Spectrophotometric Determination of Chlorpromazine Hydrochloride in Pharmaceutical Preparations by Oxidative Coupling reaction.

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Abstract
An easy and simple spectrophotometric method was described for estimating chlorpromazine Hydrochloride drug in aqueous solution. Where The method was adopted on oxidative coupling reaction of the drug with p-nitro aniline in the presence of ceric(IV) ammonium nitrate and hydrochloric acid solution an orange-brown product dye was obtained with maximum absorption at 525 nm. with molar absorptivity of \(9.24 \times 10^3\) l. mol\(^{-1}\). cm\(^{-1}\) and sandell's sensitivity of 0.0385 µg.cm\(^{-2}\). Beer's law is obeyed over the concentration range of (12-46) µg.ml\(^{-1}\). The method was applied successfully for the estimating the drug it's on pure condition or in pharmaceutical preparations (Largactil drug).

Keywords: chlorpromazine Hydrochloride, oxidative coupling, spectrophotometric.
التقدير الطيفي للكلوربرومازين هيدروكلورايد في المستحضرات الصيدلانية

وسيطة تفاعل الاقتران ألتاكسدي

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الملخص

تم وصف طريقة طيفية سهلة وبسيطة لتقدير عقار الكلوربرومازين هيدروكلورايد في المحلول المائي، حيث اعتمدت الطريقة على الاقتران ألتاكسدي للعقار مع بارا- دايتروترييلين ووجود نترات السيريك الأمونياكي وحلول حامض الهيدروكلوريك وتم الحصول على ناتج برتقالي- بني. يمتلك امتصاصية عظمى عند 525 نانوميتر بلغت قيمة معامل الامتصاص المولاري $9.24 \times 10^{-3}$ l. mol$^{-1}$. cm$^{-1}$ وحساسية ساندل 0.0385 µg.cm$^{-2}$ وحدود قانون بير ضمن مدى التراكيز $0.0002-10$ µg.m$^{-1}$. وطبقت الطريقة بنجاح في تقدير العقار في حالته النقية أو في مستحضراته الصيدلانية (دواء الارجكتيل).

الكلمات الدالة: الكلوربرومازين هيدروكلورايد، الاقتران ألتاكسدي، التقدير الطيفي.
1. Introduction

Phenothiazine is a very important class of organic compound with strong biological efficacy used as a treatment for severe and moderate psychosocial conditions [1,2]. It binds with certain receptors of dopamine D2 and affects its function and thus affects many processes in the body such as metabolism [3] and is used for epilepsy [4], stomach, liver and bowel diseases [5] and tetanus treatment [6]. Many phenothiazine derivatives is formally found in the British pharmacopoeia [7] and the Indian pharmacopoeia [8]. Chlorpromazine is one of the most important phenothiazine and the scientific name of chlorpromazine according to the IUPAC system is: 2-chloro-10-[3-(dimethylamino)propyl] phenothiazine monohydrochloride.

![Molecular Formula](image1)

**Fig. 1:** Molecular formula (C₁₇H₁₉ClN₂S.HCl), M.wt 355.33 g.mol⁻¹, M.p 196°C.

Several spectral methods have been used to estimate chlorpromazine hydrochloride, which is generally based on the oxidation and reduction reaction [9] oxidative coupling [10,11] and chromatographic methods used to estimate chlorpromazine, such as high performance liquid chromatography [12,13] Gas chromatography [14,15], and flow injection[16,17]. In this research a new method was developed, simple, rapid, and sensitive, to estimate micrograms of chlorpromazine based on the oxidative coupling reaction with p-nitroaniline in the presence of ceric(IV) ammonium nitrate in a strong acid medium.

2. Experimental part

[Apparatus Used]

Spectral measurements were performed using double-beam spectrophotometer UV-Visible cintra 6 with matched 1cm quartz cells.

[Solution of the materials used]

All the chemicals used were highly purified

[Chlorpromazine hydrochloride solution 300µg.ml⁻¹]
Prepare the solution by dissolving 0.1000 g of chlorpromazine hydrochloride powder provided by (S.D.I) in a quantity of distilled water and then complete the volume to the mark in 100 ml volumetric flask with the same solvent to obtain a 1000 µg/ml concentrated solution. The required solution was diluted by diluting 30 ml of the standard solution 1000 µg/ml, in a 100 ml volumetric flask.

**[Oxidant factor solution 0.01 Molar]**

This solution was supplied by (Beijing Solarbio Science& Technology) by dissolving 0.548 g of pure material in a volumetric vial similar to that is used in preparation standard solution of drug [18].

**[Hydrochloric acid solution 0.5 Molar]**

This solution was supplied by (Fluka) by diluting 4.24 ml of acid in a volumetric bottle like that employee in oxidant solution and full size to the brand with distilled water[18].

**[Reagent solution P-nitro aniline 0.01 Molar]**

This solution was supplied by (Beijing Solarbio Science& Technology) by dissolving 0.138 g of reagent in a quantity of ethyl alcohol and then complete the size to the limited line in a 100 ml volumetric flask[18].

**[Procedure for pharmaceutical formulations]**

Largactil(Tablet)

Ten tablet (100mg/tablet) are weighed, and mixed fully, an exactly weighed amount of powder analogous to (0.1) g of chlorpromazine hydrochloride, dissolved in hot distilled water with continuous stirring and then filtered to remove the insoluble matter, and then transferred to 100 ml volumetric vial, and full the volume with deionized water to the line. 30 ml of this solution is taken and convey to a 100 ml volumetric bottle to obtain a solution concentration of 300µg/ml.

**[Injection]**

Empty the content of two ampoule(25mg/5ml) in a 50 ml volumetric flask , then take 30 ml of this solution and dilute to a 100 volumetric flask to obtain 300µg/ml solution of chlorpromazine hydrochloride.
3. Result and Discussion

Optimal conditions were studied, which affect the resulting absorption, intensity composed and on color contrast to get the best condition 2 ml of chlorpromazine solution with a concentration of 300µg/ml was used in final volume 25 ml.

[Effect of the amount of oxidizing agent]

The effect of adding different amount of the oxidizing agent on the absorption intensity was studied. A series of volume (0.1-3) ml of oxidizing agent ceric (IV) ammonium nitrate 0.01 Molar concentration was taken with 1ml reagent p- nitro aniline and 1ml of hydrochloric acid solution. It was found that the best amount (0.5) ml was given the absorption intensity, and this volume was selected in all subsequent measurements.

[Effect of the Amount of coupling Reagent]

The effect of reagent quantity p- nitro aniline was studied on the absorption intensity. A series of volume of reagent (0.5-3) ml 0.01 Molar were taken using 0.5 ml of oxidizing reagent and 0.5 ml hydrochloric acid solution. The addition of 1 ml of reagent was found to be the best to give it the highest absorption intensity and this volume was selected in all subsequent measurements.

[Effect of the Amount of acid]

Some of weak and strong acids have been used and found (1) ml of hydrochloric acid give the maximum absorption intensity and this volume was elected in all following measurements.

[Effect of Order of Addition]

It was found that the best addition sequence that gives the highest absorption intensity is (O+R+D+A) where O= Oxidative factor, R= Reagent, D= Drug , A=Acidic solution as show in Table 1.
[Stability of reaction product]

It was found that the value of absorption of the color product remained stable for a period of not less than 70 minutes and this time is suitable for completion of many measurements and results show in Table 2.

Table 1: Effect of Order of addition.

| Order Number | Order of addition | Absorbance |
|--------------|------------------|------------|
| I            | O+D+R+A          | 0.522      |
| II           | D+O+R+A          | 0.425      |
| III          | O+R+D+A          | 0.611      |
| IV           | R+D+O+A          | 0.321      |

[Stability of reaction product]

Table 2: Stability of reaction product.

| Time(min) | 5   | 10  | 15  | 20  | 30  | 40  | 50  | 60  | 70  |
|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Abs       | 0.600 | 0.610 | 0.610 | 0.612 | 0.601 | 0.600 | 0.600 | 0.610 | 0.611 |

[Solvent effect]

After all components of the reaction were added according to the method used, different solvent were used to complete the volume to the extent of the mark in 25 ml volumetric flask to obtain the highest absorption, and the figure indicates that water is a good medium for the reaction and gives the highest absorption at wavelength 525 nm.

Fig. 2: Solvent curve.
The final absorption spectra were measured after optimum conditions were established.

Table 3: Optimum Conditions.

| λ max (nm) | 525 |
|-----------|-----|
| Amount(ml) of 1*10^{-2} M p-nitroaniline | 1ml |
| Amount(ml) of 1*10^{-2} M Ceric(IV) ammonium nitrate | 0.5 ml |
| Amount(ml) of 0.5M Hydrochloric acid | 1ml |
| Temperature , Solvent | 25°C, Water |

Fig. 3: Final Absorption Spectrum

(A absorption spectrum of chlorpromazine versus distilled water) (B chlorpromazine versus Blank) ( C Blank versus distilled water).

[Procedure Construction of Calibration]

Increasing volume (1-3.8) ml of chlorpromazine 300µg/ml were added to 25 ml volumetric flask containing 1ml of p- nitro aniline 1x10^{-2} M, 0.5 ml of oxidized agent solution 1x10^{-2} M, 1ml of hydrochloric acid solution 0.5 M, then complete the volume to the mark with distilled water and then measure the absorption of all solution versus Blank solution at
525 nm. Fig. 2 represents linear calibration curve for chlorpromazine with the concentration (12–46) µg/ml, linear regression equation: \( y=0.0264x-0.0899 \) (\( R^2=0.9929 \)) where \( y \) is the absorbance and \( x \) is the concentration in µg/ml. Molar absorption coefficient \( 9.38 \times 10^3 \) L.mol\(^{-1}\).cm\(^{-1}\), sandel’s Index \( 0.0385 \) µg.cm\(^{-2}\). This indicates that the standard curve has a high linear specification.

**Fig. 4:** Calibration graph for determination of chlorpromazine hydrochloride.

*Precision and Accuracy*

The precision and accuracy for calibration curve it has been measured by determination three different concentration of 300 µg/ml chlorpromazine hydrochloride and the product were show in Table 4 which indicate good thoroughness and agreement.

| Conc. of CPH µg/ml | RE, % | Recovery, % | Average of Recovery, % | RSD, % |
|-------------------|-------|-------------|------------------------|--------|
| 18                | +0.978| 100.98      |                        | 0.382  |
| 30                | -1.000| 101.00      |                        | 0.344  |
| 42                | -0.655| 99.35       | 100.44                 | 0.335  |

*Stoichiometry of chlorpromazine hydrochloride-p- nitro aniline complex*

The stoichiometry of the reactant was investigated by Job method[19] the result obtain indicated that the existence of 1:1 chlorpromazine-p nitro aniline at 525nm.
Fig. 5: Job method for determine chlorpromazine with p-nitroaniline and ceric(IV) ammonium nitrate.

Fig. 6: Proposed equation of interaction.

4. Applications

The method can be applied to pharmaceutical preparation contain chlorpromazine:
Largactil (100mg/Tablet)
Largactil (25mg/5ml, injection)

Direct Method

Two different concentration of each solution were taken (Tablet, Injection) 24, 36 µg/ml. The solution were treated with the same steps as the calibration curve and then measured at 525 nm, and the result show in Table 5 which indicate to success the suggested method in determination of chlorpromazine in pharmaceutical preparation.
5. Statistical evaluation of the results of the proposed method

To determine the success of the suggest method for the determination of chlorpromazine in pharmaceuticals, the validity of application of the method was examined by testing f and t for the accuracy and precision of the proposed method and the results are shown in Table 6.

The results of the above table show that the calculated F and t values of the two formulas (Tablet, injections) is less than the value of the tabular F and t at 95% confidence limit and for four degrees of freedom. This indicates the accuracy of the spectral method used.
6. Comparison of method

Some of the physical variables of the proposed method were compared with the differences in the spectral methods from the literature used in the estimation of chlorpromazine. We conclude from the results shown in Table 7 that the proposed method has a wide range of estimation and has been successfully applied in estimating the compound under study in two pharmaceutical preparations, As well as good sensitivity in compared to other methods.

Table 7: Comparison Of Method.

| Reagent               | λ max(nm) | Linear range, ppm | Molar absorptivity (L.mol⁻¹.cm⁻¹) | Recovery (%) | R.S.D (%) | Number of Ref. |
|-----------------------|-----------|-------------------|-----------------------------------|--------------|-----------|---------------|
| Chloranilic acid      | 520       | 20-150            | (1.48-1.75)x10³                  | 99.54-100.4  | 1.04-1.82 | 20            |
| N-Chlorosuccinimide   | 516.5-534.5| 2.0-40           | (5.34-6.16)x10³                  | 98.31-100.84 | 1.21-3.81 | 21            |
| P-nitroaniline        | 525       | 12-46             | 9.24x10³                         | 99.35-101.00 | 0.335-0.382| Proposd method|

7. Conclusions

An easy and simple spectrophotometric method was described for estimating chlorpromazine Hydrochloride drug in aqueous solution. Where The method was adopted on oxidative coupling reaction of the drug with p-nitro aniline in the presence of ceric(IV) ammonium nitrate and hydrochloric acid solution an orange-brown product dye was obtained with maximum absorption at 525 nm. with moler absorptivity of 9.24x10³ l. mol⁻¹. Cm⁻¹ and sandell's sensitivity of 0.0385 µg.cm⁻² Beer's law is obeyed over the concentration range of (12-46) µg.ml⁻¹. The method was applied successfully for the estimating the drug it's on pure condition or in pharmaceutical preparations (Largactil drug).
Reference

[1] L. P. Posner, P. Burns, Sedative agents: tranquilizers, alpha-2 agents, and related agents. In: J. E. Riviere, M G. Papich, "Veterinary pharmacology and therapeutics", 19th Ed., Wiley-Blackwell, Ames, Iowa, USA, 337 (2009).

[2] S. C. Sweetman, "Martindale: The complete drug reference. Pharmaceutical Press" 37th Ed., London, UK, 977 (2006).

[3] C. O. Wilson, O. Gisvold, R. F. Doerge, “Text book of organic medical and pharmaceutical chemistry” 7th Ed., 384 (1977).

[4] R. PHawkins, J. PForsyth, "The behavior analytic perspective: Its nature, prospects, and limitations for behavior therapy", Journal of Behavior Therapy and Experimental Psychiatry, 28(1), 7 (1997).

[5] B. D. Roufogalis, M. Thornton, D. N. Wade, "Specificity of the dopamine sensitive adenylate cyclase for antipsychotic antagonists", Life Sciences, Life Sciences, 19(6), 927 (1976).

[6] D. Healy, "Explorations in a New World the Creation of Psychopharmacology", Harvard University Press., 77 (2004).

[7] "British Pharmacopeia CD-ROM", 7th Ed. Copyright by system simulation Ltd ., The stationery office , London , (2012).

[8] "Pharmacopoeia of India", ministry of health and family welfare, Govt. of India, New Delhi, (1985).

[9] M. J. Hamzah. AL-kaffiji, and A. M. Saeed. AL-Anbakey, "New chromogenic reagent for the spectrophotometric determination of chlorpromazine. HCL in aqueous solution and pharmaceutical formulations", International Journal of pharmacy and pharmaceutical Sciences, 5, supple 3, (2013).
[10] عمر عدنان هاشم شريف ال ابهيش، "التقدير الطيفي للترايفلبرازيه والكلوربرومازيه في المستحضرات الصيدلانية باستخدام تفاعلات الاكسدة " رسالة ماجستير، جامعة تكريت(العراق)، 48 (2012).

[11] M. J. Hamzah. AL-kaffiji, and A. M. Saeed. AL-Anbakey, " New chromogenic reagent for the spectrophotometric determination of chlorpromazine.Hcl in aqueous solution and pharmaceutical formulation", International Journal of pharmacy and pharmaceutical Sciences, 5, supple 3 (2013).

[12] P. Shetti and A. Venkatachalam, "Stability indicating HPLC method for simultaneous quantification of trihexyphenidyl hydrochloride, trifluoperazine hydrochloride and chlorpromazine hydrochloride from tablet formulation", E-Journal of Chemistry, 7(1), 299 (2010).

[13] S. Venkatesh, M. B. Kumar , S. Ramachandran, and N.G Sameer, "Hplc method development validation and its application to stability studies of chlorpromazine hydrochloride tablets", international research journal of pharmacy, (1), 225 (2012).

[14] L. Zhang, P. Wu, Y. Zhang, Q. Jin, D. Yang, L. Wang, and J. Zhang, "A GC/MS method for the simultaneous determination and quantification of chlorpromazine and diazepam in pork samples", Anal. Methods, 6(2), 503 (2014).

[15] G. Zhang, G. Michael and V. Alvin, "Sensitive liquid chromatography/tandem mass spectrometry method for the determination of the lipophilic antipsychotic drug chlorpromazine in rat plasma and brain tissue", Journal of Chromatography. B, , 854 (1-2), 68 (2007).

[16] S. Feng, cunhong Li, J. fan,xingguo chen "Sequential injection technique for the determination of chlorpromazine hydrochloride in pure form and pharmaceutical formulations" Journal of Analytical chemistry, 62 (3), 233 (2007).

[17] I. M. A. Shakir and M. J. H. Al-kaffiji, “Flow-injection spectrophotometric determination of chlorpromazine HCl based on releasing of sodium persulphate from
hydrogel beads, study and applications”, Wasit Journal for Science & Medicine, 7(3), 110 (2014).

[18] أ.د محمد مجدى عبدالله واصم, "أسس الكيمياء التحليلية”, دار الفجر للنشر والتوزيع ص 113.

[19] R. Delevie, "principles of Quantitative chemical Analysis", the Mc. Graw-Hill Companies, Inc. Singapore (1997).

[20] P. Nagaraja,; Dinsh, N. D. ; Gowda, N. M. ; Ranjappa, K. S. "A Simple spectrophotometric determination of some phenothiazine drugs in pharmaceutical samples", J-STAGE Analytical Science, 16, 1127 (2000).

[21] S. M. Al-Talib, T. N. Al-Sabha, "Spectrophotometric determination of some phenothiazines using N-chlorosuccinimide", Journal of Raf. Science., 20, 27 (2009).