Department of Food Science and Technology, Tarbiat Modarres University, Tehran, Iran

Kinetic-spectrophotometric determination of propylthiouracil based on its inhibitory effect on the reduction of neutral red by hypophosphite

M. Barzegar, A. Rahmani, A. Jabbari, M. F. Mousavi

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Dr. M. Barzegar, Department of Food Science and Technology, Tarbiat Modarres University, P.O. Box 14115–336, Tehran, Iran
mbb@modares.ac.ir

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A new kinetic-spectrophotometric method for determination of propylthiouracil (PTU) is described. The proposed method is simple, rapid, inexpensive and sensitive for the determination of PTU in pure and tablet forms. This method is based on the inhibitory effect of propylthiouracil on the palladium(II)-catalyzed reaction between neutral red and hypophosphite ions. The effect of various parameters such as: dye, hypophosphite, and Pd(II) concentrations, pH, ionic strength, and temperature were optimized. Two distinct linear calibration graphs were observed in the ranges 0.006–0.033 ($n = 6$, $r = 0.9991$) and 0.033–0.300 ppm ($n = 10$, $r = 0.9980$). The variable time method was used. The limit of detection was 0.004 ppm. The proposed method was applied for the determination of propylthiouracil in pure and tablet forms.

1. Introduction

Propylthiouracil (6-propyl−2-thiouracil) inhibits the formation of thyroid hormones and is used for the treatment of hyperthyroidism [1, 2]. Several different analytical procedures have been described for the determination of propylthiouracil: potentiometric [3, 4], conductometric [5], titrimetric [6–8], and spectrophotometric [9–11] methods. In biological fluids propylthiouracil has been determined by HPLC [12–14] and by spectrophotometric methods [15, 16].

Most of the proposed methods are either not sensitive enough, or require complicated and expensive instruments, or are time consuming, or provide high detection limits. Therefore, there is still a need for a sensitive, simple and relaible method for the determination of propylthiouracil.

Recently, our research group was involved in the introduction of some kinetic-spectrophotometric methods for the determination of some species such as nitrite, selenium, palladium and etc. [17–20]. In an earlier work [21], the catalytic effect of Pd(II) on the reduction of neutral red (NR) by the hypophosphite ion was studied. It was found that some ions show an inhibitory effect on this catalysed reaction. Furthermore, a report [22] has shown that Pd(II) reacts with sulphur-containing ligands to produce stable complexes.

This paper describes a kinetic-spectrophotometric method for the determination of trace quantities of propylthiouracil based on its inhibitory effect on the reaction of neutral red with hypophosphite in the presence of Pd(II). The reaction was monitored spectrophotometrically at the maximum wavelenght of neutral red (530 nm) while measuring the change in the absorbanse with time.

2. Investigations, results and discussion

Our previous work [21] has demonstrated that in the presence of trace amounts of Pd(II), hypophosphite reduces neutral red rapidly. A report [22] has shown that Pd(II) reacts with sulphur-containing ligands to produce stable complexes. Therefore, PTU decreases the catalytic effect of Pd(II) on the reaction of the neutral red-hypophosphite system. The reaction rate of the catalyzed reaction decreased with increasing amounts of PTU and time required for the absorbance to decrease to a predetermined value increased. The reaction was monitored spectrophotometrically by measuring the time required for the absorbance of the neutral red to decrease by 0.1 (at $\lambda_{max} = 530$ nm) by means of variable time method.

2.1. Influence of experimental variables

The studies were carried out by altering each variable in turn whilst keeping the other constant. All concentrations described here are the initial concentrations in the reaction mixture at $t = 0$ after mixing. Each kinetic result is the average of three determinations.

To obtain the maximum sensitivity in the determination of PTU, we investigated the effect of several variables on the rate of catalyzed and inhibited catalyzed reactions, as follows.

The effect of pH on the catalyzed and inhibited catalyzed reaction was studied in the range of 2.5–7.0 (by adding 3.0 ml of Britton-Robinson buffer solution) and the results are shown in Fig. 1. As can be seen, the inhibitory effect of PTU reached a maximum at $pH = 4.0$ and remained constant up to pH 5.3. All subsequent investigation were performed at $pH = 4.5$.

The effect of neutral red concentration was investigated in the range 1.1×10^{-5}–5.6×10^{-5} M. Fig. 2 shows the per-

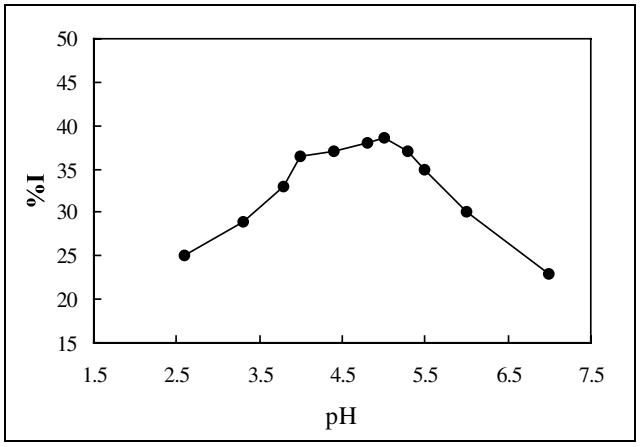

Fig. 1: Influence of pH on the inhibitory effect of PTU
Conditions: [Pd] = 1.1 ppm; [NR] = 3.2×10^{-5} M; [HP] = 0.4 M; [PTU] = 0.14 ppm; T = 25 °C

centage of inhibition (I%) versus different neutral red concentrations. A concentration of 3.2×10^{-5} M, at which the inhibitory effect of PTU is maximum, was selected as optimum dye concentration.

The influence of the hypophosphite (HP) concentration was studied over the range 0.11–0.56 M. The rates of catalyzed and inhibited reactions increased with increasing concentrations of HP up to 0.40 M, above which they decreased slightly.

The influence of ionic strength on the reaction rate was studied using KNO_3 solution (2M) in the concentration range of 0.0–0.9 M. The percentage of inhibition remained constant when the KNO_3 concentration was in the range of 0.0–0.4 M and decreased with the concentrations larger than 0.4 M.

An important variable is the concentration of Pd(II). It was observed that when the concentration of the PTU was constant the rate of the process increased considerably with increase in the concentration of Pd(II). The inhibitory effect of the drug decreased when the concentration of Pd(II) increased. Fig. 3 shows the results obtained in the presence of 0.14 ppm of PTU and increasing amounts of Pd(II). Moreover, the linear range of propylthiouracil concentrations determined and the slope of the calibration graph obtained under optimum conditions depended on the concentration of the catalyst. A concentration of

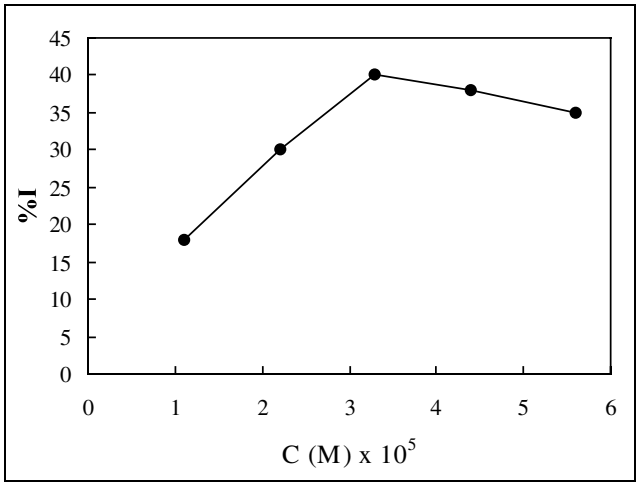

Fig. 2: Effect of dye concentration on the percent of inhibition
Conditions: [Pd] = 1.1 ppm; [HP] = 0.4 M; [PTU] = 0.14 ppm; T = 25 °C; pH = 4.5

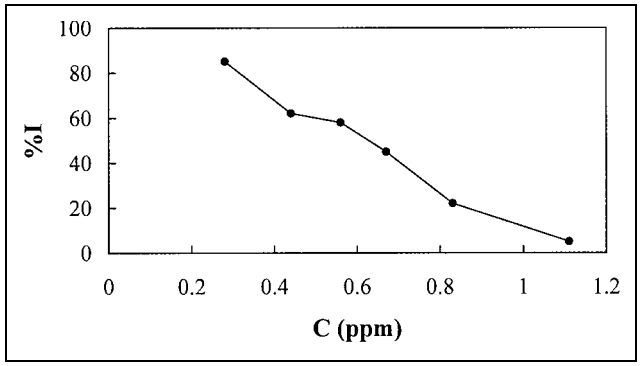

Fig. 3: Influence of Pd(II) concentration on the inhibitory effect of PTU
Conditions: [NR] = 3.2×10^{-5} M; [HP] = 0.4 M; [PTU] = 0.14 ppm; T = 25 °C; pH = 4.5

0.56 ppm of Pd(II) was selected as the most suitable concentration.

The effect of temperature on the rate of catalyzed and inhibited catalyzed reaction was studied in the range 25–55 °C. The results show that 25 °C was best, since at higher temperatures the inhibition effect of PTU was decreased, causing very increasing in the rate. Thus, 25 °C was used as the most convenient temperature.

2.2. Features of the proposed method

Plotting the percentage of inhibition as a function of the inhibitor concentration, as described by Perez-Bendito [23], we commonly ran the method used to construct calibration plots in the kinetic determination of inhibitors. Under the selected experimental conditions two linear relationship between the PTU concentration and the percent of inhibition (I%) were obtained. Table 1 gives the equations obtained and other typical figures of merit, such as the limit of detection, the correlation coefficients and the ranges of linearity. In order to estimate the precision (RSD%) of the method, replicate samples (n = 10) containing 0.01 and 0.15 ppm of PTU were measured individually.

2.3. Interference studies

The influence of commonly used excipients and additives (lactose, magnesium stearate, starch, saccharose and glucose) was investigated befor the determination of PTU in dosage forms. No interference was observed.

2.4. Application

To evaluate the analytical applicability of the method, the method developed was applied to the determination of

Table 1: Analytical charactristic of the elaborated method

	Calibration curve 1	Calibration curve 2
Linear range (ppm)	0.006–0.033	0.033–0.30
Equation of calibration curve	I% = 1.322C + 0.429	I% = 0.159C + 41.45
Correlation coefficient	0.9991	0.9980
RSD%	1.0*	0.6**
Limit of detection (ppm)	0.004 (S/N = 3)	

* For determination of 0.01 ppm of PTU (n = 10).
** For determination of 0.15 ppm of PTU (n = 10)

Table 2: Determination of authentic samples of PTU by the proposed method

Taken (mg)	Found* (mg)	Recovery (%)
2	1.98	99.00
4	4.01	100.25
6	6.05	100.83
8	8.03	100.38
10	10.00	100.00
	Average = 100.10 ± 0.68	

* Average of five different determinations

Table 3: Determination of PTU in tablet in mg per tablet*

Decleared content	Refrence method	Proposed method	RSD%
50.00	49.90 ± 0.30	49.70 ± 0.15	0.70

* Average of five determinations ± S.D.

authentic PTU and its tablet form. Table 2 shows the results obtained for the determination of authentic PTU. As can be seen, the results demonstrate good precision. To complete the validation of the described method, an analysis of the content PTU in tablet was made and for comparison, the reference method adopted in the USP [24] was applied. The results obtained for the determination of PTU in tablet form are summarized in Table 3. Applying the F-test and the t-test at the 95% confidence level compared the results obtained in both methods. The calculated F and t values did not exceed the theoretical values ($F = 6.39$, $t = 2.31$) indicating that there is no significant difference between the two methods with respect to accuracy.

Finally, the kinetic-spectrophotometric method proposed for the determination of PTU is simple, sensitive, inexpensive, and rapid. This method is comparable in accuracy and precision with the reference pharmacopocia method. The developed method is suitable for the analysis of the PTU as a substance and in tablet form, as there are no interferences from the excipients normally found in commercial preparation.

3. Experimental

3.1. Reagents

All chemicals were of analytical-reagent grade and the solutions were prepared with doubly distilled water. Working solutions of lower concentrations were prepared by appropriate dilution of the respective stock solutions.

Palladium chloride (1000 ppm, 0.1670 g) was dissolved in 2.0 ml concentrated HCl (Merck) and the solution was transferred into a 100 ml standard flask, and then diluted to volume with water to make Pd stock solution. Neutral red (NR)solution (2×10^{-4} M) was prepared by dissolving 0.0290 g of NR (Merck) and diluting to 500 ml in a calibrated flask. Hypophosphite (2 M) solution was prepared by dissolving 20.8180 g of potassium hypophosphite (Merck) in 100 ml water.

The purity of the propylthiouracil (PTU, Sigma, USA) was proved to be 100.1% according to the USP method [24]. The stock solution was prepared by dissolving 0.0100 g of PTU in 1 ml of 0.01 M NaOH and diluting to 100 ml with distilled water. Britton-Robinson buffer solutions were used for pH adjustment.

3.2. Apparatus

A 662 probe-type photometer (Metrohm), and a Grant thermostatically controlled bath/circulator (Grant Instrument, Ltd., Cambridge) were used. During the all experiments, solutions were stirred with a magnetic stirrer (Zag-Chemie Co., Ltd., Tehran, Iran). All pH measurements were carried out with a Metrohm pH-meter model 691. The glass cell was cleaned after use by immersion into HNO_3 (6 M) in order to remove any traces of Pd(0) adsorbed onto the walls.

3.3. General procedures for the determination of propylthiouracil

For calibration curves: After a suitable aliquot of solution containing 0.006–0.30 μg PTU was transferred into a 10 ml volumetric flask, 2.0 ml of 2.0 M hypophosphite, 3.0 ml Britton-Robinson buffer (pH = 4.5), and 1.6 ml of 2.0×10^{-4} M neutral red solution were added. The solution was diluted to 10 ml with water. After well mixing, the aliquot transferred into a glass cell (termostated at 25 °C). Then, the A-t curve was recorded just after the instaneous addition of Pd(II) using a microsyringe. The reaction was monitored spectrophotometrically at 530 nm. The time scan was recorded by computer-interfaced probe-type.

Assay of PTU tablets (50 mg): Ten tablets were weighted and crushed in a mortar. A mass of powder equivalent to the average mass of one tablet was dissolved in water, 0.4 ml of NaOH (0.2 M) was added and diluted with water to 50 ml. The solution was filtered through a Millipore filter, and filtrate was diluted with water in a 100 ml calibrated flask. A 2.0 ml aliquot of solution was diluted to 100 ml with water and the proposed procedure was applied.

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