Drug Development and Industrial Pharmacy, 27(8), 831–835 (2001)

RESEARCH PAPER

Development and Validation of a High-Performance Liquid Chromatographic Method for the Analysis of Propylthiouracil in Pharmaceuticals

Ahmad M. Abdul-Fattah and Hridaya N. Bhargava*

Department of Pharmaceutical Sciences, Massachusetts College of Pharmacy and Health Sciences, Boston, MA 02115

ABSTRACT

A simple, rapid, and stability-indicating high-performance liquid chromatographic (HPLC) method was developed and validated for the assay of propylthiouracil (PTU). The method was used to quantify PTU in topical formulations and in tablets. Excellent linearity was observed between PTU concentration and the peak area ($R^2 = 0.999$). The limit of detection was 1 ng, and the limit of quantitation was 1.2 ng. The method proved to be selective. Selectivity was validated by subjecting a stock solution of PTU to acidic, basic, and oxidative degradations. The peaks of the degradation products did not interfere with the peak of PTU. Excipients present in the dosage forms did not interfere with the analysis, and the recovery of PTU from each dosage form was quantitative.

Key Words: *High-performance liquid chromatography; Liquid chromatography; Propylthiouracil; Selectivity; Stability indicating*

INTRODUCTION

Propylthiouracil (PTU) (Fig. 1) belongs to a class of antithyroid drugs called *thionamides* or *thioureylenes* for the nondestructive therapy of hyperthyroidism. Thionamides are potent inhibitors of thyroid peroxidase enzymes responsible for the iodination of tyrosine residues of thyroglobulin and the coupling of iodotyrosine residues to form iodothyronines. In addition, PTU is the drug of choice in the treatment of thyroid storm because of its ability to inhibit 5′-deiodinase, which is

*Corresponding author.

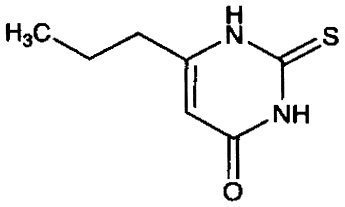

Figure 1. Structure of PTU.

responsible for the peripheral deiodination of tetraiodothyronine to triiodothyronine (1). PTU is currently available as tablets and is also dispensed as an enema and suppositories, but not in a topical dosage form (2).

PTU and other thioureylenes have potential in treating psoriasis. A topical preparation containing 5% PTU given to patients with plaque psoriasis produced significant resolution (3). The mechanism of action is possibly related to effects of PTU on the immune system (4). PTU therapy has also been reported to reduce the clinical severity of atopic dermatitis (5).

No method for the assay of PTU in topicals is reported. A simple, rapid, and stability-indicating high-performance liquid chromatographic (HPLC) method for the analysis of PTU in both tablets and topicals was developed and validated.

EXPERIMENTAL

Chemicals and Reagents

All the chemicals and reagents used were HPLC, USP-NF, or ACS grades and were used without further purification. The PTU USP reference standard was purchased from the U.S. Pharmacopoeia, Incorporated (Rockville, MD). Propylthiouracil Tablets® (Lederle Pharmaceutical) were purchased locally. Three PTU creams (2.5%, 5%, and 10% w/w) and a placebo cream were supplied by Stiefel Research Laboratories (Oak Hill, NY).

Apparatus

A Hewlett Packard series 1100 (HP 1100) system equipped with a multiple-wavelength ultraviolet (UV) detector and an HP 3395 integrator were used. The stationary phase was a Zorbax® SB-CN (Cyano) column (5 μ, 150 × 4.6 mm internal diameter, Mac Mod Analytical, Inc.).

Chromatographic Conditions

The mobile phase consisted of 5% v/v of acetonitrile and 0.025 M potassium phosphate monobasic aqueous buffer (pH 4.6 adjusted with 0.5 N sodium hydroxide solution). The flow rate was 1 ml/min. The wavelength of detection was 282 nm; the chart speed was 0.5 cm/min; the column temperature was maintained at 35°C; and the injection volume was 20 μl.

Preparation of Stock and Standard Solutions

For the solvent, filtered deionized-distilled water was mixed in a 1 : 1 ratio with HPLC grade methanol. The stock solution of PTU was prepared by dissolving 50.0 mg of PTU in 100.0 ml solvent, creating a 50-μg/ml solution of PTU. This solution was diluted with solvent as needed to prepare different standard solutions. Three different stock solutions of PTU were prepared. Each of the stock solutions was used to prepare standard solutions. Each standard solution was injected into the liquid chromatographic system.

The mean peak areas of all the tested concentrations were used to construct a standard calibration curve to test the linearity and regression coefficient R^2 of the HPLC method. The precision of the method was also tested by injecting a standard solution of 2 μg/ml PTU eight times.

Oxidation of Propylthiouracil

For oxidation of PTU, 2.0 ml of the stock solution of PTU (50 μg/ml) were transferred to a 10-ml volumetric flask, and the volume was made up to 10 ml with 3% hydrogen peroxide (H_2O_2) solution. The mixture was heated and kept at 80°C for about 1 h, cooled to room temperature, filtered through a 0.45-μ syringe filter, and injected into the liquid chromatographic system to detect peaks of oxidation.

Degradation of Propylthiouracil

Degradation by Acid

For degradation of PTU by acid, 2.0 ml of the stock solution of PTU (50 μg/ml) were transferred to a 10-ml volumetric flask, and the volume was made up to 10 ml with 1 N hydrochloric acid (HCl).

The mixture was heated and kept at 80°C for about 1 h, then cooled to room temperature. The pH of the solution was adjusted to neutrality by adding 1 N sodium hydroxide (NaOH); the solution was filtered through a 0.45-μ syringe filter and injected into the liquid chromatographic system to detect peaks of degradation products.

Degradation by Base

For degradation of PTU by base, 2.0 ml of the stock solution of PTU (50 μg/ml) were transferred to a 10-ml volumetric flask, and the volume was made up to 10 ml with 1 N NaOH. The mixture was heated and kept at 80°C for about 1 h, then cooled to room temperature. The pH of the solution was adjusted to neutrality by adding 1 N HCl; the solution was filtered through a 0.45-μ syringe filter and injected into the liquid chromatographic system to detect peaks of degradation products.

Extraction of Propylthiouracil from Tablets

For extraction of PTU, 20 PTU tablets USP (each containing 50 mg of PTU) were accurately weighed and ground. A portion of the powder corresponding to 50 mg of PTU was dissolved in 100 ml of solvent and filtered through a 0.45-μ syringe filter. The first 10 ml of the filtrate was discarded. Then, 10 ml of the filtered solution was further diluted with solvent and injected into the liquid chromatographic system in triplicate for analysis of PTU content.

Extraction of Propylthiouracil from Creams

For extraction of PTU from creams, 0.5 g of each cream (placebo, 2.5%, 5.0%, and 10.0% w/w PTU) was accurately weighed into a 100.0-ml volumetric flask and dissolved in 100 ml of solvent by heating at 70°C in a water bath, followed by sonication. The solution was appropriately diluted, filtered through a 0.45-μ syringe filter, and injected into the liquid chromatographic system for analysis of PTU.

Assay Procedure and Calculations

A 20-μl quantity of the assay solution was injected into the liquid chromatographic system using the conditions described. For comparison, an identical volume of the standard solution was injected. Since the ratio of peak areas was related to the concentrations of the drug, the results were calculated using the following equation:

$$\text{Percentage of label claim found} = \left[\frac{(R_{\text{par}})_a}{(R_{\text{par}})_s}\right] \times 100$$

where $(R_{\text{par}})_a$ is the peak area of the drug, and $(R_{\text{par}})_s$ is the peak area of the standard solution.

RESULTS AND DISCUSSION

The developed HPLC assay method is accurate and precise with a percentage relative standard deviation (%RSD) of not more than 2.1% based on three readings (Table 1) for a concentration range of 0.25 to 25 μg/ml of PTU. Linearity was obtained with a correlation coefficient of not less than 0.999. The developed method proved to be specific since the peaks of the decomposition products (via oxidation and alkaline hydrolysis) were separate from the peak of PTU (Fig. 2). PTU was found to be stable to acid hydrolysis.

Under the developed HPLC conditions, the limit of quantitation was determined to be 0.06 μg/ml with a %RSD of not more than 1.3% for three successive injections of the sample. Also, the limit of detection was determined to be 0.05 μg/ml. The %RSD for the precision test for eight successive injections of a standard solution of 2 μg/ml was determined to be 0.811 (Table 2).

The results (Table 3) indicate that the developed method can be used to quantify PTU in tablets, as well as creams. The recovery of PTU from the synthetic mixtures was quantitative, and there was no interference from the excipients present in either dosage form (Fig. 3).

CONCLUSIONS

A simple, rapid, and stability-indicating HPLC method was developed and validated according to USP 24 guidelines (6) for the assay of PTU. The method is a new, easy, and reliable method that utilizes a cyano column as a stationary phase. Reported methods in the literature only use C8 and C18 columns. In addition, it is also the first method reported in the literature to quantify PTU in topical formulations. Excellent linearity was observed

Table 1

Linearity of Propylthiouracil (PTU) Response

Concentration (μg/ml)	Mean Peak Area for 3 Injections (Day 1)	%RSD	Mean Peak Area for 3 Injections (Day 2)	%RSD	Mean Peak Area for 3 Injections (Day 3)	%RSD
0.25	226335	2.1	229436	0.2	225432	1.4
0.5	468208	0.46	452130	0.34	447840	0.2
1.0	925138	0.42	926653	0.29	914169	0.23
2.0	1850861	0.32	1868646	0.08	1845605	0.4
5.0	4674102	0.23	4759767	0.31	4579173	0.25
10.0	9337658	0.01	9525572	0.19	9142025	0.18
25.0	23315504	0.3	23939040	0.17	22642645	1.3

RSD, relative standard deviation.

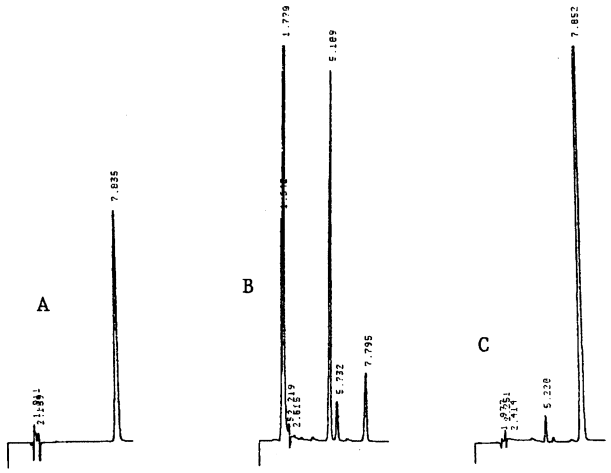

Figure 2. Sample chromatograms. Chromatogram A is from PTU standard solution; chromatogram B is from a solution subjected to oxidative degradation; chromatogram C is from a base-decomposed solution.

Table 2

Precision of Propylthiouracil (PTU) Assay for 2 μg/ml

Peak Area	Mean Peak Area	Standard Deviation	%RSD
1867187			
1880883			
1883874			
1885559	1889641	15333.2	0.811
1891144			
1920909			
1893214			
1894357			

RSD, relative standard deviation.

Table 3

Assay Results

Sample	Percentage of the Label Claim Fund	Inactive Ingredients
PTU Placebo Cream	0	Proprietary information
2.5% PTU Cream	98.20	
5.0% PTU Cream	99.14	
10.0% PTU Cream	98.00	
Propylthiouracil tablets, USP, 50 mg	103.60	Lactose, sodium lauryl sulfate, starch, stearic acid, talc

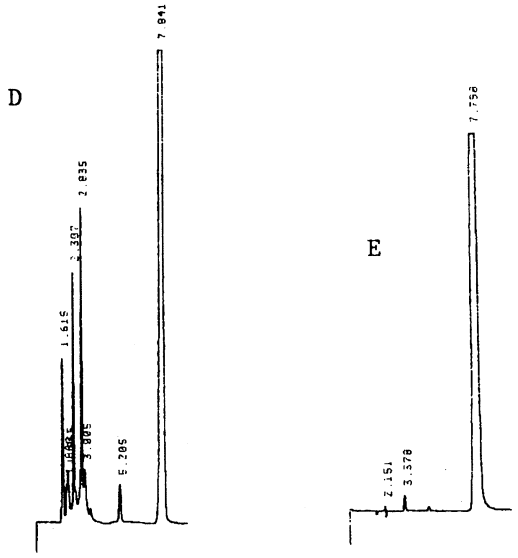

Figure 3. Sample chromatograms. Chromatogram D is from 2.5% PTU cream (Stiefel Research Laboratories); chromatogram E is from PTU tablets.

between PTU concentration and the peak area ($R^2 = 0.999$). The limit of detection was 1 ng, and the limit of quantitation was 1.2 ng. The method proved to be selective and stability indicating. The peaks of the degradation products, as well as those of the excipients in the dosage forms, did not interfere with the peak of PTU. The recovery of PTU from each dosage form (creams and tablets) was quantitative.

ACKNOWLEDGMENT

We wish to acknowledge Stiefel Research Laboratories for generously supplying the PTU creams and for their financial support.

REFERENCES

1. Banjamali, A. Thyroid Function and Thyroid Drugs. In *Principles of Medicinal Chemistry*, 4th Ed.; Foye, W.O., Lemke, T.L., Williams, D.A., Eds.; Williams and Wilkins: Media, PA, 1994; 696.
2. U.S. Pharmacopeial Convention. Antithyroid Agents: Propylthiouracil. In *Drug Information for the Health Care Professionals (USP DI)*, 20th Ed.; U.S. Pharmacopeial Convention, Inc.: Rockville, MD, 2000; Vol. 1, 465.
3. Elias, A.N.; Goodman, M.M.; Liem, W.H.; Barr, R.J. Propylthiouracil in Psoriasis: Results of an Open Trial. J. Am. Acad. Derm. **1993**, *29* (1), 78–81.
4. Elias, A.N.; Dangaran, K.; Barr, R.J.; Rohan, M.K.; Goodman, M.M. A Controlled Trial of Topical Propylthiouracil in the Treatment of Patients with Psoriasis. J. Am. Acad. Derm. **1994**, *31* (3), part 1, 455–458.
5. Chung, J.H.; Bang, H.D.; Moon, S.H.; Cho, K.H.; Kim, K.H.; Youn, J.I. Propylthiouracil Therapy Reduces the Clinical Severity of Atopic Dermatitis: Results of an Open Trial. Clin. Exp. Derm. **1998**, *23*, 290–299.
6. U.S. Pharmacopeial Convention. Validation of Compendial Methods. In *United States Pharmacopeia and National Formulary (USP 24/NF 19)*; U.S. Pharmacopeial Convention, Inc.: Rockville, MD, 2000; 2149–2151.