m)	0.0445
Intercept (C)	-0.0522
Correlation coefficient (r2)	0.9984
(RSD%)	0.53
(SD)	0.12
Color	pink
LoD (μg mL^{-1})	8.9*10-2
LoQ (μg mL^{-1})	2.6 * 10-2

Table 2: Effect of order addition

1	*CPZ+NaIO4+ PHCl*	*0.665*
2	CPZ+PHCL+NaIO4	0.544
3	PHCL+NaIO4+CPZ	0.211
4	PHCL+CPZ+NaIO4	0.011
5	NaIO4+PHCL+CPZ	No colour

Table 3: Determination of CPZ in some formulations using the official and proposed method.

Pharmaceutical preparation	*Conc. Of CZP (mg L^{-1})*		*E %*	*Rec%*
	Present	*Found*		
CZP tablet 5 mg , Iraq	5	5.011	0.219	100.21
	10	10.012	0.119	100.11
	20	20.1	0.497	100.4
CZP tablet 5 mg , Iran	5	5.11	2.15	102.1
	10	9.89	-1.112	98.8
	20	20.11	0.54	100.5

volume, the oxidizing agent's volume and the solution temperature. Where the time required to obtain high color stability of the azo dye and give the best absorption is after (10 minutes),[25,26] we note that the color is stable for at least one hour, So 10 minutes was adopted in all experiments as shown in Figure 5.

Pharmaceutical Applications

The proposed ways were useful successfully on solutions pharmaceutical preparation for CZP drug. The proposed ways have the best precision and accuracy for estimating pharmaceutical tablets with CZP Table 3. The standard way to determine CZP in British pharmacopeia was useful in pure and pharmaceuticals for study drug and compared with proposed ways to evaluate the data for determine CZP drug.[27,28] The results appear in Table 3 no significant differences were found between the two ways. The standard method for determination of CZP was used on pharmaceuticals and pure preparation for studied drugs for evaluating the results for determining CZP drug. The data epitomized in Table 3 show no considerable differences between pure and pharmaceuticals.[29,30]

CONCLUSION

The oxidation coupling process is one of the utmost important methods for the spectrophotometric determination of pure pharmaceutical preparations. The color of the formed azo dye was stable for at least one hour. when the volume of reagent Ph-HCl increased of the intensity of color and gave the best absorbance and the time required to obtain high color stability of the azo dye after 10 minutes. When the proposed method was successfully applied to pharmaceutical preparations such as tablets, it gave the best accuracy and precision.

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