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Note

Simple and sensitive method for the determination of propylthiouracil in blood by high-performance liquid chromatography

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Propylthiouracil (PTU) has been used in the treatment of hyperthyroidism and liver disease. Despite widespread use of PTU, pharmacokinetic studies in man and laboratory animals have been hampered by the lack of a specific method of sufficient sensitivity to determine PTU in biological fluids. The colorimetric method [1] lacks specificity and sensitivity. The use of radioactive isotopes [2, 3] and a method of high-performance ion-exchange chromatography [4] are not feasible for extensive clinical studies. A gas chromatographic technique [5] which involves the conversion of PTU to its salt form with tetrapropylammonium hydroxide is time consuming and technically difficult. A recent method using high-performance liquid chromatography (HPLC) [6] offers specificity and high sensitivity, but did not include an internal standard.

A simple and sensitive method for the determination of PTU in blood using HPLC is described, along with a basic pharmacokinetic analysis after a single intraperitoneal administration to rats. The blood samples were deproteinized with phosphotungstic acid (PTA) reagent and the clear supernatant obtained after centrifugation was directly analysed in the HPLC system.

EXPERIMENTAL

Materials

PTU (H3 420-3, Aldrich, Milwaukee, WI, U.S.A.), 4-hydroxy-6-methyl-2-thiopyrimidine (HMTP, H-2502, Sigma, St. Louis, MO, U.S.A.), HPLC grade acetonitrile (A-998, Fisher, Fair Lawn, NJ, U.S.A.), potassium monobasic phosphate (P-284, Fisher) and PTA reagent (RO-1196, BDH, Toronto, Canada) were used. Water was deionized and then triple distilled.

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Apparatus

HPLC determinations were performed with a Beckman Model 330 isocratic liquid chromatograph, A Model 110A pump, a Model 160 ultraviolet detector operating at 280 nm with the absorbance detector sensitivity set at 0.005 a.u.f.s., and a Hewlett-Packard 3390A recording integrator. Separation of PTU was performed on a 250 mm × 4.6 mm I.D. Altex Ultrasphere ODS column (C_{18} reversed phase, particle size 5 μm) with isocratic elution. A mixture of acetonitrile and 0.025 *M* potassium monobasic phosphate in water, pH 4.6 (20:80, v/v) was used as a mobile phase. The phosphate buffer was filtered through a 0.45-μm filter (Millipore, Bedford, MA, U.S.A.) prior to mixing. A flow-rate of 2.0 ml/min (17 MPa) at ambient temperature was employed in the present study.

Preparation of standard solutions

Standard solutions of PTU (0.1 mg/ml) and HMTP (0.1 mg/ml) were prepared in water in volumetric flasks by immersing them in a hot water bath and stored subsequently at 4°C. the appropriate concentrations of standard solutions were prepared by diluting the stock solutions with water.

Analytical procedure

A series of spiked rat blood samples (0.4 ml) in polypropylene tubes was prepared by mixing 0.1 ml blood with 0.3 ml water containing varying amounts of PTU, from 0.031 to 4.0 μg (corresponding to 0.31—40.0 μg/ml blood), and 1.0 μg HMTP as an internal standard. A spiked human blood sample containing 0.063 μg PTU (corresponding to 0.63 μg/ml blood) and 1.0 μg HMTP was prepared as above. The samples were deproteinized with 25 μl PTA reagent for 90 min at room temperature and then centrifuged for 15 min at 31,550 *g*. A 20-μl aliquot of the clear supernatant obtained (pH 4.4) was injected into the HPLC system with a 50-μl Hamilton syringe. The same procedure was followed using varying amounts of PTU in 0.425 ml water with no blood and PTA reagent.

Standard curve

Known amounts of PTU (0.031—4.0 μg) in 0.4-ml aliquots of the spiked blood samples were taken through the entire procedure, HMTP (1.0 μg) being added to each sample as an internal standard. An identical set of PTU samples was made up in 0.425 ml water with no blood or PTA reagent. To construct the standard curve, the PTU/HMTP response ratios were plotted against the concentrations of PTU in μg/ml. Actual amounts of PTU injected into the HPLC system were 1.5—188.2 ng, while the amount of HMTP was 47.1 ng.

Animal study

To study the suitability and applicability of the present method to animal studies, male rats weighing 430—470 g (CBL, Montreal, Canada) were administered intraperitoneally (i.p.) with 20 mg/kg PTU (in 2.0 ml saline per 100 g body weight, pH 6.7). Rats were housed under constant environmental conditions and food was removed 24 h prior to the administration of PTU. Blood samples (0.1 ml) from the tail vein were collected into polypropylene

tubes containing 0.3 ml water at various time intervals after administration. The samples were analysed as described above for the spiked samples.

RESULTS AND DISCUSSION

Clearly separated peaks representing PTU and its internal standard, HMTP, were seen in water and rat blood (Fig. 1). The chromatogram obtained with a human blood sample showed the same result. No interfering endogenous compounds were found on the chromatograms obtained with the blank rat or human blood. Under the present experimental conditions, the elution volumes for PTU (retention time, 3.6 min) and the internal standard HMTP (retention time, 1.9 min) were 7.2 and 3.8 ml, respectively. Maximum absorbance of both PTU and HMTP were found to occur at 275 nm, and in all subsequent studies these were analysed at 280 nm.

The linearity of the concentration and response relation was established over the range of 0.31—40.0 μg/ml blood (r = 0.9998; slope, 0.050; y-intercept, 0.0028). Analytical recovery of PTU (0.031—4.0 μg) added to the spiked blood samples calculated for peak areas by comparison with results obtained with water samples was 74 ± 3% ($\bar{x}$ ± S.D.). The mean between-run coefficient of variation recovered from blood samples was 3.5% over the range of 0.31—40.0 μg/ml (in each concentration, n = 6), whereas the within-run coefficient of variation at 0.31 μg/ml (n = 6) was 5.6%.

The in vivo study illustrates that the method is suitable for the pharmacokinetic study of PTU following administration of a single dose (Fig. 2). The blood level of PTU was measurable (0.2 ± 0.04 μg/ml, $\bar{x}$ ± S.E.M., n = 5) within 1 min after i.p. administration. The peak level of 27.8 ± 0.3 μg/ml occurred at 90 min. The blood PTU appeared to have a $t_{1/2}$ of about 6 h.

The presence of PTA reagent in samples of blood and water was found to interfere with the maximum absorption of PTU and HMTP at 280 nm. Under the present conditions, the values obtained for peak areas of PTU and HMTP

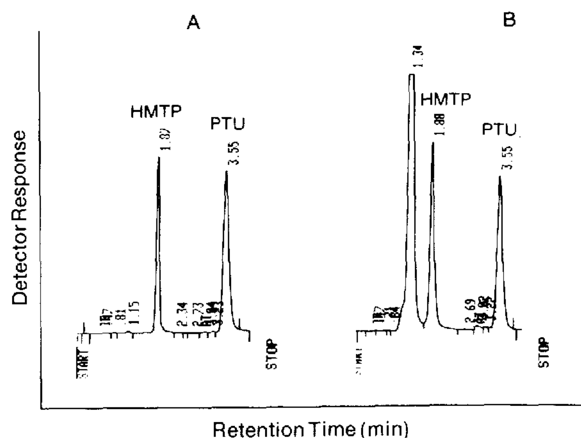

Fig. 1. Isocratic separation of PTU and its internal standard, HMTP. (A) Chromatogram of PTU and HMTP standards in water. (B) Chromatogram from rat blood sample showing peaks of PTU (11.8 ng) and of internal standard, HMTP (47.1 ng).

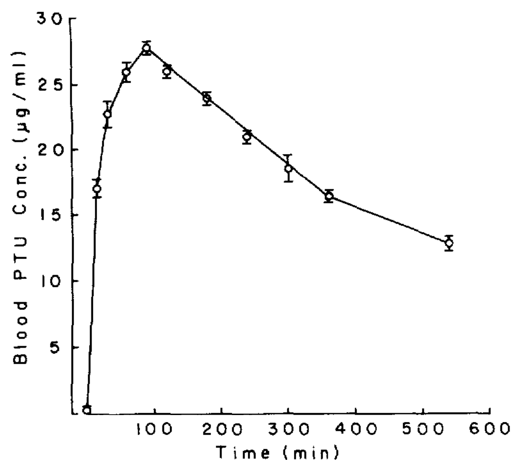

Fig. 2. The concentration of PTU in blood of rats after i.p. administration of 20 mg/kg. Results are the means ± S.E.M. for five rats at each time interval.

from blood samples with PTA reagent were 26% and 8% lower than those obtained with water samples with no PTA reagent. However, the ratios of peak area (PTU/HMTP) remained constant, although 25% lower than the absolute values. Concentrated PTA reagent (25 µl) added to water samples containing PTU and HMTP obscured the PTU and HMTP peaks, and addition of PTA reagent diluted 1:10 to water samples resulted in a significant decrease but detectable response for PTU (60% or higher). The amount of PTA reagent used in the present study was sufficient to deproteinize blood samples, but less than 25 µl (10–20 µl) did not produce complete deproteinization. Addition of PTU before or after the deproteinization of blood samples with PTA reagent showed the same quantitative results which indicated that there appeared to be no protein-binding interactions. Storage of blood samples at 4°C for up to two months did not alter the quantitation of PTU.

The present method is simple and sensitive, and comparable to the method of Giles et al. [6]. However, inclusion of HMTP as an internal standard for the determination of PTU improved the quantitation, i.e., an average drop of the between-run coefficient of variation from 4.2 to 3.5%. Use of PTA reagent for the deproteinization of blood further simplified the sample preparation. In the present study, no extraction, purification and concentration procedures of samples before the HPLC system were found to be necessary.

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