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Spectrophotometric Determination of some Phenothiazines Using N-Chlorosuccinimide

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ABSTRACT

A simple and sensitive spectrophotometric method has been developed for the determination of some phenothiazine derivatives as pure and in dosage form. The method is based on oxidation of phenothiazines by N-chlorosuccinimide in hydrochloric acid medium to give red coloured species having maximum absorptions in the range 516.5-534.5nm. Beer's law is obeyed over the concentration ranges 2-28, 2-32 and 4-40µg.ml^{-1} with apparent molar absorptivity of 6.16×10^3, 5.89×10^3 and 5.34×10^3 l.mol^{-1}.cm^{-1} for phenothiazine, promethazine hydrochloride and chlorpromazine hydrochloride respectively. Additives and excipients normally found in dosage forms do not interfere.

Keywords: phenothiazines, determination, N-chlorosuccinimide, spectrophotometry.

التقدير الطيفي لبعض الفينوثايازينات باستخدام N - كلوروسكسينميد

الملخص

تم تطوير طريقة طيفية بسيطة وحساسة لتقدير الفينوثايزينات بأشكالها النقية وفي مستحضراتها الصيدلانية. تعتمد الطريقة على أكسدة الفينوثايزينات بوساطة N–كلوروسكسينميد في وسط حامض الهيدروكلوريك لإعطاء أصناف ملونة تمتلك أقصى امتصاصات عند أطوال موجية بحدود 516.5-534.5 نانوميتر. وجد أن قانون بير ينطبق ضمن مدى التراكيز 2-28 و 2-32 و 4-40 مايكروغرام.مل$^{-1}$ وبامتصاصية مولارية 6.16×10^3 و5.89×10^3 و5.34×10^3 لتر.مول$^{-1}$.سم$^{-1}$ لكل من الفينوثايزين وبروميثازين هيدروكلوريك وكلوربرومازين هيدروكلوريد على التوالي. كما تراوح الخطأ النسبي بين -3.72% و +2.29% في حين كان الانحراف القياسي النسبي أفضل من 3.81%. إن نتائج تقدير الفينوثايزينات في مستحضراتهما الصيدلانية أثبتت عدم وجود تداخل لمواد السواغ المضافة إلى تلك المستحضرات.

INTRODUCTION

Phenothiazines which were introduced in the1950's as antipsychotic drugs are still widely used in the treatment of moderate to severe mental illnesses including schizophrenia. Phenothiazines also possess antiemetic, sedative, antipruritic, antidyskinetic, analgesic and antihistaminic properties (Gordon, 1964). The therapeutic importance of these drugs has prompted many workers to develop methods for their determination in body fluids as well as pharmaceuticals. Many direct spectrophotometric methods for the determination of phenothiazines have been suggested based on the oxidation of the drugs to the radical cations and subsequent measurement of absorbance, with the use of such oxidizing agents as molybdate (Basavaiah and Krishnamurthy, 1998), chloramine-T (Basavaiah and Swamy, 2001 ; Revanasiddappa and Veena, 2008), hexacyanoferrate (III) and Ferriin (Basavaiah and Swamy,2002), vanadate (Misiuik and Tarasiewicz, 1993), N-bromosuccinimide (Taha *et al.,* 1983), cerium(IV) (Misiuik and Tarasiewicz,1996), 2-iodobenzoate (Hassan *et al.*,1989) and p-benzoquinone (Murthy and Seetharamappa, 2000). Some of these methods, unfortunately, suffer from several disadvantages like use of heating step (Basavaiah and Krishnamurthy, 1998), low sensitivity (Misiuik and Tarasiewicz, 1996), strong acid medium (Taha *et al.,* 1983), narrow range of determination and critical working conditions (Misiuik and Tarasiewicz, 1993).

Procedures based on charge-transfer complex formation using chloranilic acid as acceptor (Basavaiah, 2004) and ion-association complex formation with many acidic dyes such as bromocresol green (Basavaiah and Krishnamurthy, 1998), chrome azurol S (Basavaiah *et al.,* 1999), bromophenol blue (El-Kerdawy *et al.,* 1993), eriochrome cyanine R (Starczewska and Karpinska, 1996), amaranth (Bhongade and Kasture, 1993) and brilliant blue (Bhongade and Kasture, 1993), have also been reported. The chief limitations of these methods are: low sensitivity, tedious and time consuming extraction step, use of organic solvents, strict pH control and significant blank absorption. A couple of indirect spectrophotometric methods utilizing metavanadate (Singh *et al.*, 1988) and diphenylpicrylhydrazyl (DPH) (Emara,1992) are also used for the assay of phenothiazines. But even these procedures are unsuitable for routine analysis, since the vanadate method requires heating the reaction mixture in a boiling water bath for 10 min, and the method is poorly sensitive (range 50–1000 µg ml^{-1}). The method using DPH also involves heating the methanolic solution at 60°C for 15–20 min before measuring the absorbance of unreacted reagent, besides being applicable for 10–300 µg ml^{-1} concentrations.

The present work reports the use of N-chlorosuccinimide as oxidant reagent for the spectrophotometric determination of some phenothiazine derivatives.

EXPERIMENTAL

Apparatus

A CECIL Model CE-3021 UV-VIS spectrophotometer with a 1.0-cm cell was used for spectral measurements.

Reagents

All the reagents used were of analytical reagent grade where otherwise not mentioned. Standard solutions of phenothiazines (1000 µg ml^{-1}) were prepared by dissolving 100 mg

each of phenothiazines, listed in Table (1) and supplied by State Company for Drug Industries and Medical Appliance-(SDI) Sammara-Iraq, in absolute ethanol and diluting to the mark in a 100-ml calibrated flask. A working standard solution of phenothiazines containing 50 µg.ml^{-1} was prepared by further dilution. N-chlorosuccinimide solution (0.005M) and hydrochloric acid solution (1 M) were prepared in distilled water .

Recommended procedure

In a series of volumetric flasks of 25-ml, aliquots of standard solutions of phenothiazine, promethiazine hydrochloride and chlorpromazine hydrochloride with concentrations of 2-28, 2-32 and 4-40 µg ml^{-1} , respectively in final volume were added separately, followed by addition of 4 ml N-chlorosuccinimide and 2 ml of hydrochloric acid, the contents were diluted to the mark with distilled water and mixed thoroughly, the solutions were left for 15 minutes at room temperature and the absorbance was measured at 516.5, 517 and 534.5 nm for the above drugs, respectively against their respective reagent blank and a calibration graph was constructed.

Procedure for Tablet

Ten tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 50 mg of the phenothiazine or chlorpromazine hydrochloride was transferred into a 100-ml calibrated flask and diluted to the final volume with absolute ethanol. Using a mechanical stirrer, the powder was completely disintegrated and the solution was filtered. A suitable aliquot of this solution was treated as described in the recommended procedure.

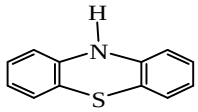
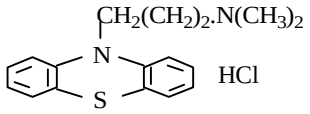
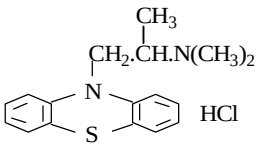
Procedure for injection

An accurately measured volume was appropriately diluted with absolute ethanol to get 50 µg ml^{-1}of promethazine hydrochloride solution. A suitable aliquot of this solution was taken and treated as described in the recommended procedure.

RESULTS AND DISCUSSION

The spectrophotometric method for the determination of phenothiazines described in Table 1 is based on the oxidation reaction of the drug with N-chlorosuccinimide to form a red colored species. The factors affecting the color development, reproducibility, sensitivity and adherence to Beer's law were investigated with phenothiazine as the model compound, since the other phenothiazine derivatives behaved similarly to it.

Table 1: Phenothiazines

Sample	Synonyms	Proprietory Names	Structure
Phenothiazine (PT)	Thiodiphenylamine	Agrazine Phenosame Phenovis	
Chlorpromazine Hydrochloride (CPH)	Aminazin	Hibernal largactil Megaphen Thorazine	
Promethazine hydrochloride (PH)	Diprazine Proazamine-Chloride	Fargan Phenergan Prothazine	

Spectral characteristics

Red-complexes are formed when phenothiazines were allowed to react with N-chlorosuccinimide in hydrochloric acid medium with maximum absorptions at 516.5, 534 and 517 nm for phenothiazine, chlorpromazine hydrochloride and promethazine hydrochloride, respectively (Fig.1). The colorless reagent blank has practically negligible absorbance at these wavelengths.

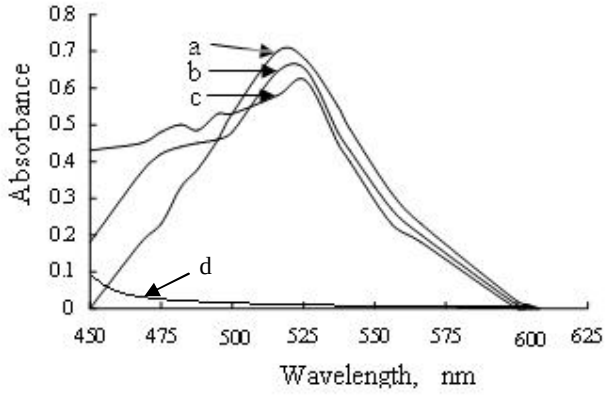


Fig.1 Absorption spectra of the colored species obtained by the reaction of N-chlorosuccinimide and (a)phenothiazine,(b)promethazine hydrochloride, (c)chlorpromazine hydrochloride versus reagent blank and (d) reagent blank versus distilled water.

Optimum conditions for products formation

The spectrophotometric properties of the colored species formed between phenothiazines and N-chlorosuccinimide reagent, as well as the different parameters affecting the colour development by varying one and fixing the other parameters at wavelengths, shown in Table 2, were extensively studied. The optimum conditions for the assay procedures have been established by studying the reactions as a function of the reagent concentration, the temperature and the stability of the colored species.

It was found that a 4.0 ml of 0.005 M N-chlorosuccinimide and 2.0 ml of 1 N hydrochloric acid were necessary to achieve the maximum color intensity of the product. However the oxidation of phenothiazines by N-chlorosuccinimide was completed within 30 minutes and developed color was stable for at least 25 minutes in final dilution at room temperature in the absence of hydrochloric acid, but in the presence of hydrochloric acid these oxidation reactions were found to be instantaneous and completed within 15 minutes at room temperature and the color stability remains constant for more than 25 minutes.

Table 2 : Parameters for the spectrophotometric determination of phenothiazines.

Parameters	**Phenothiazine**	**Chlorpromazine Hydrochloride**	**Promethazine Hydrochloride**
Color	Red	Red	Red
λ_{max} **(nm)**	516.5	534.5	517
Stability period (min)	25	25	25
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	6.16×10^{3}	5.34×10^{3}	5.89×10^{3}
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.03235	0.06654	0.05446
Beers law range ($\mu g.ml^{-1}$)	2-28	4-40	2-32
Regression equation (y)* Slope (b) Intercept (a) Correlation coefficient	 0.1392 -0.0501 0.9933	 0.1230 -0.0194 0.9882	 0.1335 -0.0135 0.9914

*A = a + bC where "A" is the absorbance for concentration "C " in µg ml^{-1}.

Quantification

In order to investigate the range in which the colored species adhere to Beer's law, the absorbences of the complexes were measured at their respective λ_{max} values after developing the color by following the suggested procedure for a series of solutions containing increasing amounts of phenothiazine drugs. Beer's law limits, Sandell's sensitivity and molar absorptivity values were evaluated and are given in Table 2, which indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficients for the phenothiazines determined for the proposed method, represents excellent linearity. (Table 2)

The accuracy and precision of the method, were checked by determining phenothiazines at two different concentrations. The results represented in Table 3 indicate that the method is satisfactory.

Table 3: Accuracy and precision of the proposed method.

Phenothiazines	Amount of phenothiazine taken (µg ml $^{-1}$)	Relative error* (%)	RSD* (%)
Phenothiazine	5.0	-2.61	2.16
	20	+1.95	1.73
Chlorpromazine hydrochloride	5.0	-3.72	3.81
	30	+2.29	3.03
Promethazine hydrochloride	5.0	+0.93	1.88
	20	-2.77	2.96

* Average of six determinations.

INTERFERENCE

In order to assess the possible analytical application of the proposed method the effect of some foreign substances which often accompanied pharmaceutical preparations were studied by adding different amounts, up to 400 µg.ml^{-1}, of foreign substances to 20 µg.ml^{-1} of chlorpromazine hydrochloride in final volume of 25 ml. The color was developed following the recommended procedure described earlier. It was found that the studied foreign species do not interfere in the present method. Typical results are given in Table 4.

Table 4 : Determination of 20 µg ml $^{-1}$chlorpromazine hydrochloride in the presence of excipients.

Excipient	Fold excess	Recovery %*
Glucose	10	101.9
Lactose	20	99.5
Sucrose	20	102.8
Starch	10	103.4
Acacia	10	97.2

* Average of three determinations.

Application of the method

The applicability of the method for the assay of pharmaceutical formulation was examined. The result of assay for available formulations of phenothiazins drugs are summarized in Table 5.

Table 5 : Assay of phenothiazines in bulk and dosage forms .

Phenothiazines	**Average recovery %⋆**	
	Proposed method	**Official method ⋆⋆**
Phenothiazine (Tablet)	99.25	99.71
Promethazine hydrochloride (Ampoule)	100.84	99.64
Chlorpromazine hydrochloride (Tablet)	98.31	100.25

⋆ Average of five determinations.

⋆⋆British Pharmacopoeia , HMSO , London , 1988 , 996 .

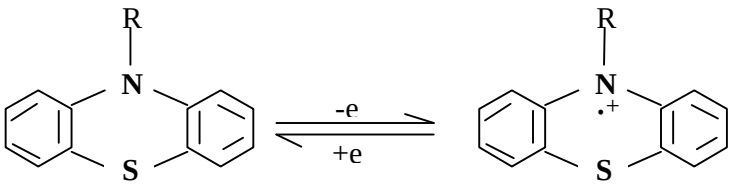
The results were reproducible and the assay of formulations was cross checked by the official method. The proposed method is found to be simple, selective and sensitive in comparison with other reported methods (Table 6).

Table 6 : Comparison of some spectrophotometric methods for the determination of phenothiazines .

Reagent	λ_{max} (nm)	Linear range ($\mu g.ml^{-1}$)	Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	Recovery (%)	R.S.D (%)	
Chloramine-T	550-565	1.6 - 20.0	–	99.7-101.2	±0.9-±5.2	
Molybdoarsenic acid	525-540	4.0 - 47.2	4.9×10^3 - 7.09×10^3	98.4-99.4	±0.92-±1.46	
4-aminoazobenzene with potassium dichromate	620	0.2 - 20	1.88×10^4	99.49-101.63	0.62-1.35	A
Iron(III)with ferricyanide	700	0.1- 8.0	3.66×10^4- 2.1×10^4	–	1.12-1.4	
Chloranilic acid	520	20 - 150	1.48×10^3- 1.75×10^3	99.54-100.04	1.04-1.82	Ba
N-chlorosuccinimide	516.5-534.5	2.0 - 40	5.34×10^3- 6.16×10^3	98.31-100.84	1.21-3.81	

Proposed reaction mechanism

N-chlorosuccinimide instantaneously oxidizes phenothiazines at room temperature in hydrochloric medium to give a red colored species having maximum absorptions in the range 516.5 - 534.5 nm, as shown in Fig. 1, where the reagent blank do not absorb appreciably. This is agreed with oxidation product of phenothiazines with vanadium (V) (Dwivedi *et al*., 1975) which is believed to be a radical cations. The formation of radical cations were confirmed by passing an aliquot of the solution through cation and anion exchange resins (Mahaveer *et al*.,2001). Only the cation exchange resin retained, indicating the cationic nature of the red colored species. The stability and sensitivity of the red radical cation depend on the nature and the concentration of the oxidizing reagent and acid medium used. However; according to these observations, the reaction mechanism is proposed in illustration 1.



Red colored species

Phenothiazine , R= H

Chlorpromazine, R= $CH_2(CH_2)_2.N(CH_3)_2$

Promethazine , R= $CH_2.CH(CH_3).N(CH_3)_2$

Illustration 1: Proposed reaction mechanism

CONCLUSION

The proposed spectrophotometric method for the determination of phenothiazines is simple, accurate, precise and cheap. The statistical analyses show that the data from the proposed method are in good agreement with those of the official method. The color reaction does not require stringent conditions nor any specific reagent or buffer. The color is stable more than 25 min, which is sufficient time for the analyst to perform the analysis. A comparison of the present method with the existing spectrophotometric methods is given in Table 6, which demonstrates the agreeable of the proposed method.

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