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A Sensitive Kinetic Spectrophotometric Method for Trace Amounts Determination of Promethazine in Drug Formulation and Biological samples.

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ABSTRCT

In this research, a new strategy was introduced for quantitative determination of promethazine. The procedure was based on the catalytic effect of promethazine on the Orange G-bromate reaction system. The change in absorbance was followed spectrophotometrically at 478.5 nm. The maximum sensitivity was obtained by optimizing the reagents concentration, temperature and time. Under optimum experimental conditions, calibration graph was linear over the range 0.07 to 50.0 µg mL^{-1} of promethazine including two linear segments. The relative standard deviations (n = 5) for 2.0, 10.0 and 35.0 µg mL^{-1} of promethazine were 1.21, 1.18 and 1.07%, respectively. The limit of detection was 0.05 µg mL^{-1} of promethazine. As many as 17 species including cations, anions and some of co-exist compounds that may be presence in real samples was investigated which more of them (13 species) did not show interfering effect. The developed procedure was successfully applied for the determination of promethazine in drug formulation and bioloical samples that the recovery results were near to 100%.

Keywords: Promethazine; Kinetic spectrophotometry; Drug formulation; Biofluides

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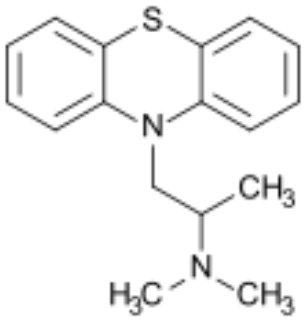
INTRODUCTION

Promethazine, see scheme 1 for molecular structure, is a neuroleptic medication of the phenothiazine family. Promethazine is widely used as an antiallergic, a sedative to reduce nervousness, an adjunct treatment for anaphylactoid conditions, a motion sickness and treatment for migraines. It was also recommended by National Aeronautics and Space Administration for space sickness treatment due to its effectiveness and long duration. It has also adverse effects such as endocrinal, cardiac and reproductive alterations [1, 2]. Therefore, quantitative determination of promethazine in commercial formulations and biological fluids can be extremely important.

Scheme 1. Molecular structure of promethazine.



There are a number of analytical techniques in the literature which focus on the determination of promethazine including electrochemical methods [3-5], high performance liquid chromatography-mass spectrometry [6], gas chromatography [7], capillary zone electrophoresis [8], spectrophotometry [9] and flow injection spectrophotometry [10, 11]. Shortages such as low repeatability are the characteristic of electrochemical methods. Chromatographic methods have disadvantages such as high cost, hard operation and uses considerable amount of high quality expensive solvents. Along with the limitations such as low selectivity in comparison to other methods, simple procedure, necessity of less expensive apparatus and sufficient accuracy are the advantages of kinetic spectrophotometric method that make it attractive for the determination of various species in different matrices [12-14].

The aim of this work was the development of a simple, rapid, sensitive and cost effective kinetic method for the determination of promethazine in pharmaceutical and biological samples. Beside, to the best of our knowledge, there is no published report relating to the quantitative determination of promethazine using kinetic spectrophotometric method. The preliminary studies showed that promethazine had a strong catalytic effect on the Orange G - bromate reaction system in acidic media. Wide linear dynamic range, low detection limit introduces the proposed method as a new strategy for the quantification of promethazine in pharmaceutical and biological samples.

EXPERIMENTAL

Instrumentation and chemicals

A double beam Unique UV-Vis spectrophotometer (T80+, UK) with 1cm matched glass cells was used to measure the absorbance. A thermostated water bath (Hieldolph, Germany) was used to keep the temperature of all solutions at the working temperature (25±0.1 °C). A stop-watch was used to record the reaction time.

Redistilled water and analytical grade chemical reagents were used. 50 mL of 50.0 µg mL-1 of promethazine solution was prepared by dissolving 0.0025 g of promethazine.HCl (Sigma) in water daily. 10.0 µg mL-1 of working solution was prepared by diluting appropriate amount of the solution in 50 mL volumetric flask. A solution of Orange G (7.0×10-4 mol L-1) was prepared by dissolving 0.3166 g of it in water, and then diluting to 1 L in volumetric flask. Sulfuric acid (4.0 mol L-1) was prepared by appropriate dilution of

concentrated acid solution (Merck). 1.0×10-1 mol L-1 of potassium bromate solution was subsequently prepared by dissolving 16.7080 g of KBrO3 (Merck) in water and diluting to 1 L in volumetric flask.

General procedure

After initial kinetic spectrophotometric investigations of the selected reaction system, the concentrations of reagents (except the catalyst) were judiciously selected for the analytical procedure. The catalyzed reaction was studied spectrophotometrically by monitoring the change in absorbance of the reaction mixture at 478.5 nm. To a series of 10 mL volumetric flasks, 2.0 mL of 4.0 mol L^{-1} sulfuric acid solution, 0.9 mL of 7.0×10^{-4} mol L^{-1} Orange G solution and 1 mL of 50.0 µg mL^{-1} of promethazine solution were added. It was mixed and diluted with water. Then, 0.5 mL of 1.0×10^{-1} mol L^{-1} bromate solution was added and diluted to the mark with water. The time of measurement started just after adding the last drop of the oxidant solution. The solution was thoroughly mixed and a portion of it was transferred to a glass cell. The absorbance of catalyzed reaction (ΔA_s) was measured against water at 478.5 nm at 25 °C and time interval 30-240 s. The measurement in the absence of promethazine was repeated to obtain the values for the uncatalyzed reaction (ΔA_b). Finally, the difference in the absorbance change was considered as the response ($\Delta A=\Delta A_s-\Delta A_b$). Under optimum experimental conditions, calibration curve was constructed by plotting the response against promethazine concentration in working standard solutions.

Analysis of real sample

Pharmaceutical sample preparation

Promethazine injection solution (50 µg mL-1) was used as pharmaceutical sample. The vial content was transferred to a 25 mL flask, diluted to the mark and an appropriate amount of it was used in each analysis.

Biological sample preparation

Human serum and urine were used as biological samples for the determination of promethazine. Real samples were prepared from a person who had not taken the drug. They were spiked with promethazine and solid phase extraction technique with C18 cartridge (Supelco Inc., 10 mL) was used for purification and pre-concentration of promethazine from the samples [15]. The extracted promethazine was determined by the developed method.

RESULTS AND DISCUSSION

Orange G, acid orange 10, is an orange crystal powder. It can be used for staining of keratin, color marker and pH indicator. Oxidizing agents can be oxidize it in acidic media at a slow reaction and colorless oxidized form was produced.

The comparison of the absorption spectra of catalyzed and uncatalyzed reactions confirmed that the change in absorbance was increased in presence of trace amounts of promethazine. The proposed mechanism of the reaction for the oxidation of Orange G can be described by the following reactions:

$$\text{Orange G}_{(Red)} + BrO_3^- + 6H^+ \rightarrow \text{Orange G}_{(Ox)} + Br^- + 3H_2O \quad (1)$$

$$5Br^- + BrO_3^- + 6H^+ \rightarrow 3Br_2 + 3H_2O \quad (2)$$

$$Br_2 + H^+ + \text{Orange G}_{(Red)} \rightarrow 2Br^- + \text{Orange G}_{(Ox)} \quad (3)$$

Since promethazine has a catalytic effect, bromide generation was increased. It may be attributed to the following reaction:

$$\text{Promethazine}_{(Red)} + BrO_3^- + 6H^+ \rightarrow \text{Promethazine}_{(Ox)} + Br^- + 3H_2O \quad (4)$$

The reduced and oxidized forms of Orange G and promethazine defined by Red and Ox, respectively.

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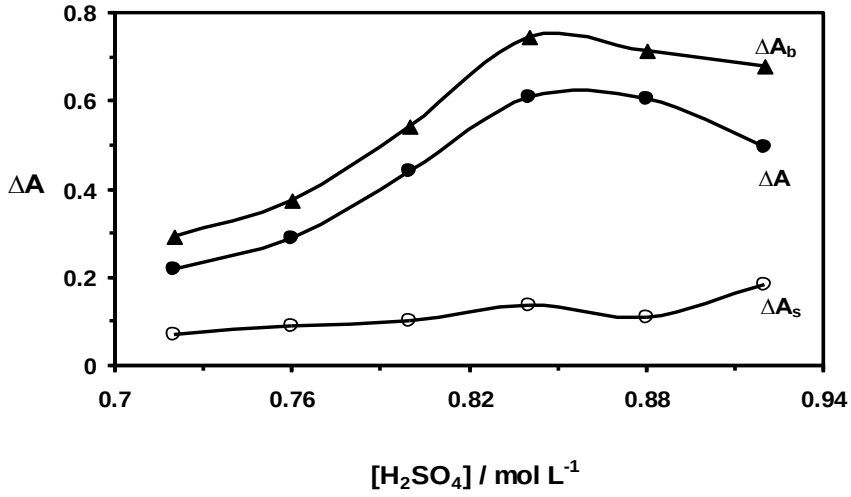
Reaction Variables Optimization

In order to establish the experimental conditions under which the catalytic effect of promethazine and therefore, the sensitivity in its determination to be at maximum, the dependence of the reaction rate to reagents concentration, temperature and time were studied. The change in absorbance after a fixed time as a measure of initial rate was used to plot the graph for each variable. Optimum conditions were taken from the graphs for the subsequent study of the variables. The reagent concentration optimization was carried out on the catalyzed and uncatalyzed reactions for a constant time of 240 s in the presence of 50.0 µg of promethazine.

Effect of Sulfuric acid Concentration

The effect of sulfuric acid concentration on the uncatalyzed and catalyzed reactions was studied in the concentration range 0.72 to 0.92 mol L^{-1}. As shown in Fig. 1, the reaction rate was increased with increasing the concentration of sulfuric acid up to 0.84 mol L^{-1}. At higher concentrations, the reaction rate was decreased. It may be attributed to the protonation of Orange G which might stop oxidation or make oxidation quite difficult to occur. Thus, 0.84 mol L^{-1} of sulfuric acid was used for further study.

Fig. 1: Effect of sulfuric acid concentration on the rate of uncatalysed (ΔA_b) and catalysed (ΔA_s) reactions and response (ΔA). (Conditions: sulfuric acid 0.72 - 0.92 mol L^{-1}, Orange G, 63.0 µmol L^{-1}; promethazine, 5.0 µg mL^{-1}; bromate, 5.0 mmol L^{-1}; 25 °C and 480.0 s).



Effect of Orange G Concentration

The experimental results on the study of Orange G concentration effect in the range of 63.0 to 91.0 µmol L^{-1} indicated that difference in absorbance increased with the concentration of Orange G up to 84.0 µmol L^{-1} (Fig. 2). Therefore, 84.0 µmol L^{-1} of Orange G was selected as optimum value.

Effect of Bromate Concentration

The dependence of oxidation reaction rate to bromate concentration was studied in concentration range of 4.0 to 8.0 mmol L^{-1}. As shown in Fig. 3, under optimum concentrations of H_2SO_4 and Orange G, the reaction rate increased up to 6.0 mmol L^{-1} of bromate. Therefore, the optimum value of 6.0 mmol L^{-1} of bromate was selected for following the procedure.

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Fig. 2: Effect of Orange G concentration on the rate of uncatalysed (ΔA_b) and catalysed (ΔA_s) reactions and response (ΔA). (Conditions: sulfuric acid 0.84 mol L^{-1}, Orange G, 63.0 – 91.0 µmol L^{-1} promethazine, 5.0 µg mL^{-1}; bromate, 5.0 mmol L^{-1}; 25 °C and 480.0 s).

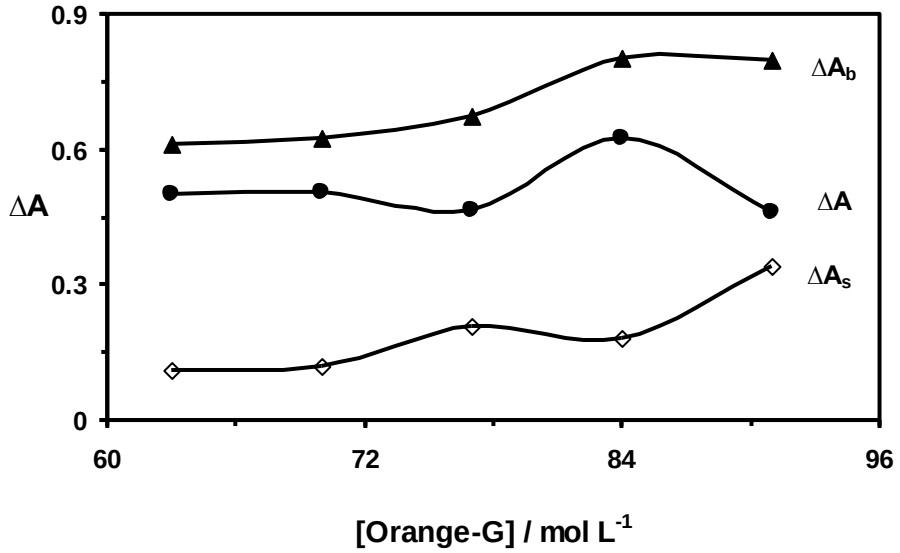
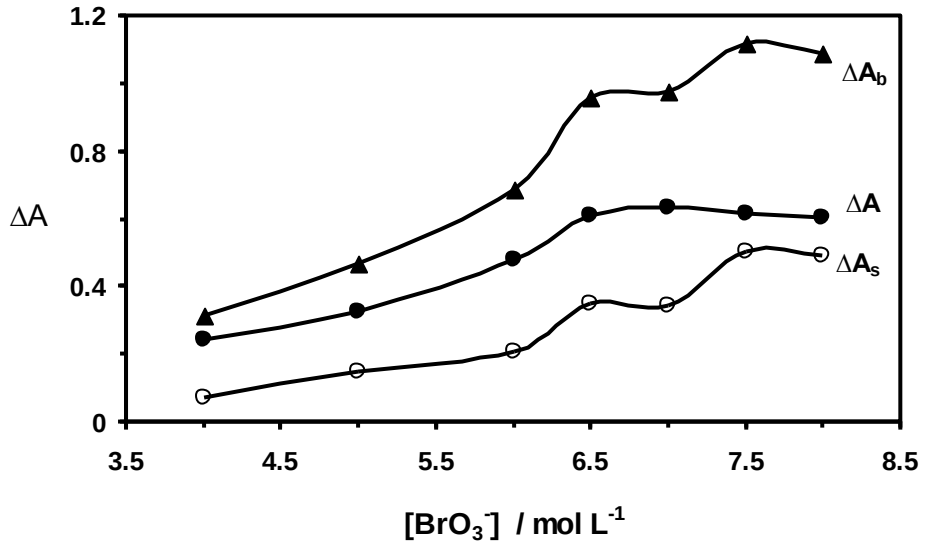


Fig. 3: Effect of bromate concentration on the rate of uncatalysed (ΔA_b) and catalysed (ΔA_s) reactions and response (ΔA). (Conditions: sulfuric acid 0.84 mol L^{-1}, Orange G, 84.0 µmol L^{-1}; promethazine, 5.0 µg mL^{-1}; bromate, 4.0 – 8.0 mmol L^{-1}; 25 °C and 48.0 s).



Effect of Temperature

Under optimum reagents concentration, temperature effect on the rate of reaction was studied in the range of 15 to 40 °C. The maximum sensitivity was obtained at 30 °C and selected as optimum.

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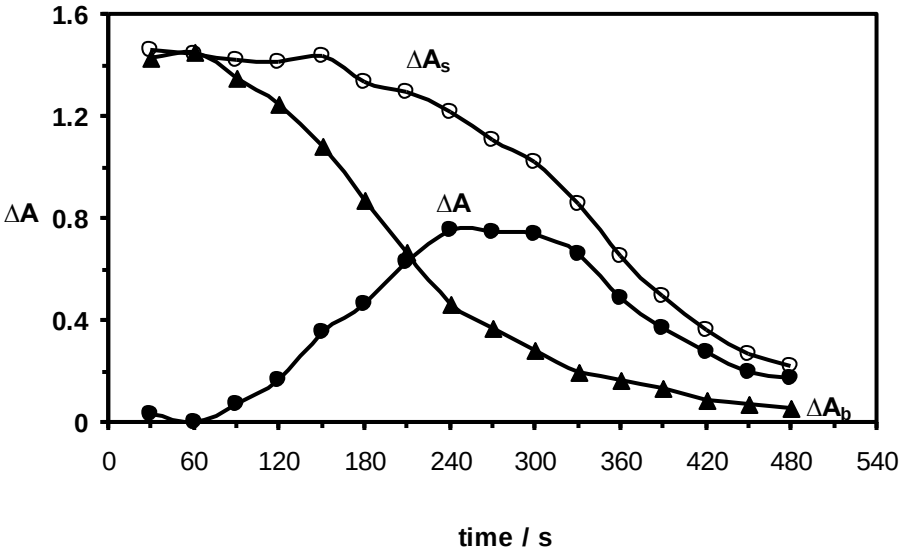
Effect of Reaction Time

The optimum time was found by measuring the change in the absorbance during 30 to 480 s. As it can be seen in Fig. 4, the reaction rate was increased up to 240 s, and for longer times was reduced. Therefore, 240 s was selected as optimum time for further study.

Fig. 4: Effect of time on the rate of uncatalysed (ΔA_b) and catalysed (ΔA_s) reactions and response (ΔA). (Conditions: sulfuric acid 0.84 mol L^{-1}, Orange G, 84.0 µmol L^{-1}; promethazine, 5.0 µg mL^{-1}; bromate, 6.0 mmol L^{-1}; 30 °C and 0 - 480 s).



Analytical Parameters

Under optimum experimental conditions, calibration curve was obtained over the range 0.07 - 50.0 µg mL^{-1} of promethazine including two linear segments of 0.07-5.0 µg mL^{-1} and 5.0-50.0 µg mL^{-1}. An analysis of the data gave the following regression equations: ΔA = 0.223 [Promethazine] + 0.0721 (R2= 0.9981) and ΔA = 0.0075 [Promethazine] + 1.1777 (R2= 0.9982) for first and second linear segments, where ΔA was the difference in absorbance between the blank and the sample response and [Promethazine] was the promethazine concentration in µg mL-1. The detection limit (3Sb/m) was 0.05 µg mL^{-1} of promethazine based on the slope of the first linear segment. The relative standard deviations (n = 5) were 1.12 and 1.04% for 4.0, 15.0 µg mL-1 and 0.97% for 20.0 µg mL-1 of promethazine, respectively.

Interference studies

The interfering effect of foreign species in the determination of 1.0 µg mL^{-1} of promethazine was investigated. The tolerance limit was defined as the concentration of the added species causing an error (analytical signal) more than ± 5%. The results are given in Table 1. The obtained results showed that nitrite and halide ions had seriously interfering effect, whereas not exist in real sample matrix.

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Table 1. Tolerance limit for foreign species on the determination of 1.0 µg mL^{-1} of promethazine.

Foreign species	Tolerance limit (WPromethazine/Wspecies)
Na^+, K^+, NH_4^+	>1000
SO_4^{2-}	>1000
Saccarose	1000
Fructose, glucose	900
Ethanol	900
HCO_3^-, CO_3^{2-}, NO_3^-	900
Urea	800
Uric acid	800
I^-, Br^-, Cl^-, NO_2^-	<1

Real Sample Analysis

The accuracy and applicability of the proposed method has been investigated and confirmed by the determination of promethazine in pharmaceutical and biological samples.

Pharmaceutical sample preparation was performed using the mentioned procedure. The results of three replicate determinations are given in Table 2. The accuracy of the proposed method was checked by statistical t-test. The difference of experimental and critical *t* –values (2.79<4.30; DF: 2 and P: 0.05) suggest that there is no evidence of systematic error. Also, the precision (RSD%) was 1.02%.

Table 2. Determination of promethazine in injection solution.

Sample	Found[a] (mg/vial)	Labled (mg/vial)	RSD (%)	Statistical t-test	Pharmaceutical Co./Batch No.
1	50.32 ±0. 49	50	0.97		Tehran shimi/496

[a] Mean ± standard deviation (n=3)
[b] Tabulated *t*-value for two degrees of freedom at P(0.05) is 4.30.

Table 3. Determination of promethazine in human serum and urine samples using the developed procedure.

Sample	Added (µg mL^{-1})	Found[a] (µg mL^{-1})	RSD (%)	Recovery (%)
Serum	—	<D.L		
1	1.0	1.03±0.01	1.02	101.0
2	4.0	3.98±0.04	1.00	99.7
3	10.0	10.03±0.12	1.20	100.3
4	20.0	20.02±0.21	1.05	100.0
Urine	—	<D.L		
1	1.0	1.03±0.01	0.97	98.0
2	4.0	3.98±0.04	1.00	100.7
3	10.0	10.03±0.12	1.20	100.5
4	20.0	20.02±0.21	1.05	99.7

[a] Mean ± standard deviation (n=3)

Moreover, the procedure was used for the determination of promethazine in human serum and urine samples that were collected from the patient who has not taken the drug. After sample preparation, the samples were spiked with different amounts of promethazine including two linear segments of the calibration curve and analysed using recommended procedure. The obtained results of three replicate determinations are

given in Table 3. The recovery results were obtained between 99.7 to 101.0% and also, the values of RSD% varied over the range 0.95 to 1.19%. The reported results confirm the accuracy and precision of the proposed method. Therefore, it can be used as an alternative method for quantitative determination of promethazine in different samples.

CONCLUSIONS

This study reports a sensitive and relatively selective kinetic spectrophotometric method for the detrmination of promethazine. The developed method possesses distinct advantages such as simplicity, ease of operation and applicable to real samples analysis. Moreover, the reliability of the developed method permits the analysis of pharmaceutical and biological samples with satisfactory results.

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