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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2018, Vol. 34, No.(1):
Pg. 1604-1610

Development of Spectrophotometric Method for Estimating Clonazepam in its Pure Form and in Pharmaceutical Tablets

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http://dx.doi.org/10.13005/ojc/340354

(Received: January 15, 2018; Accepted: March 01, 2018)

ABSTRACT

A study aimed to recommend spectrophotometric method for the determination of clonazepam (CLZ.) in pure form and their pharmaceutical tablets by the reaction of sited drug with ferric chloride in presence potassium ferric cyanide in acid medium to form prussian blue complex and determine it by UV-Vis spectrophotometric at 526 nm. The variables that affect the completion of reaction have been carefully optimized. The linearity range was (2-35µg. mL^{-1}) with a molar absorptivity of 2.8035×10^4 $L.mol^{-1}.cm^{-1}$. The limit of detection was 0.5703µg. mL^{-1} and Sandell's sensitivity value was 0.0103µg. cm^{-2}. The suggest method was successfully used to estimate clonazepam in commercial formulations.

Keywords: Clonazepam, Prussian blue, Ferric chloride, Potassium ferric cyanide, Spectrophotometry.

INTRODUCTION

Clonazepam (CLZ.) is a medication of benzodiazepine[1], belongs to a group of psychoactive drugs used in the controlling of epilepsy, is primarily used as a panacea to prevent certain types of seizures.[2] Clonazepam works on receptors in the brain called GABA receptors, leading to its release, a neurotransmitter that calms the nerves.[3, 4] (CLZ.) is a white powder, soluble in alcohol. When it exposed to air and light decomposes.[5] Its (IUPAK) name: 5-(2-Chlorophenyl)-7-nitro-1, 3-dihydro-2H-1,4-benzodiazepin-2-one1, Figure 1.

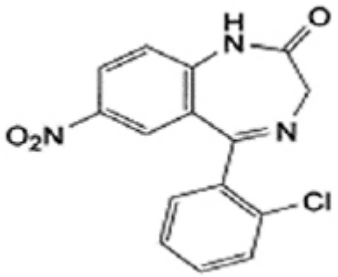

Fig. 1. Structure of (CLN.).1

The literature review revealed that many analytical methods were used to quantify (CLZ.) in their bulk and pharmaceutical

formulations including spectrophotometry[6,7,8,9,] GC-MS chromatography[10,] HPLC[11,12] capillary electrophoresis[13,] electrochem-iluminescence sensors[14], Voltammetry[15], potentiometry[16,17]. The purpose of this work is to develop a simple, rapid and accurate spectrophotometric method to measure the quantities (CLZ.) in bulk and Pharmaceutical Tablets.

EXPERIMENTAL

Apparatus

- A JASCO V-650 UV-Visible double beam spectrophotometer (UK) equipped with 10 mm quartz cell was used.
- A FA2004 electronic balance was used for weighing the samples.
- A LSB-015S Thermostatic Shaker water bath, LabTech.

MATERIALS AND METHODS

All chemicals used in this study were obtained from BDH and Sigma-Aldrich. The standard received in pure form (99.99%) from the State :Company: for Drug: Industries and Medical Appliances: Samara-Iraq (S.D.I.). Pharmaceutical formulation-assayed in this study; Rivotril 0.5 mg/tablet and 2 mg/tablet (Roche Farma-Spain) was purchased from commercial source.

Clonazepam Standard Solution [1000µg.mL^{-1}]

Standard (CLZ.) solution (1000 µg.mL^{-1}) was prepared by dissolution exactly 0.05 gm of (CLZ.) in 5 mL of methanol, and diluted to 50 mL with some solvent. The stock solution was protected from light, and store at 4 ^{0}C. Working standard (CLZ.) solution (200 µg.mL^{-1}) was obtained by dilution.

Ferric Chloride, [0.3M]

Prepared by dissolving 4.8660 gm of $FeCl_3$ in a suitable volume of distilled water then the volume was made up to 100 mL.

Potassium Ferricyanide, $K_3[Fe(CN)_6]$, [0.2M]

Prepared by dissolving 6.5848 gm of $K_3[Fe(CN)_6]$, in a suitable volume of distilled water and the volume was made up to 100 mL.

Sulfuric acid, H_2SO_4, [~10M]

Prepared by adding 27.2 mL of concentrated sulfuric acid to 22.8 mL distilled water in 50 mL volumetric flask.

Clonazepam tablets solution [1000µg. mL^{-1}]

Separately, 10 tablets from (CLZ.) was grinded and mixed well. equivalent to 0.3705 g and 0.1612 from tablets powder (each containing 0.0100 g of the drug (CLZ.)) for Rivotril® 0.5 mg and 2 mg respectively were weighed, dissolution in about 5 mL of methanol,in a separate flask. The solutions were stirred for more than 15 min. and complete to 10 mL with methanol to get 1000 µg. mL^{-1}. There after, By Whatman filter paper No. 41 each solution was filtered, avoidedany un-dissolved material before use and stored at 4 °C, further dilution in subsequent use.

General recommended procedure for calibration

In a series of standard flasks 10 mL, 1 mL of (CLZ.) (20- 350) µg. mL^{-1} and 1 mL of 10M H_2SO_4 were added to each flask. Followed by 1 mL of 0.3 M $FeCl_3$ and 1 mL of 0.2M $K_3[Fe(CN)_6]$ with shaking, and allowed to stand in water bath for 15 min. at 45 °C .The volume was made up, with distilled, waterto 10 mL and mixed well. After 10 min. the absorbance of the resulted complex was measured at 526 nm versus the blank prepared in the same manner without the analyte.

RESULTS AND DISCUSSION

Absorption Spectra for primary test

A 1 mL of H_2SO_4 (10M) is added to 1 mL of (CLZ.) (200 µg. mL^{-1}) solution in the presence of 1mL of $FeCl_3$ (0.3M) and 1 mL of $K_3[Fe(CN)_6]$ (0.2M) and diluted with distilled water in a 10 mL standard flask, a blue color product was formed. The absorbance and λmax of the product was measured against reagent blank Fig. 2 shows that the maximum absorption was obtained at a wavelength of 525 nm at which the reagent blank shows a negligible absorption.

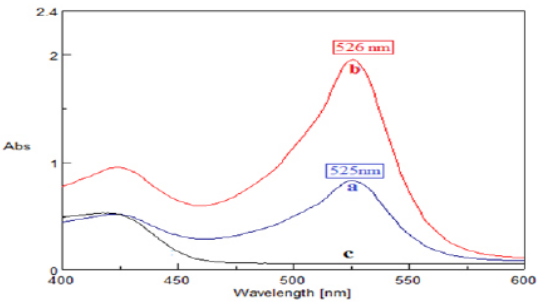

Fig. 2. Absorption spectra of:(a) Blue color product of 20 µg.mL-1 (CLZ.) vs reagent blank, underprimary test. (b) Blue color product of 20 µg.mL- 1 (CLZ.) vs reagent blank, under the optimum conditions. (c) Blank Solution against distilled water

Optimization of reaction variables

All important parameters that effect on the absorption intensity of the formed product have been studied and the optimum conditions have been selected.

Effect of sulfuric acid concentration

The influence of the different concentrations of sulfuric acid was investigated by adding 1 mL of various concentration [1.0 to 12.0]M. Fig. 3 shows that 10.0M of sulfuric acidwas chosen as optimal concentration to obtain the maximumabsorbance.Then, 10M of H_2SO_4 was selected for the subsequent studies.

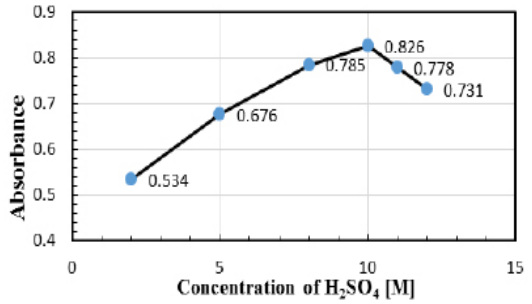

Fig. 3. Effect of sulfuric acid concentration

Effect of Ferric Chloride concentration

Various concentrations of $FeCl_3$[0.10 to 0.60] M have been tested. The results shown in Fig. 4 indicated that 1 mL of 0.30M of $FeCl_3$ was the optimum concentration and recommended in the subsequent experiments according to the highest intensity of the colored product.

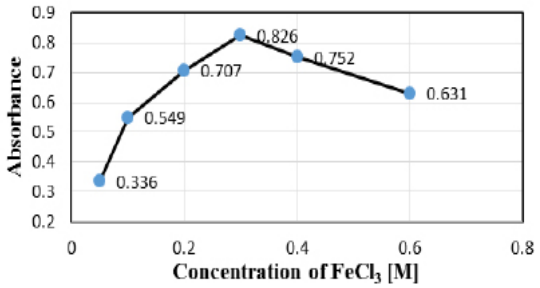

Fig. 4. Effect of variable concentration of $FeCl_3$

Effect of Potassium Ferricyanide concentration

Potassium Ferricyanide concentration effect on the intensity of the product colorwas examined by adding 1 mL of different amounts of the reagent [0.050- 0.50]M to fixed concentration of 20 µg mL^{-1} of (CLZ.) drug Fig. 5. It is obvious that using 0.20 M of $K_3[Fe(CN)_6]$ is the suitable concentration for quantitative estimation of (CLZ.) drug. That means, maximum and reproducible color intensities are obtained, and higher reagent concentration do not affect the color intensity.

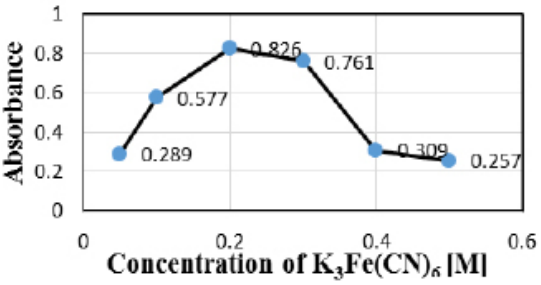

Fig. 5. Effect of different concentrations of$K_3[Fe(CN)_6]$.

Effect of time of reaction

Optimum coupling reaction time was determined by choosing different time periods (0 - 25) min. for development the colored complex. As shown in Fig. 6 the absorbance reached its maximum after 15 min. Therefore, 15 min. was selected as optimal period in the analytical procedure.

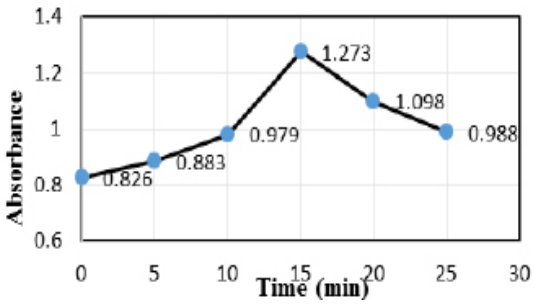

Fig. 6. Effect of reaction time

Effect of temperature

The influence temperature on the charge-transfer complex between (CLZ.) and Fe (III) was checked by changing temperature for the range (20-45) °C, Fig. 7. It was clear from the results that the absorbance attains maximum color intensity at 45 °C and at higher temperatures gave no satisfactory results because the product showed a slight turbidity.

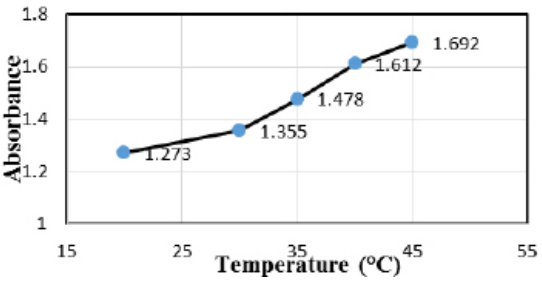

Fig. 7. Effect of temperature

Effect of addition order

The addition order influence of the reactive materials was also examined to form the charge transfer complex. The best result in Table (1) was found to be (1) recommended for subsequent testing.

Table 1: Addition order influence

No	Sequence	Absorbance
1.	Drug +H_2SO_4 + $FeCl_3$+ [$K_3Fe(CN)_6$)]	1.692
2.	Drug + H_2SO_4 + [$K_3Fe(CN)_6$)]+ $FeCl_3$	1.510
3.	$FeCl_3$+ [$K_3Fe(CN)_6$)] + H_2SO_4 + Drug	0.231
4.	$FeCl_3$+ [$K_3Fe(CN)_6$)]+ Drug + H_2SO_4	0.528

Stability time

Optimal time for complex stability was estimated the effect of time on the formation of the colored product was examined by allowing the reaction to proceed for varying period after dilution to 10 mL. The results showed that the complex between (CLZ.) and Fe (III) reached maximum absorbance after 10 min. and remains stable at least for 60 min. Figure 8.

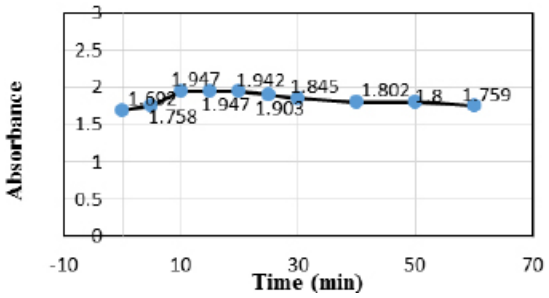

Fig. 8. The influence of varying time on stability

Calibration graph and analytical data

According to the above described analytical conditions, the final spectrum of the colored product formed exhibits a maximum at 526 nm, shows in Fig. 2. Linear calibration graph was obtained, Fig.(9). The linearity of the concentration ranged from (2-35) µg.mL^{-1} of the resulted complex. Table (2) shows the analytical parameters obtained such as slope, intercept, correlation coefficient, Sandell sensitivity, molar absorptivity (ε), limit of quantification and limit of detection.

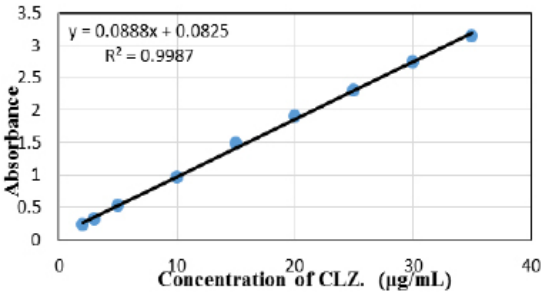

Fig. 9. Calibration graph of (CLZ.) under optimum conditions

Table 2: Optical characteristics and statistical data for the determination of CLZ

Parameter	Value
λmax (nm)	526
Color	Blue
Regression equation	Y=0.0888[(CLZ) µg/mL]+0.0825
Linearity range (µg/ mL)	2-35
Calibration sensitivity (mL/ µg)	0.0888
Correlation coefficient (r)	0.9993
Correlation of linearity (R^2)	0.9987
Molar absorptivity (L/ mol.cm)	$2.8035x10^4$
Sandell's sensitivity*(µg.cm^{-2})	0.0103
L.O.D.(µg/ mL)*	0.5703
L.O.Q.(µg/ mL)*	1.8005

Mechanism of the Reaction

The stoichiometry of the reaction between (CLZ.) drug and Fe(III) was investigated using the moleratio(18) and continuous variation(19) methods under the analytical conditions. The results obtained Fig. 10, show a 1:2 (CLZ.): Fe(III)) product was formed .The suggest mechanism for formation the complex, as follows:

CLZ (reductant) + $2Fe^{+3} \rightarrow 2Fe^{+2}$ + CLZ (oxidant)

$Fe^{+2} + 3K_3[Fe(CN)_6] \rightarrow Fe_4[Fe(CN)_6]_3.xH_2O$ (x=14-16)

Potassium ferricyanide Prussian blue

Accuracy and Precision

The accuracy and precision of the estimation of (CLZ.) via the studied method which were established by found the values of the relative error (RE %) and relative standard deviation (RSD %), for five replicate analyses at three different levels of analytes concentrations in the range of (5-20) $\mu g.mL^{-1}$. The calculated analytical results show good accuracy with acceptable precision, as reported in Table (3).

Table 3: Validation of the suggest method for the estimation of (CLZ.) in pure form

Conc. of (CLZ) (µg/ mL) Taken	Found*	Mean (µg/ mL)	Er%	RSD%
5.000	4.992	4.985	-0.3000	0.0701
	4.978			
	4.985			
10.000	9.994	9.973	-0.2700	0.2864
	9.941			
	9.986			
20.000	19.995	20.017	0.0865	0.2377
	19.985			
	20.072			

*Average of five measurements.

Interference study

The effect of various foreign species, which may be present in pharmaceutical products and affecting the reaction between (CLZ.) and Fe(III), was studied. Optimum experimental conditions, were employed to determine 20 $\mu g.mL^{-1}$ concentration of (CLZ.). Table (4) shows the presence of 500$\mu g.mL^{-1}$ of the studied interfering. It was observed that the studied foreign species did not interfere in the proposed method.

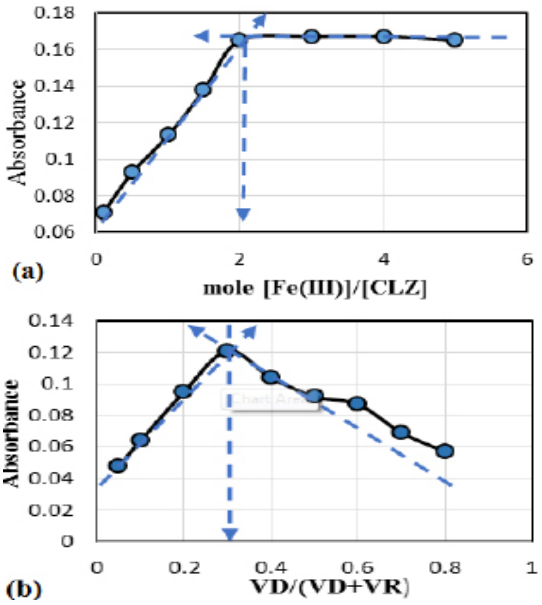

Fig.10. (a) mole ratio and (b) job methods

Table 4: Percent recovery for 20 µg.mL-1 of (CLZ.) in the presence of excipients

Excipients Name	Conc. (µg/ mL)	(CLZ.) Conc. Found (µg/ mL)	Recovery (%)
Glucose	50.000	20.081	100.405
Sucrose	50.000	20.059	100.295
Starch	20.000	20.104	100.520
Magnesium Stearate	10.000	19.951	99.755

Application

Commercially available tablets of (CLZ.) Rivotril 0.5mg and 2mg were subjected to analysis by the proposed procedure. The quantitative determination of two concentration levels of (CLZ.) was carried out via the proposed method. Table (5) shows the values of recovery percentage obtained for the analyzed samples by the application of the mentioned method on the pharmaceutical tablet. The results of the analysis were satisfactory, as indicated by the good recovery percent.

Statistical evaluation

The results obtained were statistically compared with those obtained using other methods

as shown in Table (6). The F test was used to estimate the difference in variance between the two methods, while student's t-test was used to compare the mean.

Table 5: Application of the proposed spectrophotometry to the (CLZ.) concentration measurements in their pharmaceutical tablet

Sample	Weight Found* (mg)	Concentration ($\mu g.mL^{-1}$)		Recovery%	RSD%
		Taken	Found*		
Rivotril® -0.5 mg	0.502	5.000	5.026	100.520	0.249
	0.502	10.000	10.040	100.400	0.242
Rivotril® -2 mg	2.018	5.000	5.046	100.920	0.419
	2.023	10.000	10.117	101.170	0.160

*Average of five measurements.

Table 6: Statistical comparison of the results obtained by the proposed method and other methods

Proposed Method	t- values[a]	F-values[b]	Other Methods			Ref. No.
			N	X	S.D	
N=5	0.3167	1.5319	5	9.980	0.0094	8
X =9.973	4.2924	2.4652	3	9.875	0.0355	9
S.D =0.0285	0.9224	6.3541	3	10.030	0.1203	20
tc =1.6476						

a-Theoretical value for t-test, for N=4(2.776) at 95, N=2(4.303) at 95% confidence limit.
b-Theoretical values for F-test, for N = (4, 4), at 95% is (5.72), N= (2, 4) at 95% is (6.9443), respectively.
c-(CLZ.) concentration = 10µg.mL.

CONCLUSION

The present study describes a simple and cheap method for determination of CLZ in its pharmaceutical Tablets depend on charge*transfer reaction. The method described here have many advantages such as simplicity, specificity, sensitivity and in addition it does not need expensive apparatus. After studying all the chemical conditions of the method and optimized, the method applied successfully for estimation of CLZ in tablets, with good recoveries. The proposed method was compared with other methods of CLZ determination and by using two statistical methods (t- and F tests) and both methods proved accuracy and precision of the method.

ACKNOWLEDGEMENT

I am grateful to Dr. Najwa Issac for the critical and continuous control, discussion and help during the tenure of the work.

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