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High-performance liquid chromatographic determination of propylthiouracil in plasma

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SUMMARY

A high-performance liquid chromatographic method for the determination of the antithyroid drug propylthiouracil in dog plasma has been developed. Propylthiouracil and the internal standard, methylthiouracil, are extracted from plasma with methylene chloride at pH 6 and the organic layer is evaporated to dryness. The residue is chromatographed on a Chromspher C_{18} reversed-phase column using Pic B-7 (0.005 *M* 1-heptanesulphonic acid in water)–1% acetic acid–methanol (40:45:15, v/v/v) as the mobile phase. Quantification is achieved by monitoring the UV absorbance at 300 nm. The response is linear (0.1–15 μg/ml) and using 100 μl of plasma the detection limit is 50 ng/ml. The within-run coefficient of variation is $\leqslant 5\%$ and the accuracy is within 10% of the theoretical value at concentrations between 0.1 and 15 μg/ml plasma.

INTRODUCTION

Propylthiouracil (PTU) (Fig. 1) is an antithyroid drug. In humans less than 10% of the drug is excreted unchanged in the urine, and about 60% is excreted as a glucuronic acid conjugate of PTU (for references, see ref. 1).

Serum or plasma concentrations of propylthiouracil were first measured by Ratliff *et al.*[2] by spectrophotometry, which lacks specificity and sensitivity. This problem was solved by the development of a gas chromatographic (GC) method[3] using alkylation of propylthiouracil. Several high-performance liquid chromatographic (HPLC) methods were later developed, using an ion-exchange column with UV detection[4] or a reversed-phase column with UV detection, without or with addition of an internal standard after extraction[5,6]. McArthur and Miceli[7] used reversed-phase chromatography with UV detection and an internal standard incorporated in the extraction solvent. Another reversed-phase HPLC method[8] requires a lengthy deproteinization step for the determination of blood concentrations of PTU. A sensitive radioimmunoassay has also been published[9].

We have developed a sensitive HPLC method using ion-pair reversed-phase chromatography with UV detection at 300 nm. A structurally related compound,

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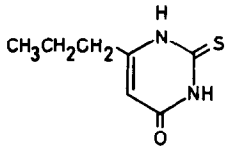
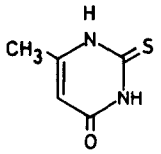


PROPYLTHIOURACIL



METHYLTHIOURACIL
(internal standard)

Fig. 1. Structures of propylthiouracil and the internal standard methylthiouracil.

methylthiouracil (Fig. 1) is used as an internal standard. The method was applied to dog plasma, as the dog is a suitable model for propylthiouracil disposition in man[10].

EXPERIMENTAL

Reagents and standard solutions

Propylthiouracil and the internal standard, methylthiouracil, were kindly supplied by Sanders Pharma (Brussels, Belgium). Methanol (HPLC grade) was obtained from Alltech Europe (Eke, Belgium) and Pic B-7 from Waters Assoc. (Milford, MA, U.S.A.). All other reagents were of analytical-reagent grade from Merck (Darmstadt, F.R.G.). Water doubly distilled in glass was passed through a 0.45-μm filter (Type HA, Millipore, Bedford, MA, U.S.A.).

Stock solutions of propylthiouracil (5 mg/ml) and methylthiouracil (5 mg/ml) were prepared by dissolving accurately weighed samples in methanol. These solutions were stable for at least 1 month when stored at 4°C. Working standard solutions of propylthiouracil and the working solution of methylthiouracil were made by diluting the stock solutions with water.

Analytical procedure

A 100-μl plasma sample was pipetted into a 7-ml brown, glass-stoppered, silanized centrifuge tube and adjusted to pH 6 with 0.1 *M* hydrochloric acid. To this were added 25 μl of internal standard solution (10 μg/ml in water) and 3 ml of methylene chloride. The mixture was shaken horizontally for 10 min, followed by centrifugation at 3015 *g* for 5 min. The organic phase was transferred into a 6-ml brown, silanized conical tube, evaporated to dryness under nitrogen and reconstituted in 50 μl of water in the dark. An aliquot (20 μl) of this solution was injected onto the column.

Chromatography

Measurements were made using a Spectra-Physics 8700 high-performance liquid chromatograph equipped with a Perkin-Elmer LC 235 diode-array detector. A

Chromspher C_{18} reversed-phase column (250 × 4.6 mm I.D.) (Chrompack, Antwerp, Belgium) was maintained at 40°C.

The mobile phase was Pic B-7 (0.005 *M* 1-heptanesulphonic acid in water)–1% acetic acid–methanol (40:45:15, v/v/v). The flow-rate of the helium-degassed mobile phase was 1 ml/min. The column temperature was 45°C. The samples were injected into the chromatograph via a Rheodyne (Berkeley, CA, U.S.A.) Model 7125 injector equipped with a 20-μl loop. Chromatographic separation was monitored by UV detection at 300 nm. Peak heights were measured with an HP 3390 A integrator.

Calibration graphs

Standards for the calibration graph were prepared by adding known amounts of propylthiouracil to blank dog plasma to provide concentrations from 0.1 to 15 μg/ml. Calibration graphs were obtained by plotting the peak-height ratio of propylthiouracil to the internal standard (y) against the concentration (x) of propylthiouracil in plasma and calculating the regression line by least-squares linear regression analysis.

Stability of propylthiouracil

The stability of propylthiouracil in dog plasma (5 μg/ml) was studied for various storage times at −20 and −80°C.

RESULTS AND DISCUSSION

Extraction

Using methylene chloride, the recovery of propylthiouracil from plasma was highest at pH 6. The extraction yield (mean ± S.D.) was 77.3 ± 4.6, 80.0 ± 1.8 and 81.6 ± 2.3% for propylthiouracil at concentrations of 0.1, 5 and 15 μg/ml, respectively (n = 5 for each concentration), and 52.1 ± 3.2% for methylthiouracil at a concentration of 2.5 μg/ml (n = 5).

Chromatography

Propylthiouracil has a UV absorbance peak maximum at 275 nm. However, at this wavelength there is a peak of an endogenous substance which nearly coelutes with the PTU peak and with a UV absorbance peak maximum at about the same wavelength (272 nm). This peak was also observed with blank plasma, but it was absent if water was used instead of plasma. Therefore, a detection wavelength of 300 nm was selected, which resulted in a diminished absolute sensitivity for PTU, but the interference was avoided.

Fig. 2 shows sample chromatograms of extracts of (A) blank dog plasma, (B) blank dog plasma spiked with PTU and methylthiouracil and (C) a plasma sample from a dog that had received a 300-mg oral dose of PTU. Under the chromatographic conditions used, the retention times for the internal standard and PTU were 3.9 and 10.8 min, respectively.

The limit of detection was 50 ng/ml, with a signal-to-noise ratio of 3:1, using 100 μl of plasma. This detection limit is comparable to that of the micro-HPLC method[7] and is better than that of the method of Kim[8]. Other HPLC methods[4–6] require relatively large volumes of plasma (1–3 ml).

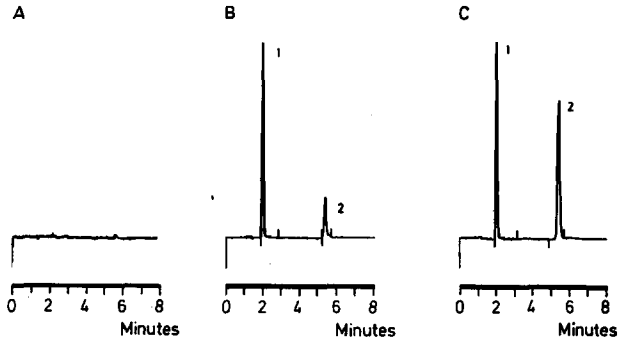


Fig. 2. Representative HPLC traces for extracts of 100 μl of dog plasma. (A) Blank plasma; (B) blank plasma spiked with 1 = internal standard (2.5 μg/ml) and 2 = PTU (0.5 μg/ml); (C) plasma from a dog 8 h after on oral dose of 300 mg of PTU: 1 = internal standard (2.5 μg/ml); 2 = PTU (1.20 μg/ml).

Calibration graphs

The calibration graphs for PTU showed a linear response over the range evaluated (0.1–15 μg/ml). A typical calibration graph gives a regression of $y = -0.0028 + 0.3145x$, $r = 0.9993$, where y = peak-height ratio, x = concentration of propylthiouracil and r = correlation coefficient.

Within-run precision and accuracy

The within-run precision and accuracy were evaluated from the analyses of five plasma samples for each of five PTU concentrations (range 0.1–15 μg/ml). The within-run coefficient of variation (C.V.) was $\leqslant 5\%$. The accuracy was within 10% of the theoretical value at each concentration (Table I).

Stability

Propylthiouracil was observed to be stable for at least 14 days at −20°C and for at least 1 month at −80°C (stability is defined as $\geqslant 95\%$ of the initial amount remaining).

In conclusion, the proposed HPLC method is sufficiently sensitive and selective for monitoring unchanged propylthiouracil in pharmacokinetic studies.

TABLE I

WITHIN-RUN PRECISION AND ACCURACY FOR THE DETERMINATION OF PROPYLTHIOURACIL IN PLASMA (n = 5)

Concentration added (μg/ml)	*Precision (C.V.) (%)*	*Accuracy (%)*
0.1	2.5	110.0
0.5	3.7	90.8
1.0	2.3	110.5
5.0	3.5	100.6
15.0	2.6	98.1

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