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Application of reinecke salt and alizarin *S* for the determination of promazine

Helena Puzanowska-Tarasiewicz, Ludmiła Kuźmicka *, Ewa Kleszczewska

Institute of Chemistry, University of Białystok, Al. Pilsudskiego 11/4, 15-443 Białystok, Poland

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Abstract

Reinecke salt and alizarin *S* have been tested as reagents for the determination of promazine. They react in neutral and acidic media with promazine forming a reddish and brown crystalline compounds. The compounds are sparingly soluble in water but fairly soluble in acetone and methanol. The quantitative extraction of compound of promazine with alizarin *S* has been used for the extractive spectrophotometric determination of promazine. Formation of promazine ion-association complex with Reinecke salt has been applied for indirect determination of low concentrations of promazine by AAS measurement of the chromium content of the reineckate counter — anion. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Promazine hydrochloride; Alizarin *S*; AAS; Spectrophotometry

1. Introduction

Promazine (PM) belongs to phenothiazines, important family of compounds from a medical point of view. Phenothiazine derivatives are widely used among the tricyclic antidepressants. Their wide application in medicine requires the methods for their determination in body fluids and pharmaceuticals. Among the methods adopted for the determination of promazine are chromatographic [1–4], electrochemical [5–8], spectrophotometric [9,10] and others [11–13]. The official methods normally involve titration in non-aqueous medium or a spectrophotometric procedure [14,15].

Several HPLC methods are now widely used in routine application owing to their sensitivity, specificity and low cost [16]. The methods have adequate sensitivity in order to quantify low concentrations of the drug in biological fluids.

Promazine dissociates in aqueous solution and creates large cation which is chemically active. This cation reacts in acidic media with thiocyanate and halide complexes of metals forming ion-association compounds [17–19]. These compounds are insoluble in water but can be quantitatively extracted into organic solvents, e.g.

* Corresponding author. Tel.: +48-85-7457580; fax: +48-85-7457581.

E-mail address: chemia@noc.uwb.edu.pl (L. Kuźmicka).

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chloroform, trichloroethylene, benzene. These properties were applied for the extractive spectrophotometric determination of metals. We found that promazine reacts with Reinecke salt forming a reddish ion-association compound. The present paper deals with the utility of Reinecke salt for the determination of promazine. Formation of promazine reineckate allows indirect determination of low concentration of promazine by AAS measurement of the chromium content of reineckate counter-anion. The complex is dissolved in acetone giving the final chromium concentration within the linear range of the calibration graph and the solutions are then aspirated into an air–acetylene flame. We have also found that alizarin *S* reacts in sulphuric acid medium with promazine forming a brown, crystalline compound. The compound is sparingly soluble in water but fairly soluble in acetone and methanol. The quantitative extraction of this compound from aqueous phase with chloroform can be applied for the extractive spectrophotometric determination of promazine.

Reviews of the methods for the determination of promazine presented by Blažek [20], Fairbrother [21] and Puzanowska-Tarasiewicz [22] show that the literature on the extractive spectrophotometric determination of PM is very limited. These methods are very useful for the determination of promazine in pharmaceuticals and body fluids. Most of proposed oxidants for spectrophotometric determinations of promazine are unsatisfactory for different reasons, e.g. some of them require heating, long time for maximum colour development, lengthy procedure, narrow ranges of determinations or lack sensitivity and specificity.

As a continuation of our previous studies on the tricyclic psychotropic drugs [9,13,17,22–24] this paper describes a simple and sensitive spectrophotometric method for the determination of promazine hydrochloride. The proposed method has been successfully applied to the assay of this drug in pharmaceuticals. Spectrophotometric methods are preferred in analytical laboratory because their procedures are very simply and the apparatus used is not expensive.

2. Experimental

2.1. Reagents

A solution of promazine hydrochloride, 10-(3-dimethylaminopropyl)-phenothiazine hydrochloride, from Polfa (Jelenia Góra, Poland) was prepared by dissolving the required amount of sample in distilled water. The solution was prepared fresh every day and protected from light.

Reinecke salt, $NH_4[Cr(NH_3)_2(SCN)_4]$ Dolder–Switzerland — 2×10^{-2} M solution.

Alizarin *S* — 1×10^{-3} M solution.

All chemicals used were of analytical grade.

2.2. Apparatus

Unicam SP 800 and Spekol-11 (Carl Zeiss, Jena, Germany) spectrophotometers were used with 1 cm cells.

Unicam SP 200 spectrophotometer.

Perkin Elmer 460 — atomic absorption spectrometer equipped with a chromium hollow cathode lamp was used under the following operation conditions: lamp current, 29 mA; slit width, 0.7 nm; wavelength, 358 nm; observation height above burner, 1 cm; single slot type burner; air flow-rate, 21.5 l min^{-1}; acetylene flow-rate, 3.4 l min^{-1}.

2.3. Determination of promazine using atomic absorption spectrometry

A 2 ml solution of promazine hydrochloride transfer to a 50 ml beaker, treat with 2 ml of 2×10^{-2} M aqueous ammonium reineckate and the mixture dilute to a total volume of 10 ml with water. The mixture stir for 2–3 min. The drug reineckate precipitate collect on a G-3 sintered glass crucible and wash with the 5 ml portions of water. The drug reineckate precipitate dissolve in 50 ml of 80–90% acetone in standard flask. The solution nebulize in an air–acetylene flame for AAS measurement of chromium at 358 nm. The absorbance was compared with a calibration graph prepared from the pure drug–reineckate solid complex under identical conditions. The drug–reineckate solid was obtained in accordance

with the following procedure: 20 ml of aqueous 1×10^{-2} M promazine hydrochloride solution mixed with 20 ml of 1×10^{-2} M ammonium reineckate solution and stirred for 3 min. The reddish precipitate of promazine reineckate was collected on a G-3 sintered glass crucible, washed several times with water, dried at room temperature and ground to obtain a fine powder. The sample for the determination was prepared by dissolving the requisite amount in acetone.

The use of acetone increases the nebulization efficiency and sensitivity of measurement, due to the low specific heat of vaporization and the heat released in the combustion of acetone vapour in the flame [25]. The acetone solutions are stable about 3 days.

2.4. Extractive spectrophotometric determination of promazine using alizarin S

Take 0.5–4 ml of promazine hydrochloride solution (concentration 5×10^{-4} M) in 50 ml separatory funnels. Add 1 ml of 0.5 M H_2SO_4 and 5 ml of 1×10^{-3} M alizarin *S* solution and dilute to 10 ml with water. The mixture stir and extract with two succesive $3-4$ ml portions of chloroform. Transfer the extracts to 10 ml volumetric flask, add 1 ml of methanol and dilute to the mark with chloroform. Measure absorbance at 420 nm against the blank. Under the described experimental conditions, the standard calibration graph for promazine were constructed. The results obtained are presented in Table 1.

Table 1
Characteristic of the method for the determination of promazine hydrochloride with alizarin *S*

Parameters	Values
Linearity range, $\mu g \cdot ml^{-1}$	7–70
ε_{420}, 1 $mole^{-1}$ cm^{-1}	8.5×10^3
Equation of calibration curve	$y = 8.5 \times 10^3 + 0.011$
Correlation coefficient	0.9984
Detection limit, $\mu g \cdot ml^{-1}$	0.15

2.5. Effect of interferences

In order to evaluate the selectivity of the developed methods for the analysis of pharmaceutical preparations, the effect of the presence of several species which can occur in the real samples with promazine hydrochloride was investigated.

The level of interferent was considered to be acceptable if the error was not larger than 2%. No interferences were observed in the determination of the drug studied in the presence of the matrix of the tablets (e.g. talc, sodium chloride, saccharin, starch, lactose, and glucose).

2.6. Assay of pharmaceutical preparations

Transfer an accurately weighed amount of the finely powdered tablets equivalent to 100 mg of promazine hydrochloride into a 100 ml standard flask and dissolve in distilled water and filter. Ten milliliters of filtrate dilute to 100 ml with distilled water and further 10 ml of the solution dilute to 100 ml with acetone. A 1.5 ml of the obtained solution use for the determination of promazine by elaborated AAS method. The 2–5 ml of the above mentioned solution use for the determination of promazine with the alizarin *S*. The amount of the drug in tablets was also determined by pharmacopeial methods [14,15]. The results obtained are presented in Table 2.

3. Results and discussion

Promazine hydrochloride reacts with ammonium reineckate [ammonium tetrathiocyanatodiammine chromate (III)] forming a reddish ion-association complex. This complex is sparingly soluble in water but fairly soluble in acetone, acetonitrile and dimethylformamide. The effects of pH, reaction time, temperature, excess of reagent and solvent on the stoichiometry of the reaction were examined. In series of experiments, solutions of 1×10^{-2} M promazine hydrochloride were allowed to react with different molar ratios of ammonium reineckate reagent at temperatures ranging from 20 to 60°C at pH 1–10. It was found that quantative reaction occurs at pH 2–7,

Table 2
Determination of promazine in Promazin's tablets[c]

Sample	Found by method (mg)				Error, %		RSD, %	
	AAS	With alizarin *S*	P.P [a]	BP[b]	AAS	With alizarin *S*	AAS	With alizarin *S*
Promazine HCl tablets 100 mg	100.3	99.4			0.2	0.7	0.3	0.9
	99.8	99.9	100.1	100.2	0.3	0.2	0.6	0.3
	100.0	100.5			0.1	0.5	0.2	0.6

[a] P.P, Polish Pharmacopoeia.
[b] BP, British Pharmacopoeia.
[c] Values RSD are given with respect to PP method.

for molar ratio at least one mole of Reinecke salt per mole of promazine hydrochloride at room temperature. No effect was noticed due to the increase of reaction temperature up to 60°C or the increase of the concentration of the reagent to a 20-fold molar excess. The amounts of promazine reineckate were isolated and their chromium contents were determined by atomic absorption spectrometry.

Calibration graphs for determination of the drug obtained under the optimised conditions and from these results is linear over the range 10–20 μg ml^{-1} of promazine, correlation coefficient of 0.9993. The regression equation was $7.90x + 1.35$.

Since the drug reineckate complex is obtainable in pure crystalline form (by recrystallization from ethanol), its solution in acetone can be used as standard solution and is readily prepared by direct weighting. The results obtained with pure samples of drug (1–4 mg) show an average recovery of $98.7\% \pm 0.6\%$ ($n = 10$).

The reproducibility of the measurements expressed as relative standard deviation (RSD) was to 0.6% for the concentrations of the drug at the examined levels.

Elemental analysis of the promazine reineckate was performed by using the standard semi-micro method. Results of elemental analysis data of the complex agreed with the formation of $[PM{\cdot}H]^+[Cr(NH_3)_2(SCN)_4]^-$. The infrared spectrum of the complex shows common absorption bands at 2160 and 3400 cm^{-1} assigned to stretching vibrations of SCN^- and NH^+ groups, respectively. The stretching vibration band of 1400 cm^{-1} due to presence of NH_4^+ in the reagent disappeared in the spectrum of the complex. All fundamental stretching vibration bands of the functional groups of promazine appear at almost the same positions in the spectrum of its reineckate complex. The electronic spectra of promazine reineckate in acetone, acetonitrile and dimethylformamide solvents display two absorption maxima at 390–400 nm and 520–525 nm. Ammonium reineckate reagent exhibits similar spectral characteristic in the same solvents, revealing that the reineckate counter-ion is the chromophoric group. On the basis of these results it can be concluded that the compound studied is ion-association complex. The amino nitrogen of aliphatic chain of promazine is responsible for the formation of the complex (Fig. 1).

We have also found that promazine hydrochloride reacts in acidic media with alizarin *S* forming a brown, crystalline compound ($\lambda_{max} = 420$ nm). The composition of the compound was established by Job's continuous variation method and by spectrophotometric titration. The molar ratio was promazine:alizarin $S = 1{:}1$.

Infrared spectra of the compound were examined (KBr discs) in the region 650–5000 cm^{-1}.

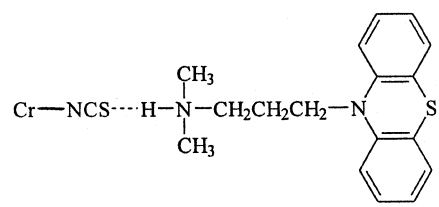
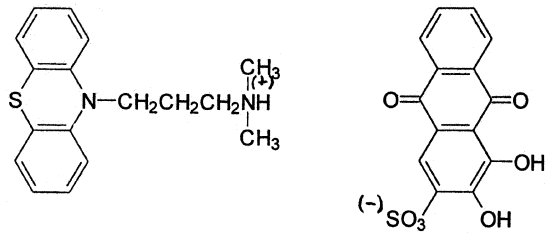


Fig. 1. The amino nitrogen of aliphatic chain of promazine with thiocyanate complex of chromium (III).



Scheme 1. Possible structure of promazine-alizarin *S* complex.

The spectra of promazine and alizarin *S* in the region 650–1700 cm^{-1} are the sum of the spectra of the reagents. Significant changes in the spectrum of the compound are observed in the region 2300–3700 cm^{-1}. A wide band, appearing in promazine hydrochloride spectrum in the region 2400–2700 cm^{-1}, and characteristic for vibrations of the NH^+ group, is shifted towards higher frequencies in the spectrum of the compound and its intensity decreased. On the basis of the data obtained it has been established that the compound studied is ion-association complex (Scheme 1).

This interpretation is in good agreement with the results of the examination of phenothiazines with other organic substances [23,24].

The compound of promazine precipitated from acidic aqueous solutions in the form of crystalline, brown sediment, can be quantitatively extracted into chloroform. Taking advantage of these properties the extractive spectrophotometric method for the determination of promazine has been elaborated. The Beer's law is obeyed in the range 7–70 μg ml^{-1} (Table 1). The reproducibility of the measurements, expressed as relative standard deviation, is 0.9%. The absorbance of the extracts is stable for at least 3 days.

The elaborated method was applied successfully to the determination of promazine hydrochloride in pharmaceutical preparations (Table 2). As indicated, the assay results for tablets using elaborated methods were in accordance with the declared amounts and the pharmacopoeial results.

The proposed method gives satisfactory agreement with official methods. The results obtained using the non-aqueous titrimetry and spectrophotometry of the British and Polish Pharmacopoeias [14,15], show an average recovery of 98.8%. Comparison with above mentioned suggested assay methods [14,15] reveals that the proposed extractive spectrophotometric and AAS procedures offer the advantages of simplicity, precision and sensitivity.

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