Spectrophotometric Micro Determination of Promrthazine Hydrochloride in Pharmaceutical Preparations Via Oxidative Coupling Reaction with Sulphanilamide and in the Presence of Ferric Chloride

Hind S. Al-Ward *

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Abstract

A simple, accurate and sensitive spectrophotometric method for the determination of promethazine hydrochloride is described, this method is based on the oxidative coupling reaction of promethazine hydochloride with sulphanilamide in the presence of ferric chloride and hydrochloric acid to form a green-water-soluble dye, which become more intense and stable at a temperature of 60 C° and has a maximum absorption at 600 nm. A graph of absorbans versus concentration shows that Beer's law is obeyed over the concentration range of 25-900 μg of promethazine hydrochlorid in a final volume of 25 ml (i.e., 1-36 p.p.m) with a molar absorpitivity of 1.74×10^{4} Lit.mol^{-1}.cm^{-1}, a Sandall sensitivity of 0.018 μg .cm^{-2}, a relative error of (-2.16--0.62%) and a relative standard deviation of less than 0.515% depending on the concentration.The optimum conditions for full colour development are described and the proposed method was applied satisfactorily to pharmaceutical preparations containing promethazine hydrochloride.

Key words:- Promethazine Hydrochloride, Oxidative coupling reaction, Spectrophotometry.

Introduction

Promethazine hydrochloride is currently used for its antipsychotic and ansiolytic effects, it is a phenothiazine with anticalmoduline action, not toxic for human beings at therapeutic doses [1]. Various methods have been reported for the determination of prmethazine hydrochloride, these include colorimetric [2-6], chromatographic [7-10] and titrimetric methods [11-13], Spectrophotometric methods seems to be the most common methods [14-20] used for its determination. The objective of the investigation reported in this paper was to evaluate a spectrophotometric method for the determination of promethazine hydrochloride based on the oxidative coupling reaction with sulphanilamide, ferric chloride and hydrochloric acid at a temperature of 60°. A stable-soluble-green dye was formed which can be measured at 600 nm. The method was applied successfully to pharmaceutical preparations containing promethazine hydrochloride.

* Assistant lecturer- Chemistry Dept.- College of Science-Baghdad University

Experimental

Apparatus:

-All spectral and absorbance measurements were carried out on a Shimadzu UV-visible -260 digital double-beam recording spectrophotometer using 1-cm sillica cell.

Reagents:

All chemicals used were of analytical reagent grade and promethazine HCl standard material was provided from the state company for drug and medical appliances industries (SDI) Sammara - Iraq.

Promethazine HCl stock solution (500 $\mu g.\ ml^{-1}$),

A 0.0500 gm amount of pure promethazine HCl was dissolved in distilled water and the solution was made up to volume of 100 ml in volumetric flask with the same solvent.

Sulphanilamide reagent ($5 \times 10^{-3} M$),

Prepared by dissolving 0.0861 gm of pure sulphanilamide reagent in 10 ml of ethanol and diluted to 100 ml in a volumetric flask with distilled water.

Ferric chloride solution ($5 \times 10^{-2} M$),

Prepared by dissolving 0.8110 gm of anhydrous ferric chloride $FeCl_3$ in distilled water and made up to 100 ml volumetric flask with the same solvent.

Hydrochloric acid solution (1M),

Prepared by simple dilution of the concentrated acid.

Procedure

In to a series of 25 ml calibrated flask, transfer increasing volumes of stock solution (500 $\mu g.\ ml^{-1}$) of promethazine HCl to cover the range of the calibration graph (25-900 μg in a final volume of 25 ml). Add 2 ml of (5×10^{-3} M) of sulphanilamide solution followed by 2 ml of (5×10^{-2} M) of ferric chloride and 0.5 ml of 1M hydrochloric acid, shake well and then dilute the solution to the mark with distilled water. Allow the reaction mixture to stand for 20 mins in a water bath at a temperature of 60C°, leave the solution to stand and become more stable at room temperature for another 10 mins and measure the absorbance at 600 nm against a reagent blank prepared in the same way but containing no promethazine HCl. The colour of the dye formed was stable for more than 90 mins. For the optimization of conditions and in all subsequent experiments, a solution of 500 $\mu g.\ ml^{-1}$ of the drug in a final volume of 25 ml was used .

Procedure for pharmaceutical preparations

Histazine tablets:- provided from the united pharmaceutical Mfg Co. Ltd./ Amman/ Jordan. Each tablets contains 25 mg of promethazine HCl.

Weigh and finally powdered 10 tablet, extract accurately weighed portion of the powder equivalent to about 50 mg of promethazine HCl in amount of distilled water. Shake well and filter the solution into a volumetric flask and dilute to 100 ml with the same solvent. 1 ml of the last solution was used for the colour formation with sulphanilamide, ferric chloride and hydrochloric acid as described under calibration procedure.

Histazine syrup:- provided from the same company of the histazine tablets. Each 5 ml of the syrup contains 5 mg of promethazine HCl.

Transfer 50 ml of the syrup into a 100 ml volumetric flask and dilute it to the mark with distilled water. 1 ml of the last solution was used for the colour formation with sulphanilamide, ferric chloride and hydrochloric acid using standard addition method[24].

Results and Discussion

Absorption spectra:

When sulphanilamide was oxidized with ferric chloride and mixed well with aqueous solution of promethazine HCl, a green colour forms which become more intense and stable when the reaction mixture was warmed up in a water bath for 20 mins at 60 C°. This green dye has a maximum absorption at 600 nm, and a less intense peak at 468 nm. The reagent blank shows no absorption over the range of 400-600 nm. Figure (1) shows the spectra of the green dye formed and of the reagent blank, the maximum absorption at 600 nm was used in all subsequent experiments.

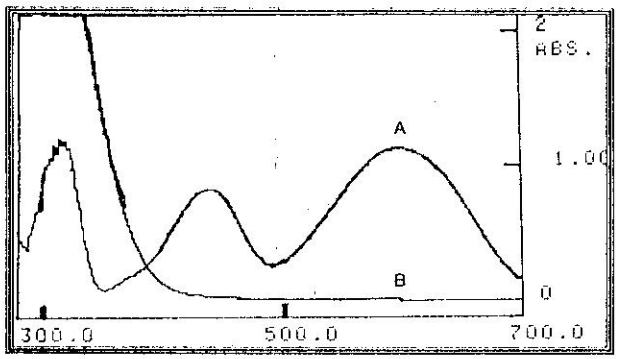


Figure (1):- Absorption spectra of A ($500\ \mu g.ml^{-1}$) of promethazine HCl. Treated as described under procedure and measured against a reagent blank, B the reagent blank measured against distilled water.

Study of the optimum reaction conditions

The effects of various parameters on the absorption intensity of the dye formed were studied and the reaction conditions were optimized.

1- Effect of temperature on the stability and absorbance of the dye.

Preliminary investigations showed that heating of the reaction mixture will increased the intensity of the colour of the formed dye. Therefore, the effect of different temperatures (35, 50, 60, 70 C°) showed an increasing in absorption with temperature at a fixed time of 10 mins, up to 60 C° which gives the optimum temperature followed by a decrease in absorption at 70 C° as shown in figure 2.

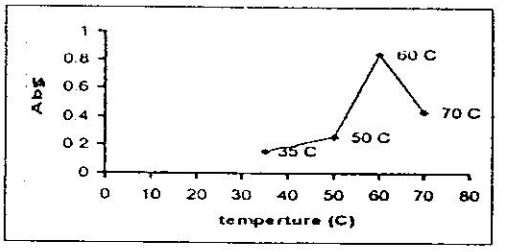


Figure (2):- The effect of temperature on absorption of the dye

2- Effect of time on the stability and absorbance of the dye.

The colour intensity reached a maximum after the drug had been reacted with sulphanilamide, ferric chloride and hydrochloric acid and the reaction mixture was warmed up in a water bath for 20 mins at 60 C° then leave the solution to stand and become more stable at room temperature for another 10 mins. this dye will remain stable for 90 mins at room temperature as shown in figure 3.

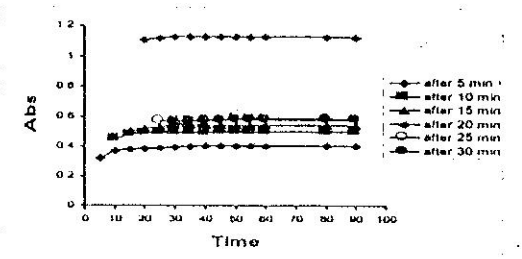


Figure (3)
The effect of heating time on the stability of the dye formed (the measurements at different time on water bath of 60 C°)

3- Effect of reagent concentration

When various concentrations of sulphanilamide solution were added to a fixed amount of the drug solution, 2 ml of 5×10^{-3} *M* solution was found enough to develop the colour to its full intensity and give a minimum blank value and was considered to be optimum for the concentration range of 25-900 $\mu g.ml^{-1}$ of promethazine HCl.

4-Effect of oxidant concentration

Various oxidizing agent were studied (sodium periodate, potassium ferricyanide cerium sulphate, ammonium persulphate, potassium dichromate and ferric chloride anhydrous) in the presence of a fixed amount of drug, cou-

pling agent and acid. Anhydrous ferric chloride was found to be the best oxidizing agent because it gave a higher intensity of the dye formed and a minimum blank value.

The dye formation reached maximum with about 2 ml of $5 \times 10^{-2} M$ of ferric chloride solution and remained at this maximum when 1-4 ml was add, therefore, 2 ml volume of the oxidizing agent solution was used in the procedure since it gave high sensitivity, minimum blank value and ensure a quantitative determination at the upper limit of the calibration graph.

5- Effect of acid

In practice, the addition of acid to the reaction mixture will increased the intensity of the colour of the dye, therefore, various acids (nitric acid, acetic acid and hydrochloric acid) were added to the mixture of promethazine HCl, sulphanilamide and ferric chloride. Hydrochloric acid was found to be the most suitable acid for this reaction. The effect of acid concentration (0.01-5 M) on the colour development of the dye was also studied and 0.5 ml of 1M of hydrochloric acid was found optimum.

6-Effect of the order of the addition

To obtain optimum results the order of the addition of the reagents should be followed as given under the procedure, otherwise a loss in colour intensity and stability were observed.

Calibration Graph

Employing the conditions described under procedure, a linear calibration graph (Figure 4) for promethazine HCl was obtained, which shows that Beer's law was obeyed over the concentration range of 25-900 $\mu g.25ml^{-1}$ or (1-36 p.p.m) with a correlation coefficient of 0.9987 and an intercept of 0.0573. The conditional molar absorptivity of the green dye formed with reference to promethazine HCl was found to be 1.74×10^4 $L.mol^{-1}.cm^{-1}$ and a Sandell sensitivity of 0.018 $\mu g.cm^{-2}$.

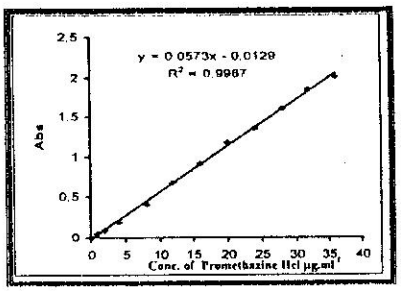


Figure (4) :-Calibration graph for Proethazine Hcl

Accuracy and precision

To determine the accuracy and precision of the method. Promethazine HCl was determined at three different concentrations. The results shown in Table (1), indicate that a satisfactory precision and accuracy could be obtained with the proposed method.

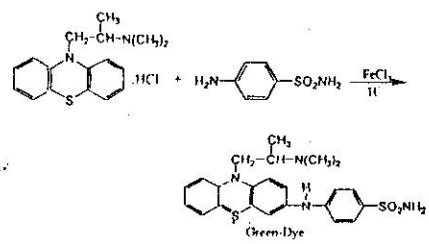
Table (1) :- Accuracy and precision of the proposed method

Concentration of promethazine Hcl µg. ml⁻¹		Error %	Recovery* %	Relative standard deviation % (R.S.D)
present	Found			
12	11.83	-1.41	98.59	0.389
24	23.85	-0.62	99.38	0.500
36	35.22	-2.16	97.84	0.515

* for five determinations.

Structure of the Dye

The stoicheiometry of the reaction between promethazine HCl and sulphanilamide was investigated using the molar ratio method. The results obtained (Figure 5) shows that a (1:1) drug to reagent complex was formed between promethazine HCl and sulphanilamide reagent at 600 nm, therefore the formation of the dye probably occurs as follows [21,22,23]:-



The dye formed was soluble in water, the apparent stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amount of sulphanilamide and promethazine HCl with that of a solution containing five-fold excess of sulphanilamide reagent. The average conditional stability constants of the dye in water under the described experimental condition was (3.25×10^7 Lit.mol^{-1})

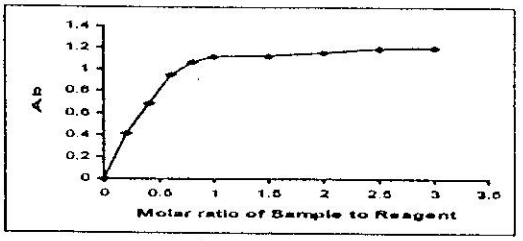


Figure (5):-Molar ratio of the promethazine HCl to sulphanilamide. The concentration of sulphanilamide was 1.900×10^{-4}M

Analytical applications

Two types of drugs containing promethazine HCl (tablets & syrup) have been analyzed and they gave the recoveries given in table 2.

Table (2):- Application of the proposed method for the determination of promethazine HCl in pharmaceutical preparations.

Drug samples	Concentration of promethazine HCl µg. ml^{-1}		R.S.D* %	Error %	Recovery %
	Present	Found			
Histazine tablets	20	20.94	0.73	+4.7	104.%
Histazine syrup	20	22.64	1.15	+13.2	113.2

* for five determinations

The proposed method was compared successfully with the British pharmacopoeia (B.P) standard method[25] for both pure histazine and histazine tablets but the histazine syrup gave a high recovery value in comparison with British Pharmacopoeia method (table 3). Therefore, the standard addition method was applied to determine the histazene in histazine syrup and a good recovery was obtained (figure 6).

Table(3) :- Comparation of the proposed method with standard method

Drug sample	Recovery %		
	Proposed method	Standard addition method	Standard method*
Pure histazine HCl	98.6%	-	99.0-101.0%
Histazine tablets	104.4%	-	95.0-105.5%
Histazine syrup	113.2%	100%	90.0-110.0%

* British standard method

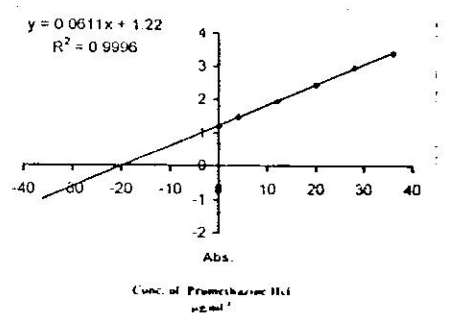


Figure (6) :- The graph of standard addition method for the determination of histazine in histazine syrup.

The Comparaison of the method

Table (4) shows a comparison between the developed method and some of spectrophotometric methods using oxidat ve coupling reaction for the determin ation of promethazine hydrochloride with various organic reagents and oxidizing agents. Some of these methods needed organic solvents for the extraction of the dye (18,20), or have a low linearity range that obeyed Beer's low (5,18,20). The proposed method have a wide linearity range (1-36 ppm) also it didn't need organic solvents and it has a good accuracy and precision.

Table 4:- A comparison between the proposed method and some of the spectrophotometric methods.

No.	Coupling reagent	Oxidising agent	λ_{max} (nm)	Linearity (µg.ml^{-1})	molar absorptivity (L.mol^{-1}.cm^{-1})	Recovery %	Ref.
1	di-phenyl amine	N-bromo succinamide	393 773	1-10 1-15	3.28×10^4 1.53×10^4	99.0	21
2	1,10-phenanthroline	Fe^{+3}	510	1-6	-	-	18
3	Morpholine	I_2/KI	662	0.2-4	7.2×10^4	99.4	5
4	Sulphanilamide	$FeCl_3$	600	1-36	1.74×10^4	98.6	Present work

Conclusions

A simple, accurate and sensitive spectrophotometric method has been proposed for the determination of trace amounts of promethazine HCl in aqueous solution based on its oxidative coupling reaction with sulphanilamide reagent and ferric chloride and hydrochloric acid at 60C°. The proposed method dose not need solvent extraction step and have a good accuracy and precision. The method was applied

successfully to pharmaceutical samples.

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التقدير الطيفي المايكروي لبرومثيازين هيدروكلورايد في المستحضرات الصيدلانية بوساطة تفاعل الازدواج التأكسدي مع السلفانيلاميد وبوجود كلوريد الحديدك

هند صادق الورد

مدرس مساعد-قسم الكيمياء-كلية العلوم-جامعة بغداد

الخلاصة

يتضمن البحث تطوير طريقة طيفية للتقدير الكمي لمقادير مايكروية من عقار البروميثازين هيدروكلورايد في المحاليل المائية بأستخدام المطياف الفوتوميتري . تعتمد الطريقة على تفاعل الأزدواج التأكسدي بين البروميثازين هيدروكلورايد وكاشف السلفانيلاميد بوجود العامل المؤكسد كلوريد الحديديك في وسط حامضي و عند درجة حرارة ٦٠ م°. حيث تتكون صبغة خضراء مستقرة وذائبة في الماء وتعطى اعلى امتصاص عند طول موجي ٦٠٠ نانوميتر. ويشير الرسم البياني الخطي للأمتصاص مقابل التركيز بأن قانون بير ينطبق ضمن مدى التركيز ٢٥-٩٠٠ مايكروغرام من العقار في حجم نهائي ٢٥ مل(١-٣٦ جزء بالمليون)، وكانت قيمة الأمتصاصية المولارية مساوية الى ١,٧٤×١٠ لتر.مول$^{-1}$.سم$^{-1}$ وقيمة حساسية ساندل ٠,٠١٨ مايكروغرام.سم$^{-2}$ والخطأ النسبي (-٢,١٦--٠,٦٢%) وانحراف قياسي نسبي اقل من ٠,٥١٥% أعتماداً على مستوى التركيز المراد تحديده . تمت دراسة الظروف المثلى للتفاعل، وطبقت الطريقة على المستحضرات الصيدلانية الحاوية على البروميثازين هيدروكلورايد.