High-Performance Liquid Chromatographic Determination of Plasma Propylthiouracil

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Abstract □ A reversed-phase high-performance liquid chromatographic analysis was developed for propylthiouracil in plasma (1 ml). After protein precipitation with acetonitrile, the solution was diluted with water and injected into a liquid chromatograph equipped with C-18 and C-8 columns in series. The peak area was linear over the 0.25–10-mg/liter range, and the recovery was 101 ± 4.5%. This assay has the advantages of specificity, simplicity, and speed over previously published methods and requires smaller sample volumes. None of 19 drugs tested interfered with the assay.

Keyphrases □ Propylthiouracil—analysis, high-performance liquid chromatography in plasma, alcoholic liver disease □ Thyroid inhibitors—propylthiouracil, high-performance liquid chromatographic analysis in plasma, alcoholic liver disease □ High-performance liquid chromatography—analysis, propylthiouracil in plasma, alcoholic liver disease

Propylthiouracil has been used in the management of hyperthyroidism for many years. Attention has been given to its analysis in plasma and to its pharmacokinetics in normal subjects and in patients with thyroid dysfunction (1–11). The drug is presently being evaluated in the treatment of alcoholic liver disease (12) where its clearance and apparent distribution volume may be altered (13).

BACKGROUND

For pharmacokinetic studies, plasma propylthiouracil concentrations have been determined. Because of normal and disease-related interindividual variations in kinetics, a rapid, specific, and sensitive method for plasma analysis would help in determining dosage adjustments for the individual patient. Previously published analysis methods lack either specificity or simplicity and are not suitable for routine determinations in a heterogeneous patient population.

One hour after a single propylthiouracil dose (200–400 mg po), the peak plasma concentration is often 10 mg/liter. After distribution equilibrium has been achieved, the plasma concentration declines exponentially, with a half-life commonly in the range of 60 min (3, 5–11), although other apparent elimination rates have been reported (1, 4). First-order kinetics have been demonstrated over a wide range of doses (10). Five hours after drug administration, the plasma concentration is commonly 1 mg/liter. During chronic propylthiouracil administration (100 mg every 8 hr) to patients with usual propylthiouracil kinetics, the mean steady-state concentration may be calculated to be ~1 mg/liter (14). Therefore, the sensitivity limit of any propylthiouracil assay should be at or below 1 mg/liter as in the previously published methods (2, 6, 7).

Although the sensitivity of the other methods seems adequate to determine pharmacokinetics, the specificity of the total radioactivity method (1) and the colorimetric method (2) has been questioned (6, 7). In particular, the lack of analytical specificity may have been responsible, in part, for some of the unusually prolonged half-lives reported (4). A further small disadvantage of the colorimetric method is that 2 ml of serum is required.

Two specific methods for plasma propylthiouracil determination have been reported. A time-consuming and technically difficult GLC method (7) employs tetrapropylammonium hydroxide as an alkylating agent for propylthiouracil after an ether extraction. One milliliter of plasma is required for this method. An ion-exchange high-performance liquid chromatographic (HPLC) analysis measures propylthiouracil after a chloroform extraction (6). The serious disadvantages of this method are the excessive volume of plasma required (5 ml), the long propylthiouracil retention time (20 min), and the column temperature control required. Neither method is suitable for routine use.

The present paper reports a sensitive and specific HPLC assay for the rapid determination of propylthiouracil in plasma.

EXPERIMENTAL

Reagents—Propylthiouracil was obtained from propylthiouracil tablets[1] after recrystallization from ethanol. The purity was checked by melting point [221–222°, lit. (15) 219–221°] and by comparison of the HPLC retention time with that of propylthiouracil obtained from another source[2]. The chromatographic peak was identified by determination of its maximum UV absorption [275 nm, lit. (16) 275 nm in methanol]. Water was triple distilled in glass. Acetonitrile was HPLC grade[3]. Phosphoric acid and monobasic sodium phosphate were ACS grade[4].

Instrumentation—The high-performance liquid chromatograph[5] was equipped with a universal injector[6], a column inlet filter[7], a column guard[8], C-18[9] and C-8[10] reversed-phase columns in series, a variable-wavelength UV detector set at 275 nm (0–0.04 absorbance unit)[11], and a recording integrator (attenuation ×8)[12]. The mobile phase flow rate was 2.1 ml/min, and the pressure was 232 bar. Overnight, the UV detector was left on and the flow rate was reduced to 0.2 ml/min.

Solutions—The stock propylthiouracil solution was made in methanol. The mobile phase was made by dissolving 1.4 g of monobasic sodium phosphate in 1 liter of water (0.01 *M*), lowering the pH to 3.0 with 5 *N* phosphoric acid, and adding 450 ml of acetonitrile.

Sample Preparation—For standards, appropriate volumes of the propylthiouracil stock solution were added to glass tubes (13 × 100 mm), and the solvent was evaporated under a nitrogen stream. Patient plasma samples were frozen at −20° until analyzed. Plasma (1 ml) was added, and the tubes were vortexed for 1 min. Acetonitrile (2.5 ml) was added to precipitate proteins, and the mixture was vortexed for 1 min and centrifuged for 5 min at 2000 rpm[13]. To 1 ml of the supernate was added 2 ml of water, and 0.25 ml of this solution was analyzed on the chromatograph.

RESULTS AND DISCUSSION

HPLC has proven to be fast, accurate, and precise for determining drugs in biological fluids. This particular analytical system uses a variable-wavelength UV detector for increased sensitivity and specificity. Detection at 254 nm resulted in a 60% loss of sensitivity.

The standard curves obtained from measurement of the propylthiouracil peak area in spiked plasma samples were linear (r = 0.99) over 0.25–10 mg of propylthiouracil/liter. The minimum detectable concentration was ~0.1 μg/liter. The assay reproducibility is shown in Table I. Recovery was calculated in two ways: by reference to the linear regression equation (Table I) and by comparison with results obtained from 1-ml water samples (n = 8) containing 4 mg of propylthiouracil/liter. The latter method gave the recovery as 101 ± 4.5%. The daily variation in the standard curve slope was 5.2% (n = 6).

Although 1-ml plasma volumes were used for convenience, smaller sample sizes are clearly possible. In the sample preparation, a 10.5-fold dilution occurs and only 8% of the final volume is injected into the liquid chromatograph. Therefore, a simple scaledown would permit the analysis

[1] Propyl-thyracil, Charles E. Frosst and Co., Dorval, Quebec, Canada.
[2] Aldrich Chemical Co., Milwaukee, Wis.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] HP 1081A, Hewlett-Packard, Boblingen, West Germany.
[6] CV-6-UHPa-N60, Valco Instruments Co., Houston, Tex.
[7] 7302, Rheodyne, Berkeley, Calif.
[8] μBondapak C_{18}, 400 mg, Waters Associates, Mississauga, Ontario, Canada.
[9] μBondapak C_{18}, 3.9 mm i.d. × 30 cm, Waters Associates.
[10] RP-8, Brownlee, 4.6 mm i.d. × 25 cm, Santa Clara, Calif.
[11] SF 700, Schoeffel, Westwood, N.J.
[12] HP 3380A, Hewlett-Packard, Avondale, Pa.
[13] Sorvall, GLC-2B, Du Pont Instruments, Newton, Conn.

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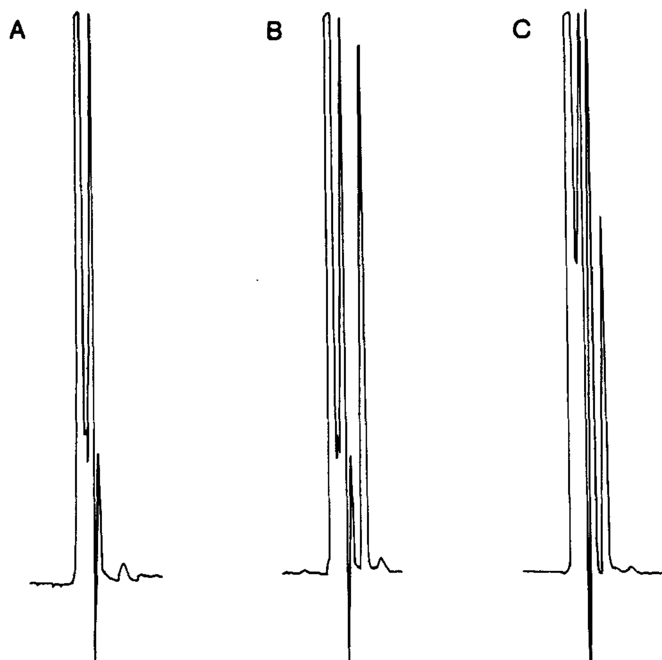

Figure 1—*Chromatograms of normal blank plasma (A), spiked normal plasma containing propylthiouracil (4 mg/liter) (B), and plasma from a patient with alcoholic liver disease, taking propylthiouracil, 150 mg twice a day, 3.67 hr after the last dose (C). The propylthiouracil retention time was 4.5 min.*

of 0.1-ml plasma samples. In addition, the good peak shape, peak separation, and noise level may allow a further reduction in sample size, which may be useful in pediatrics and in studies with small animals.

Propylthiouracil metabolism in humans is virtually unknown, although Kampmann (10) reported that most propylthiouracil in the urine is present as the glucuronide. The parent compound and this conjugate would not be expected to have similar retention times under the present chromatographic conditions. Interference tests with 19 commonly used drugs at concentrations often encountered during therapy (17) were negative. The drugs tested and the concentrations (milligrams per liter) were: amphetamine, 0.1; acetaminophen, 10.0; amitriptyline, 0.05; amobarbital, 0.1; chlordiazepoxide, 1.0; codeine, 0.1; *N*-desmethyldiazepam, 0.3; diazepam, 0.2; phenytoin, 10.0; flurazepam, 0.05; meperidine, 0.5; methadone, 0.3; methamphetamine, 0.1; morphine, 0.1; phenobarbital, 0.1; propranolol, 0.05; quinine, 0.1; sodium salicylate, 200.0; and warfarin, 1.0.

To test the method, two plasma samples (3 ml) were obtained from each of two patients with alcoholic liver disease who had been taking propylthiouracil, 150 mg twice a day, for >3 weeks. The sampling times, relative to the last dose, and the plasma concentrations were: Patient 1, 3.67 hr, 2.66 mg/liter, and 5.1 hr, 0.79 mg/liter; and Patient 2, 3.08 hr, 1.56 mg/liter, and 5.17 hr, 0.76 mg/liter. Two-point estimates of the apparent elimination half-lives are: Patient 1, 0.80 hr; and Patient 2, 2.0 hr.

The analysis of propylthiouracil in plasma from patients with alcoholic liver disease showed no serious interference from other compounds in plasma, although the peak separation was not as good as with plasma from normal subjects (Fig. 1). This finding shows the advantage of a two-column system for studies in a heterogeneous patient population. The estimated propylthiouracil apparent elimination half-lives of both patients were in the normal range. This result was supported by the fact that there was no unusual propylthiouracil accumulation in these patients during therapy. A more complete description of propylthiouracil kinetics in patients with alcoholic liver disease has not been reported but would be useful.

Table I—Propylthiouracil Assay Reproducibility Using Spiked Human Plasma (1 ml)

Amount Added, mg/liter[a]	Amount Recovered, mg/liter	Percent Recovered	*CV*, %
0	0.00		
0.25	0.26	103	5.4
0.5	0.47	94	3.7
1	0.99	99	4.7
2	2.02	101	2.7
4	3.89	97	4.5
6	6.17	103	5.7
8	7.70	96	3.3
10	10.2	102	3.6

[a] Eight samples were determined in each case.

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