Spectrophotometric Determination of Chlorpromazine HCl by Oxidative Coupling Method in Pharmaceutical formulations

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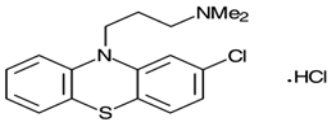
Abstract
The research includes the development of spectrophotometric method for determination microgram amounts of CPZ via oxidative coupling with the Sodium sulphacetamide reagent in presence of oxidizing agent potassium persulphate in the acidic medium (pH=1.8) to form a violet colored-dye, which is soluble in water and showed the highest intensity of absorption at λ_{max} 550 nm. The method has been obeyed Beer's Law in the concentration range (2 - 20) µg/ml, with molar absorptivity of 1.2791×10^4 L/ mol. Cm, Sandel Index value 0.0278 µg/cm^2. The detection limit 0.1727 µg/ml, with correlation coefficient 0.9983, the relative standard deviation of the method does not exceed 0.8933%, the proposed method was applied successfully for the determination of chlorpromazine hydrochloride in pharmaceutical preparation.

Kay word: Chlorpromazine .HCl , Spectrophotometer.

1. Introduction
The scientific name for the Chlorpromazine. HCl (CPZ) is 2-Chloro-10 dimethyl amino propyl) phenothiazine hydrochloride.

Its composition formula:



Molecular formula: $C_{17}H_{19}ClN_2S.HCl$, M.W= 355.33 g/mol.

CPZ is a white powder And the degree of melting 196˚m, , readily soluble in water and alcohol and partially dissolves in the ether and disintegrate when exposed to air and light and has the greatest wavelength for absorption of solution in water 239-306 nm[1,2]. Chlorpromazine is used as antipsychotic and to treat schizophrenia and other severe mental illnesses[3]. Sometimes doctors prescribe this drug for treatment of nausea and vomiting in patients, which has a calming properties so is used in anesthesia prior to surgery[4]. Different analytical method have been used for the determination of CPZ such as spectrophotometric method [5,6] HPLC [7] electrochemical method [8] and flow-injection technique[9].

2.Experimental
2-1 Apparatus:
Spectrophotometric measurements have been performed using UV-Visible Spectrophotometer model Cintra 6 from GBC scientific equipment Ltd. Company.

2-2 Reagent and chemicals used:
All chemicals and analytical reagents used in this research are pure or of high purity and supplied by companies BDF, Merck and SDI.

2-3 Preparation of solution:
A-Standard CPZ solution, (250 µg/ml):
This solution is prepared by dissolving 0.025 gm of CPZ in distilled water and the volume completed in the volumetric flask to 100 ml distilled water.

B- Sodium sulphacetamide reagent solution (0.05 M):
This solution is prepared by dissolving 1.1811 g of Sodium sulphacetamide in distilled water and the volume completed in the volumetric flask to 100 ml distilled water.

C- Potassium persulphate solution (0.05 M):
This solution is prepared by dissolving 1.3517 gm of Potassium persulphate in distilled water and the volume completed in the volumetric flask to 100 ml distilled water.

D- Sulphuric acid solution (0.5 M):
This solution is prepared by dilution 1.359 ml Sulphuric acid (36.8 M) in distilled water and the volume is completed in the volumetric flask to 100 ml distilled water.

E- Solution of CPZ tablets formulation 250 µg/ml:
Pharmaceutical formulation of Largacatil (production of company of pharmaceutical and medical supplies-Oubari Pharma-Aleppo-Syrie), every tablet contains 25 mg of CPZ and the solution has been prepared as follows: Ten tablets are weighed (1.145g) and powdered well, then a weight of 0.1145g of this powder is dissolved in an amount of distilled water, and then the solution filtered by paper filtration, the volume is completed with distilled water in a volumetric flask of 100 ml.

3-Preliminary Investigations:
A 1ml of Sodium sulphacetamide (0.05 M) is added to 1ml of standard CPZ solution in the presence of 1 ml of Potassium persulphate (0.05 M) solution in acidic medium (1ml of 0.5M, Sulphuric acid), diluted with distilled water in a 25 ml volumetric flask, a violet Color product. Absorption spectrum of the Colored dye against its corresponding blank reagent shows maximum absorption at 550 nm in contrast to blank reagent which shows few absorbance at this wavelength.

4-Optimization of the experimental conditions:
The effect of various variables on the Color intensity of 1ml of standard CPZ solution (250µg/ml),1ml of

Sodium sulphacetamide and 1ml of Potassium persulphate in acidic medium (1ml of 0.5M, Sulphuric acid), was studied to establish the optimum conditions.

4-1 Effect of the amount of oxidizing agent:

This study was conducted to select the best amount of oxidizing agent potassium persulphate (0.05 M) by adding different volumes (0.5 - 4) ml of oxidizing agent to volumetric flasks containing 1ml of CPZ (250 µg/ml) and 1ml of reagent solution (0.05 M) then addition of 1 ml of (0.5M) Sulphuric acid and the volume was completed to 25 ml with distilled water.

Table (1): Effect of the amount of oxidizing agent

ml of 0.05 M Potassium persulphate	Abs.	ml of 0.05 M Potassium persulphate	Abs.
0.5	0.201	2.5	0.189
1	0.224	3	0.183
1.5	0.207	3.5	0.171
2	0.191	4	0.179

the result are shown the volume 1ml is the optimum amount because of highest absorbance, so it was used in subsequent experiments.

4-2 Effect of the amount of coupling reagent:

The effect of the amount of coupling reagent was studied by adding different volumes (0.5 − 4 ml) of sodium sulphacetamide reagent (0.05 M) to the volumetric flasks, containing 1ml of CPZ (250 µg/ml) and 1ml of the potassium persulphate (0.05M), then the addition of 1ml of 0.5M Sulphuric acid, and the volume was completed to 25 ml with distilled water.

Table (2): Effect of the amount of coupling reagent

ml of Reagent 0.01M	*Abs.*	*ml of Reagent 0.01M*	*Abs.*
0.5	0.136	3	0.369
1	0.224	3.5	0.363
1.5	0.265	4	0.359
2	0.332	5	0.352
2.5	0.340	6	0.345

the result are shown the volume 3 ml is the optimum amount because it gave the highest absorption. So it is adopted in subsequent experiments.

4-3 Effect of the Sulphuric acid:

The effect of Sulphuric acid was studied by adding 0.7 - 4 ml of 0.5 M sulpharic acid solution. the best pH is found 1.8, (2 ml of solution sulphuric acid), was adopted in subsequent experiments. the result are shown in table (3).

Table (3): Effect of the acid:

ml of Sulphuric acid 0.5 M	*Abs.*	*pH*	*ml of Sulphuric acid 0.5 M*	*Abs.*	*pH*
0.7	0.311	2.1	2.5	0.352	1.7
1	0.369	2.0	3	0.350	1.6
1.5	0.378	1.9	3.5	0.342	1.4
2	0.391	1.8	4	0.334	1.2

4-4 Effect of Oxidation time:

To a series of volumetric flask, each containing 1ml 0f CPZ ,1ml of sodium sulphacetamide (0.05 M), 1ml potassium persulphate (0.05M) and the solution were left for different periods of time, then 5 ml (0.5M) of sulphuric acid solution were added. The volume was completed to 25 ml with distilled water, and the absorption of solution was measured at a wavelength of 550 nm versus blank, the results are in table (4) shown.

Table (4): Effect of Oxidation time.

Min min	5	10	15	20	25	30	40	45	50	60
Abs.	0.391	0.398	0.411	0.409	0.409	0.405	0.404	0.402	0.398	0.396

Table (4) shows that 15 min is sufficient for the oxidation to be completed, so it adopted in the subsequent experiments.

4-5 Order of additions:

The effect of different orders of addition on the absorption of the colored product have been studied. It is found that the addition sequence (1) achieves a higher absorption of colored product, so it is adopted in subsequent experiments and the result are in table (5) shown.

Table (5) Order of additions

Order *number*	*Order of addition*	*Abs.*
1	D + R + O + A	0.411
2	D + O + R + A	0.085
3	R + O + D + A	0.213
4	O + R + A + D	0.335
5	O + R+ D + A	0.229

D=Drug**, R**=Reagent**, O**=Oxidant**, A**=Acid

4- 6 Effect of temperature:

The temperature between (5 – 60 °C) is effect on the absorption of the formed colored product, and the result shown in table (6).

Table (6) Effect of temperature

Temp. °C	5	10	15	20	25	30	35	40	45	50	60
Abs.	0.364	0.371	0.395	0.407	0.411	0.405	0.385	0.359	0.332	0.311	0.298

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4-7 Effect of time on stability of the colored product:

The time of stability the formed colored product was studied by taking 1 ml of CPZ (250µg/ml) with addition 3 ml of sodium sulphacetamide, then 1 ml of potassium persulphate solution and 2 ml of sulphuric acid (0.5 M), the volume is completed to 25 ml in a volumetric flasks with distilled water, the value of the absorption of the colored product remains constant for not less than (50) min. The results are shown on table (7).

Table (7): Effect of time on stability of the colored product

Time min	5	10	15	20	25	30	35	40	45	50	60
Abs.	0.398	0.409	0.409	0.411	0.411	0.412	0.409	0.409	0.409	0.394	0.389

4-8 Effect of the solvent:

The effect of the solvent on the formed colored product was studied, the dilution was carried out by different solvent instead of water.

Table (8): effect of the solvent

Solvent	***Absorbance***	$\lambda_{max,}$ ***nm***
Ethanol	0.385	560
Methanol	0.363	561
Water	0.409	550

The results show that the water is a good medium for reaction and give good absorption value at the wavelength of 550 nm and due to its availability, it has been used as the best solvent in the subsequent experiments.

5- Final absorption spectrum:

The spectrum of the formed colored product by coupling of CPZ with sodium sulphacetamide in the presence of Potassium persulphate in acidic medium pH= 1.8 and temperature 25 C^0 against its corresponding reagent blank shows a maxim absorption at 550 nm in contrast to the blank reagent of a few absorbance at λ_{max}.

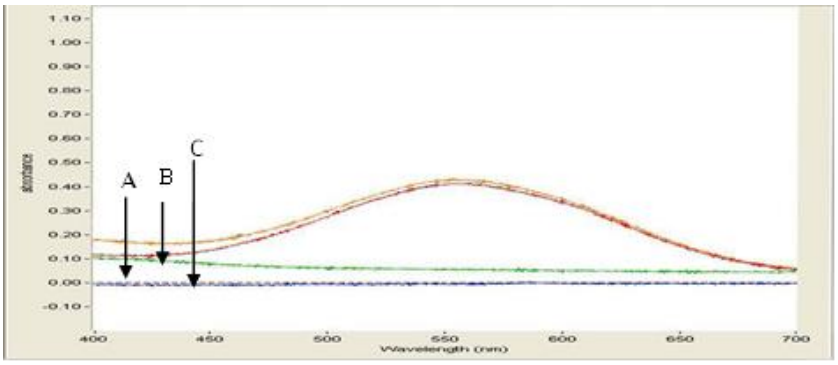

Fig. (1): Final absorption spectrum of the Determination CPZ

A: Absorption spectrum of the colored products versus blank.
B: Absorption spectrum of colored products versus distilled water.
C: Absorption of blank reagent versus distilled water.

6-procedure for construction of calibration curve:

To a series of volumetric flask (25 ml), (0.2 -2) ml (250 µg/ml) of CPZ were transferred, 3ml of sodium sulphacetamide reagent (0.05 M) and 1ml of Potassium persulphate (0.05 M), after that the solution were left for 15 min to complete the reaction, then 2 ml of sulphuric acid were added to (0.5 M), and volumes were completed to the mark with distilled water. The absorbance was measured at 550 nm against the blank. Figures (2) and (3) illustrate that the calibration curve are linear over the concentration range of (2–2) µg/ml while higher concentration show a negative deviation form beer's law. The molar absorptivity value is 12.791×10^3 Iiter/mol.cm and the sandal's sensitivity index 0.0278 µg/cm^2.

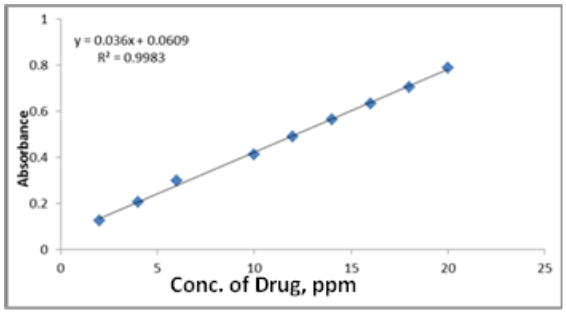

Fig. (2): Calibration curve for Determination of CPZ

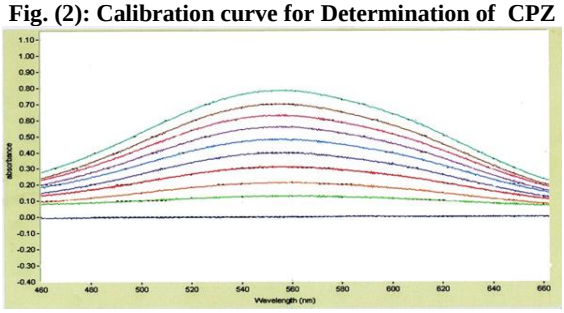

Fig. (3): Absorptions spectrum of concentration (2 - 22) µg/ml for CPZ

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7- Accuracy and precision:

Accuracy and precision were studied by measuring absorption (5 times) at 550 nm for three different concentrations of the drug (4 ،12 ،18) µg/ml within the limits of beer's law, the average recovery (%100.02) and the RSD (0.5878%) indicate that the method is of high accuracy and precision. The results are shown in table (9).

Table (9): Accuracy and precision

Conc. of Chlorpromazine . HCl µg /ml	*RE%*	*Recovery%*	*Average of Recovery%*	*RSD%*
4	0.01	101	100.02	1.554
12	- 0.333	99.67		0.489
18	0.611−	99.39		0.637

8- Detection limit:

detection limit was calculate by measuring the absorption for the lower concentration 4 µg/ml at optimal condition (5 times) at 550 nm. The results are shown in table (10).

Table (10): Detection limit. D. L $= \frac{3SC}{\bar{x}}$

Conc. of Chlorpromazine .HCl µg /ml	$\bar{x}$ *(Mean)*	*S (Standard deviation)*	*D.L (Detection Limit) µg/ml*
4	0.138	0.0035637	0.1727

9- The nature of the formed product:

To know the nature of the formed violet color product , Job's method and molar method were applied. In both methods, the concentrations of each of the standard CPZ solution and sodium sulphacetamide reagent solution equal to 0.03 M. In Job's, method ,in a series of volumetric flasks (25 ml), different volumes of the drug solution ranging from 1-9 ml and different volumes 1-9 ml of reagent solution were mixed. A 1ml of Potassium persulphate and 2 ml of sulphuric acid solution were added and volumes were completed to the mark with distilled water. The absorbance was measured at 560 nm against the blank. Fig. (4) shows that the ratio is 1:1.

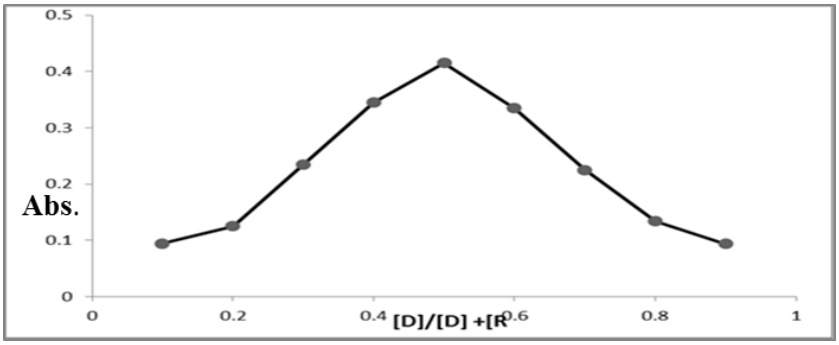

Fig. (4): Job's method of formed product by oxidative coupling of CPZ with sodium sulphacetamide reagent.

In molar ratio method, 1ml of standard drug solution in a series of volumetric flasks (25 ml) were transferred and different volume (0.2 - 1.8) ml of sodium sulphacetamide reagent solution, 1ml of potassium persulphate and 2 ml of sulphuric acid 0.5 M solution were added. The volumes were completed to the mark with distilled water and the absorbance was measured at 560 nm against the blank reagent. Molar ratio was found to be 1:1, the results are shown in fig (5) which is in agreement with the Job's method result.

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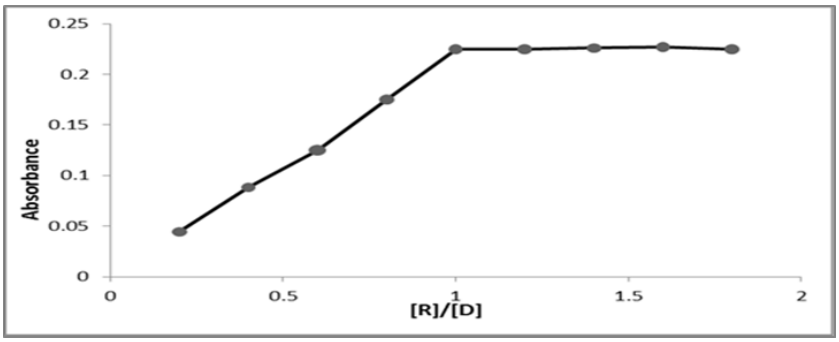
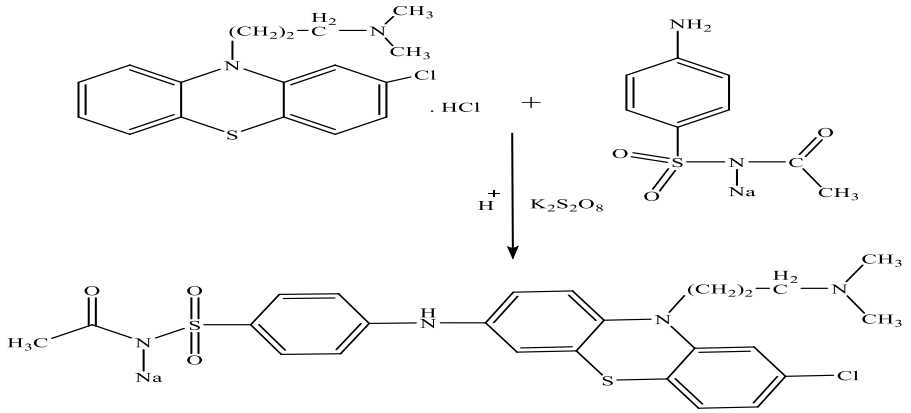


Fig (5): molar ratio method of formed product by oxidative coupling of CPZ with sodium sulphacetamide reagent

The proposed equation for reaction can be written as follow:



10-Application

10-1 Direct method:

In this method, different volumes (0.4, 1.2, 1.8) ml of a pharmaceutical formulation Largectil (250 µg/ml) were transferred to 25 ml volumetric flask and the resulting concentration (4,12, 18) µg/ml and were tread as in construction of calibration curve. The absorbance was measure at 550 nm for five times. Recovery and RSD were calculated and the results are presented in table (11).

Table (11): Direct method

Conc. Of Chlorpromazine µg/ml	*RE%*	*Recovery%*	*Average recovery %*	*RSD%*
4	4.925	104.93	103.06	1.193
12	3.817	103.82		1.537
18	0.417	100.42		1.002

Results from the above table indicate the success of the proposed method to estimate CPZ in it's pharmaceutical preparation , the value of the recovery of 103.06% in the product Largacatil (25 mg).

10-2 Standard additions method:

For the purpose of the statement of the efficiency of the proposed method, accuracy and prove that the way free from interference the standard method is applied to estimate the added CPZ in the pharmaceutical preparation Largacatil. This method can be conducted to fixed amount (0.4, 0.8) ml lotion pharmaceutical of 250 µg/ml to a series of volumetric flasks 25ml, increased volumes (0, 0.4, 0.8, 1.4, 1.8 ml) of the solution PYR of 250 µg/ml, and treated as in the calibration curve. The absorption of all solutions are measured at wavelength 550 nm, and the results are shown in the table (12) and fig (6).

Table (12): Standard additions method

Type of Drug	*Chlorpromazine . HCl present µg/ml*	*Chlorpromazine . HCl measured µg/ml*	*Recovery, (%)*
Tablets Largectil	4	3.842	96.05
	8	7.68	96

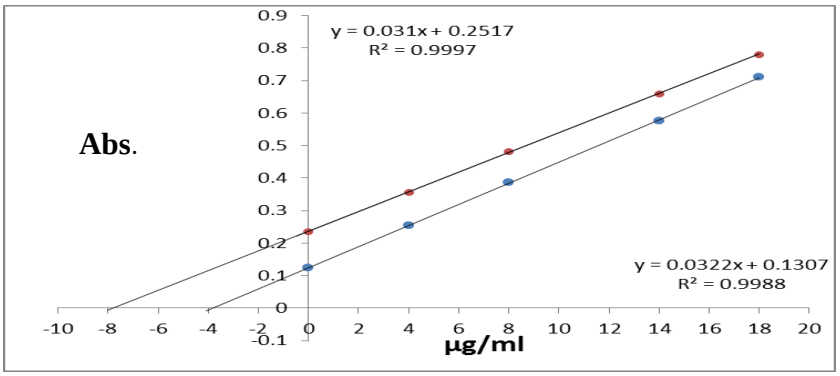


Fig (6): Standard additions method

Results of standard additions is in good agreement with the direct method within the acceptable range of error, indicating that the method is satisfactory and free from interferences.

11- Conclusions

The result obtained confirm that the proposed method is simple, rapid and of good sensitivity for the Determination of CPZ. The method is based on oxidative coupling between CPZ and sodium sulphacetamide reagent in presence of potassium persulphate in acidic medium to form violate colored dye which in water soluble, stable and show a maximum absorption at 550 nm. This method does not require temperature control, not use of organic solvents, or solvent extraction and it can be applied successfully for Determntion of CPZ in pharmaceutical formulation with recovery of not less than 98.35%.

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التقدير الطيفي لعقار لكلوربرومازين هيدروكلوريد بطريقة الاقتران التأكسدي في المستحضرات الصيدلانية

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الملخص

تم تطوير طريقة طيفية لتقدير كلوربرومازين هيدروكلوريد بالاقتران التأكسدي مع سلفاسيتاميد الصوديوم بوجود بيرسلفات البوتاسيوم كعامل مؤكسد في الوسط الحامضي (pH=1.8) لتكوين ناتج ذو لون بنفسجي ذائب في الماء ويعطي أعلى امتصاص عند الطول الموجي 550 نانوميتر. اطاعت الطريقة قانون بير في مدى من التركيز تراوح بين 2–20 مايكروغرام/ مل، وقد بلغت قيمة الامتصاصية المولارية 1.2791×10^4 لتر/ مول. سم، وقيمة دلالة ساندل 0.0287 مايكروغرام/ سم^2، وحد الكشف0.1727 مايكروغرام/مل، وقيمة معامل الارتباط 0.9983، والانحراف القياسي النسبي للطريقة لا يتجاوز0.8933%، طبقت الطريقة بنجاح على المستحضرات الصيدلانية الحاوية على الكلوربرومازين هيدروكلوريد.

الكلمات الدالة: كلوربرومازين هيدروكلوريد، مطيافية الاشعة فوق البنفسجية والمرئية.