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Spectrophotometric Determination of Chlorpromazine Hydrochloride in Pure and Pharmaceutical formulation by Using The Organic Reagent 2-5 dimethoxyaniline.

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ABSTRACT

A simple and sensitive spectrophotometric method for the determination of Chlorpromazine hydrochloride (CPZ) in pure form and in pharmaceutical dosage was developed. The method is based upon the oxidative coupling reaction between chlorpromazine hydrochloride and 2-5 dimethoxyaniline as a new organic reagent in the presence of oxidative agent NH_4VO_3. The reaction complete with a strong acidic medium to form a stable chromophore which absorbs at (717nm). The method showed a good linearity in the range (0.05– 14 µg. mL^{-1}) with molar absorptivity of $4.039*10^4$ ($L.Mol^{-1}cm^{-1}$) This method is a free from the interference of common excipients that found in pharmaceutical dosage. It was also applied for the determination of chlorpromazine hydrochloride (CPZ) in some of pharmaceutical dosage sample containing of this drug.

Keywords: Chlorpromazine hydrochloride, spectrophotometric, oxidative coupling, 2,5-dimethoxyaniline.

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INTRODUCTION

Phenothiazines belong to the oldest, synthetic psychiatric drugs, which do not have their precursor in the world of natural compounds [1]. They are heterocyclic molecules containing two benzene rings associated in tricyclic order across nitrogen and sulfur atoms. The derivatives of these compounds contain the amino alkyl side chain, and these are connected to the nitrogen atom of heterocyclic unit, plying important role in pharmaceutical chemistry [2]. These compounds are used in different diseases, for example, anti cancer [3] antihistamines, antiemetic, antipruritic [4], and they are widely used in the treatment of schizophrenia, organic psychoses and other acute or chronic idiopathic psychological diseases [5]. The important compound in the group of phenothiazine is Chlorpromazine hydrochloride (CPZ), which has the scientific name 3-(2-chlorophenothiazine-10-yl) propyl dimethyl-amine. And molecular weight of 355.33 (g/Mol) [6].

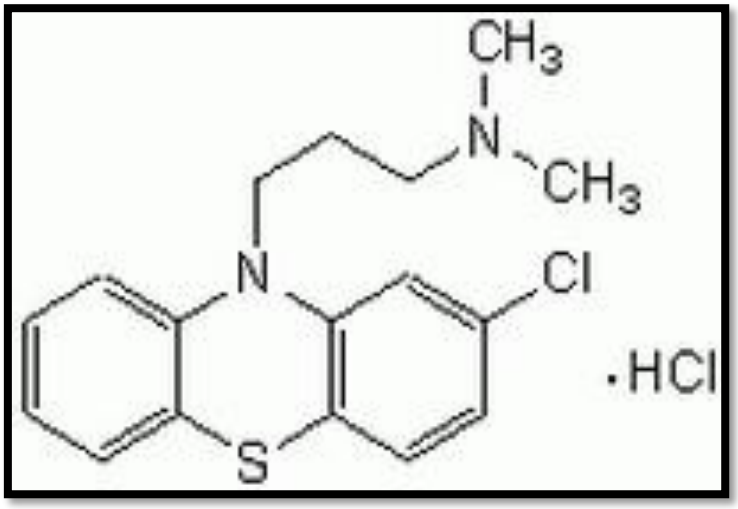

Figure (1): The chemical structure of chlorpromazine hydrochloride (CPZ)

Chlorpromazine hydrochloride known as Largactil. It was white crystalline powder has melting point 196 C^0 with a good solubility in water and ethanol, also it was dissociates when exposed to light or air [7]. There are several analytical methods that are used in the estimation of the (CPZ), spectroscopic methods are most important one, such as, UV-Vis [8], ^{13}C NMR [9,10], NMR [11], Mass spectroscopy [12], Atomic absorption [13], IR spectroscopy [14]. The other methods have been used to determination of (CPZ) are HPLC [15,16], liquid chromatography-mass spectrometry (LC-MS/MS) study [17], flow injection method [18,19], and electrochemical method [20-22]. In this paper describes a simple, rapid and sensitive method for the determined of small amounts of chlorpromazine hydrochloride (CPZ) depending on the oxidative coupling reaction between (CPZ) and reagent 2-5 dimethoxyaniline in the presence of oxidative agent (NH_4VO_3) in strongly acidic media to form a stable chromophore. And study the optimum condition for this reaction. Then applying this method on some different types of pharmaceutical Preparations which is contained (CPZ) compound with high accuracy and precision.

EXPERIMENTAL

-All spectral and absorbance measured by T80 UV-Visible Spectrophotometer PG Instrumental Ltd, UK. With quartz cells, which have (1 cm) optical path.

-Oven BS Size Two, Gallenkamp, England. With (0-300 C^0) thermal range.

Heating, Cooling Water Bath – Haak Fe, Sartorius, Balance Bp3015 –Germany.

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MATERIAL AND METHOD

All materials used have a high degree of purity, they were prepared by the following:

- hydrochloride standard material from the state company for drug industries and medical appliances Samara-Iraq (SDI). The standard stock solution of (CPZ) at (100 µg/ml) prepared by dissolving (0.005 gm) of (CPZ) by distilled water and diluting into (50 ml) with the same solvent in a volumetric flask as a stock solution, which is stable for month after saving away from the light.

- 2,5-dimethoxyanilin (0.1M): It was provided from (Sigma-UK) company. This solution was prepared by dissolving (0.7659gm) of 2,5-dimethoxyaniline by ethanol and diluting into (50 ml) with the same solvent in a volumetric flask as a stock solution.

- Sulfuric acid H_2SO_4 (1M): It was prepared from (GCC) at percentage (%98) company and used for preparation (1M) solution

- Ammonium metavanadate NH_4VO_3 Solution (0.003M) [23]: It was provided for (BDH Chemicals Ltd, Laboratory reagent) company This solution was prepared by dissolving (0.0174gm) of ammonium metavanadate by a small amount of (0.1M) of sulfuric acid, and diluting with distilled water to the mark of (50ml) volumetric flask as a stock solution.

Recommended Procedure

In a series of volumetric flasks of (10ml), taken from standard solutions of (100µg.ml^{-1}) chlorpromazine hydrochloride with concentrations of (0.05 -14 µg/ml), followed by addition (2ml) of (0.003M) of ammonium metavanadate , then addition (1ml) of (0.1 M) 2,5 dimetoxyaniline, then addition after that (0.5ml) of (1M) sulfuric acid , the contents were in a series of (CPZ) diluted to the mark with distilled water, The solutions were left for 10 minutes in a room temperature and the absorbance was measured at (717nm), against reagent blank solution and a calibration curve was constructed.

Procedure for Assay of chlorpromazine Hydrochloride in Pharmaceutical Preparations.

As some of the preparations Largactil containing (CPZ) as an active material was taken and it included the following:

- Largactil tablets (100mg): They were supplied from (Ruhsat Sahibin- Turkey, Istanbul) company under license from Aventis Pharma-France.

- Largactil injections (25mg/5ml): they were supplied from (Oubari pharma -Aleppo- Syria) company under license from: Sanofi- Aventis -France.

Procedure for Tablets [24]

Ten tablets were weighed and finely powdered from tablets. An accurately weighed amount of the powder equivalent to (0.005gm) of (CPZ), It was dissolved in (5ml) of ethanol and (5ml) of (5M) hydrochloric acid with heating and after that filtering to separate the non-dissolved components. Then transferred into a (50ml) volumetric flask and diluted to the mark with distilled water, then take the suitable amount of solution and treated in the same conditions on a calibration curve to find the concentration.

Procedure for Injection [25]

Take about (1ml) from ampule containing (25 mg/5ml) of Chlorpromazine hydrochloride and transferred into (50ml) volumetric flasks and diluted to the mark with distilled water. Then we calculated the concentration depending on the standard calibration curve.

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RESULTS AND DISCUSSION

Different conditions were studied which are affecting the absorbance of the product formed such as:

Effect of oxidizing agent volume

The effect of oxidizing agent volume on the intense absorption was studied. It was taken from (0.5 – 4ml) of ammonium metavanadate at a concentration (0.003M), with Presence (1ml) of (0.05M) of the reagent and (1ml) of acidic solution. It was found that (2ml) is the best volume of the oxidizing agent, that gives the highest absorption, which was used in the following experiments, Fig (2) explain this.

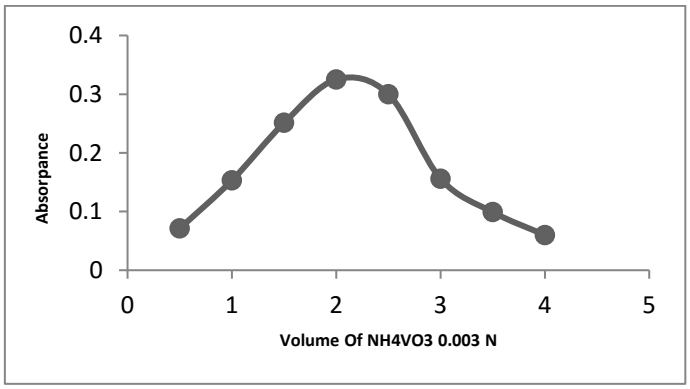

Figure (2): effect of NH_4VO_3 volume

Effect of Reagent concentration

It was taken from (0.03 –0.17M) of the reagent 2,5-dimethoxyaniline (1ml), in the Presence of (2ml) of an oxidizing agent and (1ml) from acidic solutions (1M). It was found that (0.1M) is the best concentration of the reagent, that gives the highest absorption, which was used in the following experiments, Fig (3) explain this.

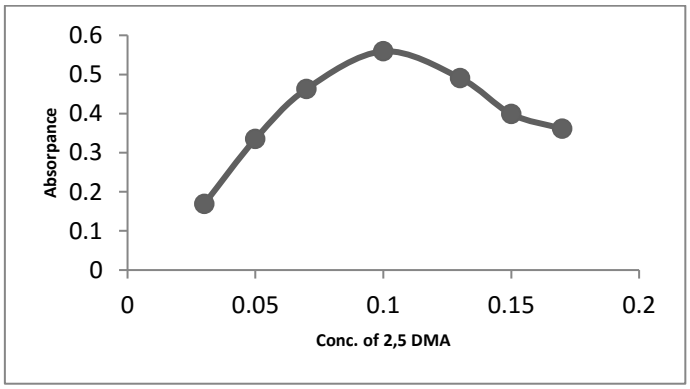

Figure (3): effect of 2,5DMA concentration

Effect of Reagent volume

The effect of Reagent volume on the absorbance was studied. It was taken from (0.2 –2ml) of the reagent 2,5dimethoxyaniline (0.1M) with Presence (2ml) of the oxidizing agent and (1ml) from acidic solutions (1M). It was found that (1ml) is the best volume of the reagent, that gives the highest absorption, which was used in the following experiments, as show in Fig (4).

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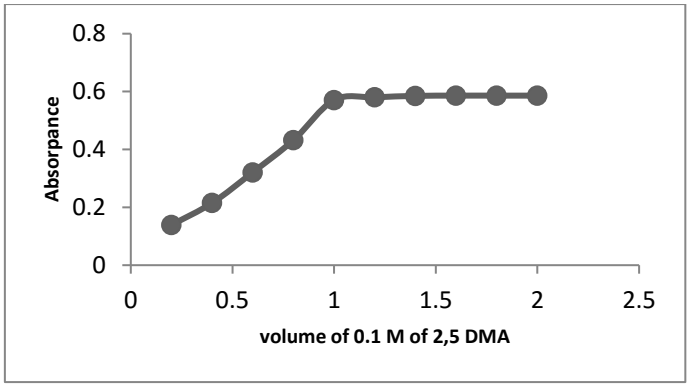


Figure (4): effect of 2,5DMA volume

Effect of acid

Some of acids such as H_2SO_4, HCl, CH_3COOH, and HNO_3 are examined, it was found that (0.5ml) of (1N) H_2SO_4 gave the high absorbance of the color product, it was selected in the following experiments. This show in Fig (5)

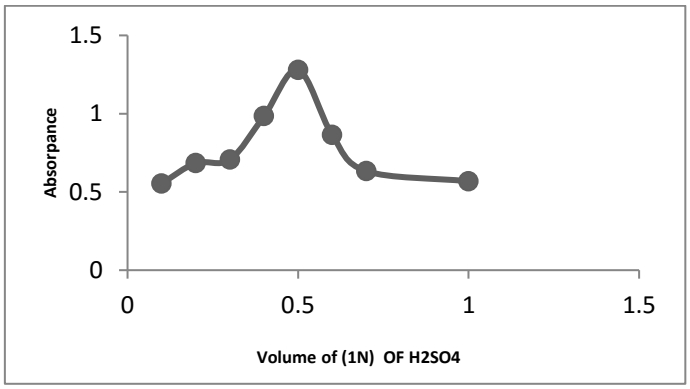


Figure (5): effect of acid

Effect of Sequence of Addition

It was found that the best sequence of addition that gives the highest absorption (D+O +R +A), where: (D = Drug, O = Oxidizing agent, R = Reagent, and A = Acidic solution) which selected in following experiments.

Effect of Temperature

The resulting product of the proposed method was studied at different temperatures. The results referred that the absorbance values remain nearly constant in the temperature range (0-35ºC), whereas, at higher temperatures the absorbance value decreases, referring the dissociation of the product when heated for a long time. The color product was stable at temperature (25ºC) which was given the highest absorbance. The room temperature was selected in this method. This explains in Fig (6).

ISSN: 0975-8585

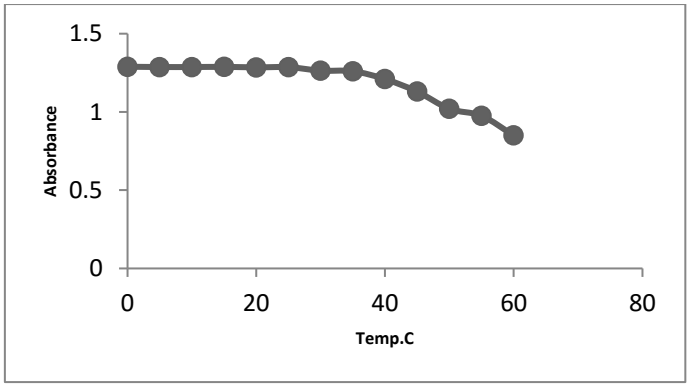


Figure (6): effect of temperature

Effect of Reaction Time

The color intensity appears its maximum after the drug (CPZ) had been reacted immediately with The oxidizing agent in the presence of 2,5dimethoxyaniline and acidic solution became stable after (10) minutes. Therefore (10) minutes was selected as an optimum time in the general procedure. The color product obtained was stable in (65) minutes. As in Fig (7).

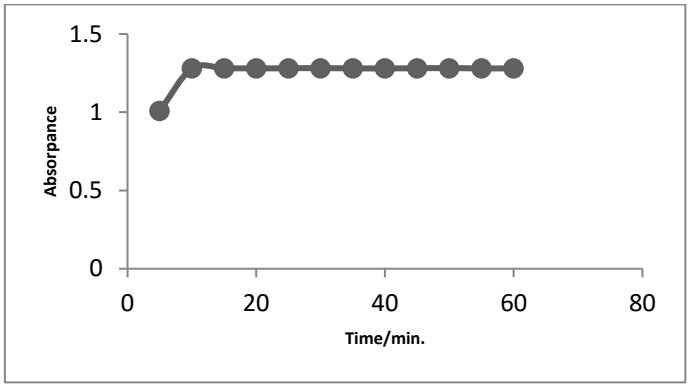


Figure (7): effect of reaction time

Absorption Spectra

The spectral scan has been done to get the highest wavelength absorption of the resulting compound getting after fixing the optimum conditions for the reaction against blank solution which contain all the additives except for the drug compound, Figure (2) shows the spectrum of complex product from the reaction between (CPZ) and 2,5-dimethoxyaniline, Fig (8) shows the spectra of color product formed and of the blank solution, where (A): solution of (100µg/ml) of CPZ against blank solution. (B): solution of blank against distilled water.

ISSN: 0975-8585

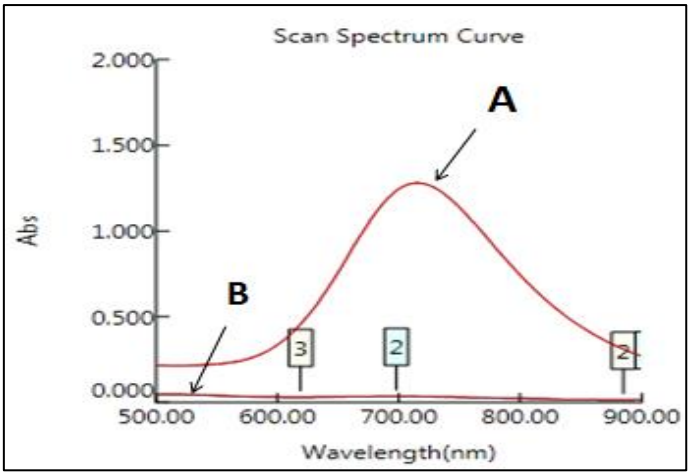


Figure (8) shows the spectra of product formed at(100µg/ml) of (CPZ) (A) and of the blank (B) at (0.1M) of reagent and oxidant and (1M) sulfuric acid.

Calibration curve

According the conditions described in the procedure, a linear calibration curve for Chlorpromazine hydrochloride is obtained (Fig 9), which shows that Beer's law is obeyed over the concentration range of (0.05–14µg/ml) with a correlation coefficient of (0.9987) and an intercept was (0.0548), the slope of the curve was (0.1137). The conditional molar absorptivity of the blue product formed was found to be ($4.039*10^4$ $L.mol^{-1}$. cm-1). The Sandell's sensitivity was (0.008µg. cm^{-2}). LOD was (0.024µg/ml^{-1}). LOQ was (0.082µg/ml^{-1}).

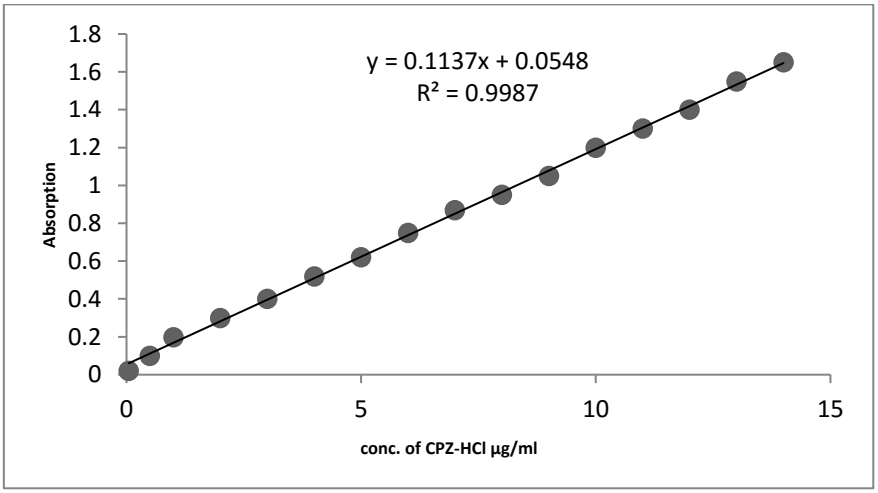


Figure (9) shows the Calibration curve of (CPZ)

Accuracy and precision

The accuracy and precision of the method were checked by determination the Chlorpromazine –HCl at three different concentrations from calibration curve (five solution for each concentration) under optimum condition. And calculation relative error (E%), recovery (Rec %), and relative standard deviation (RSD %). The results represented in Table (1) indicate that the method is satisfactory, and have high accuracy and precision.

ISSN: 0975-8585

Concentration Of CPZ µ/ml		%Error*	%Rec*	%RSD*
Taken	Found			
5	5.008	+0.16	100.16	0.213
7	6.991	-0.114	99.88	0.140
9	8.982	-0.200	99.80	0.095

***Each value, it's the average of five readings**

Table (1): Accuracy and precision of the proposed method.

Stoichiometry of reaction

The stoichiometry of the reaction between Chlorpromazine – HCl and the reagent was investigated by applying Job's method and mole ratio method; the results obtained that (1:1) drug to reagent complex was formed as (717nm) shown (Fig 10). The product formed was soluble in water. To know the stability of the product, degree of dissociation and stability constant were calculated by comparing the absorbance of a solution containing stoicheiometries amount of Chlorpromazine – HCl and the reagent at a concentration ($4*10^{-3}$ M), with other solutions containing the same amount of the drug and five times the amount of reagent. The average conditional stability constant of the color product in the water under the described experimental conditions was ($2.6*10^{8}$ L^2 Mol^{-2}).

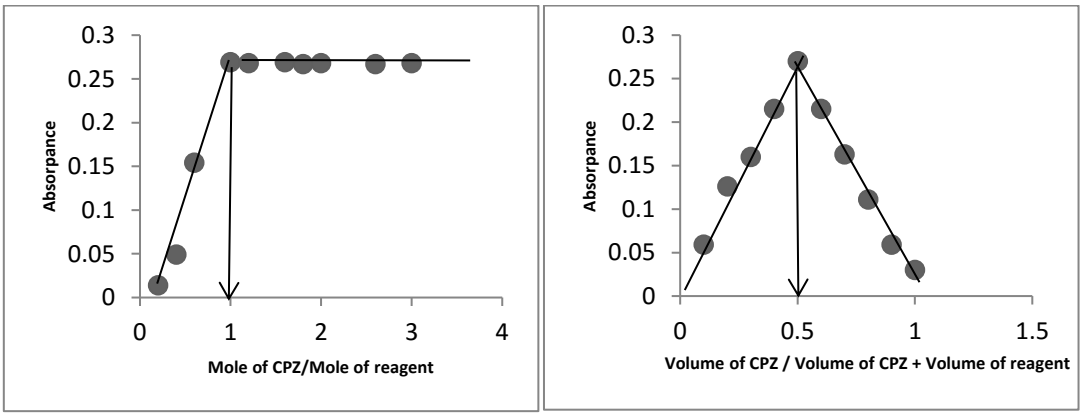
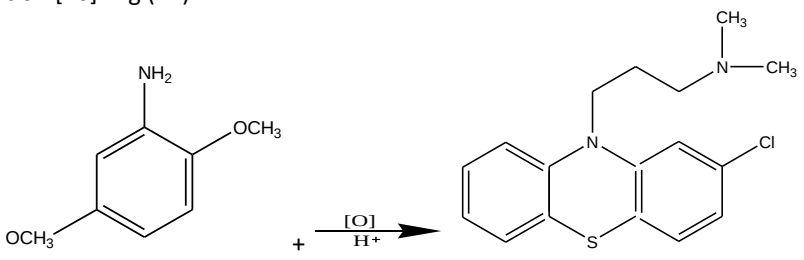


Figure (10): mole ratio and job methods, for reaction of (CPZ) with reagent in the presence of NH_4VO_3 and H_2SO_4.

The formation of the color product between Clorpromazine hydrochloride and 2,5-dimethoxyaniline in the presence of H_2SO_4 and ammonium metavanadate was suggested at the scheme of reaction probably occurs as the following equation [26] Fig (11):



2,5-dimethoxyaniline CPZ

ISSN: 0975-8585

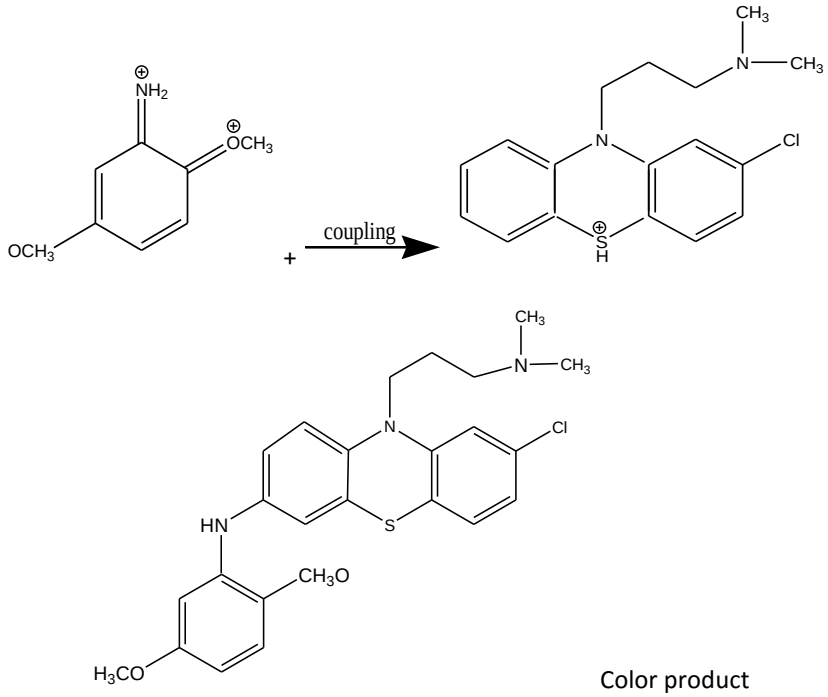


Figure (11): scheme of the oxidative coupling reaction

Interferences

The effect of additives which are found in the pharmaceutical contain (CPZ) were studied, to ensure from the selectivity of the proposed method for the purpose of application of the pharmaceutical contain (CPZ). For this study, the solution was contained (5µg / 10 ml) of (CPZ) and each one of the excipients was taken separately in concentrations, ten-times greater than of (CPZ) were analyzed under the same procedure in the Calibration curve. The level of interference was considered to be acceptable if the error was not higher than (± 2%) relative to the expected No interferences were observed in the determination of (CPZ) in the presence of the excipients studied, show in table (2) (Average of five determinations).

Excipient	Conc of CPZ µg/ml (found)	%Error*	%Rec*
Lactose	5.02	+0.40	100.4
Sucrose	4.96	-0.80	99.20
Benzoic acid	4.95	-1.00	99.00
starch	4.95	-0.05	99.95
Glucose	4.96	-0.80	99.20
Talc	4.98	-0.02	99.98
Mg striate	4.98	-0.02	99.98

***Each value, it's the average of five readings**

Table (2): Determination of (5µg / 10 ml) of chlorpromazine hydrochloride in the presence of excipients.

ISSN: 0975-8585

Application of the method

The applicability of the method for the assay of pharmaceutical formulation, it has been studied. The result of experiment for available formulations of Chlorpromazine – HCl drugs are summarized in Table (3).

Pharmaceutical preparation	Proposed method		Error%	Rec%	RSD%
	taken	Found			
Largactil : Tablet Each one contain 100 mg of CPZ	5 7 9	5.01 7.008 8.991	+0.322 +0.114 -0.009	100.32 100.11 99.90	0.196 0.099 0.067
Largactil : injection 25 mg of CPZ in 5 ml	5 7 9	4.970 6.970 8.977	-0.600 0.328 -0.255	100.16 99.85 99.74	0.112 0.140 0.093

Table (3): Assay of Chlorpromazine – HCl in bulk and dosage forms

For assessing proposed method and its success in the spectral estimation of (CPZ) in some pharmaceuticals, it has applied the standard method to estimate (CPZ) were taken from British pharmacopoeia (2009) [25]. Then comparison with The proposed analytical method. The results are summarized in the table (4).

Pharmaceutical preparation	%Rec for proposed method	%Rec for standard method
pure CPZ	99.94	99.20
Largactil : Tablet Each one contain 100 mg of CPZ	100.12	100.00
Largactil : injection 25 mg of CPZ in 5 m	99.91	99.20
T Value (exp)= 0.965 , Critical Value = 3.182 F Value (exp)= 0.054 , Critical Value = 9.552		

Table (4): A comparison between the proposed and standard methods

The results showed no significant differences between the two methods, this means the proposed method in this research with the possibility and validity of Good apply to all pharmaceuticals used.

CONCLUSION

A simple, rapid, precise and sensitive spectrophotometric method has been developed for the determination of trace amounts of chlorpromazine hydrochloride in aqueous solution based on oxidative coupling reaction with the organic reagent 2,5-dimethoxyaniline and ammonium metavanadate in the presence of sulfuric acid. The proposed method does not require temperature control or the solvent extraction step, the method was applied, successfully for the determined of small amounts commercial (CPZ) drug.

REFERENCES

[1] Agata J, Kazimierz G, Piotr S, Wiestaw M, Kataryna C, Joanna P, Bogustawa C. Pharmacological Reports 2012; 64: 16-32.

[2] Sinha S, Pandeya S N, Verma A, Xadav D. International Journal of Research in Ayurveda & Pharmacy 2011; 2: 1130-1137.

[3] Alejandro G, Li Pan, Richard W J G, Frederic B, Alex K, Jason M, Ruta G, Elena K, Casie R, Francoise P,Sandrine P, Benjamin U, Paul C, Lynn V, Frank A, Jason B,Stephanie N, Nicola T, Hanno S, Andrew PW, Huipin Y, James EB, Constantine M, Thomas L, Jon A. The Journal of Clinical Investigation 2014; 124: 644–655.
[4] Wilson O, Gisvold D ,Doerge RF, Textbook of organic medicinal and pharmaceutical Chemistry, 7th edn. (J B Lippincott) 1977; pp. 338.
[5] Gordon M, Psychopharmacological Agents; 1964, vol. II
[6] Karpinska J, Starczewska B, Puzanowska-Tarasiewicz H,. Anal Sci 1996; 12: 161-167.
[7] Minakata K, Suzuki O, Ishikawa Y, Seno H, Harada N, Forensic Sci Int 1992; 52: 199-210.
[8] Tehseen A, Rashid A, Khokh K, Javaid. Analytical Letters, 1997; 30: 109-119.
[9] Starczewska B. Analytical Letters 1996; 29: 2475-2486.
[10] Willy N, Stig G, Vidar T, Harald H, Holm H. Biochimica et Biophysica Acta 2000; 1464 :165-175.
[11] Florence A, ParfittIb T. The Journal of Physical Chemistry 1985; 35: 3554.
[12] Gruenke L, Cymerman C, Kleint F, Nguyen N, Barbara A H, Holaday J, Loh H Braff L, Ames F, Glick I, Hartmann F, Bissell M. Biomedical Mass Spectrometry 1985; 12: 707-713.
[13] El-Ansary A, W.F. El-Hawary, Y.M. Issa, A. F. Ahmed. Analytical Letters 1999; 32: 2255-2269.
[14] Warren, Eisdorfer B, Thompson, Zarembo J. Journal of Pharmaceutical Sciences 1966; 55: 144-150.
[15] Shetti P, Venkachalam A. E-Journal of Chemistry 2010; 7: 299-313.
[16] Venkatesh S, Mandal BK, Sridevi R, Navalgund SG. International Research Journal of Pharmacy 2010; 1: 225-232.
[17] Shetti P, Venkatachalam A. Journal of Pharmaceutical and Biomedical Sciences 2011; 9: 2230–7885.
[18] Daniela D, Ivano GR. Journal of Pharmaceutical and Biomedical Analysis2005; 37: 281–286.
[19] Issam MS, Mohammed J. Kerbala Journal of Pharmaceutical Sciences 2012; 3: 86-94.
[20] Saadet D, hci Biryol. Analyst 1989; 114: 525-526.
[21] Frag YZ, Omar MA, Elashery MM, Elashery EA, Mohamed GG. Int. J. Electrochem. Sci 2012; 7: 650-662.
[22] Waqar H, Edmond B, Dilshad W, Pak. J. Pharm. Sci 2013; 5: 977-984.
[23] Salah M S, Analyst 1991; 116: 177-181.
[24] US Pharmacopoeia XXIIth Rev, US Pharmacopoeia Convention, Rockville, MD, 2007; 294-295.
[25] British Pharmacopoeia, Her Majesty's Stationary Office, London, 2009; 1292-1293,8319-8324.
[26] Meger A, Ayers G. Am. Chem.Soc 1957; 63; 49.