Journal of Chromatography, 278 (1983) 464—468
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1864

Note

Micro high-performance liquid chromatographic procedure for the quantitation of serum propylthiouracil

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(Received June 8th, 1983)

Propylthiouracil (PTU) has been used for many years in the treatment of hyperthyroidism (Graves' disease). The drug is thought to prevent synthesis of thyroid hormones by disruption of thyroid peroxidase catalyzed reactions [1- 3].

Existing methodologies for measuring serum levels of PTU have a number of disadvantages. Colorimetric determinations generally require excessive volumes of blood to achieve adequate sensitivity [4] and lack specificity in differentiating between the parent drug and metabolites [5, 6]. More recent methods have employed high-performance liquid chromatography (HPLC). These methods [7, 8] appear to have the necessary sensitivity and selectivity for determining PTU; however, they still require relatively large sample volumes for analysis. In addition, neither method incorporates an internal standard throughout the entire procedure. We report here an HPLC method that requires only 100 μl of serum, making it particularly well suited for pediatric patients and routine therapeutic drug monitoring. Quantitation is based on reference to an internal standard incorporated in the extraction solvent. The method is fast, simple, and reproducible.

MATERIALS AND METHODS

Chromatography

All assays were performed on a Perkin-Elmer Series 2 liquid chromatograph equipped with an LC-75 UV/VIS variable-wavelength detector and interfaced with a Sigma 10 data system that electronically integrated the peak areas (all

0378-4347/83/$03.00

from Perkin-Elmer, Norwalk, CT, U.S.A.). A Perkin-Elmer 10 μm HC ODS/SIL-X reversed-phase column was used for the chromatography. The oven temperature was maintained at 40°C, the flow-rate was 1.6 ml/min, and the detection wavelength was 272 nm.

Reagents

PTU was obtained from Sigma (St. Louis, MO, U.S.A.). 8-Chlorotheophylline (8-CT), used as the internal standard, was obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.). The mobile phase consisted of 3% acetonitrile in a mixture of 100 m*M* monosodium phosphate and 10 m*M* pentanesulphonic acid (Eastman Kodak, Rochester, NY, U.S.A.). The solution was thoroughly mixed and degassed prior to use. All other solutions and reagents were HPLC or analytical grade.

Stock standards of PTU and 8-CT were individually prepared in methanol to yield a final concentration of 1.0 g/l. Working PTU standards were prepared in drug-free serum with appropriate dilutions from the stock solution to yield concentrations ranging from 0.1 to 10.0 mg/l. Chloroform—isopropanol (1:1), containing 0.4 mg/l of the internal standard (8-CT), was used as the extraction solvent.

Procedure

A 100-μl volume of standard, control or patient serum was placed in a 1.5-ml eppendorf centrifuge tube. Then 1 ml of extraction solvent was added. The tubes were vortexed for 1 min and then centrifuged for 5 min in a Brinkman table-top microcentrifuge. The organic phase was transferred to a 10 × 13 mm disposable glass test tube and evaporated to dryness at 40°C under nitrogen. Each dried sample was immediately reconstituted with 50 μl of distilled water. Since PTU is photosensitive, samples were stored in the dark to prevent decomposition prior to injection (10 μl).

RESULTS AND DISCUSSION

Under these conditions, PTU and 8-CT had retention times of 4.75 and 7.20 min, respectively. A typical chromatogram obtained from the analysis of a serum blank containing the internal standard is shown in Fig. 1A. Fig. 1B illustrates a chromatogram obtained from drug-free serum to which 1.0 mg/l PTU was added, and Fig. 1C is a chromatogram obtained from a patient sample in which the determined concentration of PTU was 2.0 mg/l.

PTU was added to drug-free serum to yield concentrations of 0.1 to 10 mg/l. Concentrations and peak areas were linearly related over this range as shown in Fig. 2. Recovery studies were carried out by preparing identical PTU concentrations in serum and distilled water, and then comparing the peak areas calculated during analysis. The results indicated virtually 100% recovery of PTU from serum.

Within-run precision was evaluated by processing ten aliquots of a prepared standard serum pool containing 2.5 mg/l PTU. The mean ± standard deviation was 2.50 ± 0.09 mg/l, with a coefficient of variation of 3.6%. The stability of samples kept frozen at −20°C was evaluated by analyzing aliquots of prepared

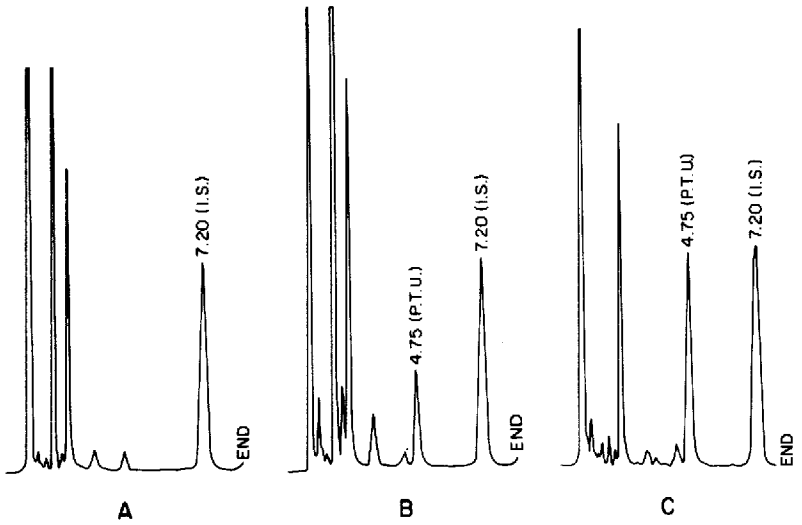

Fig. 1. Typical chromatograms of (A) blank serum containing internal standard, (B) drug-free serum reconstituted with 1.0 mg/l PTU, (C) patient's serum determined as 2.0 mg/l PTU. Retention times: PTU 4.75 min; internal standard (8-CT) 7.20 min. The peaks eluting prior to PTU are unidentified serum extractants.

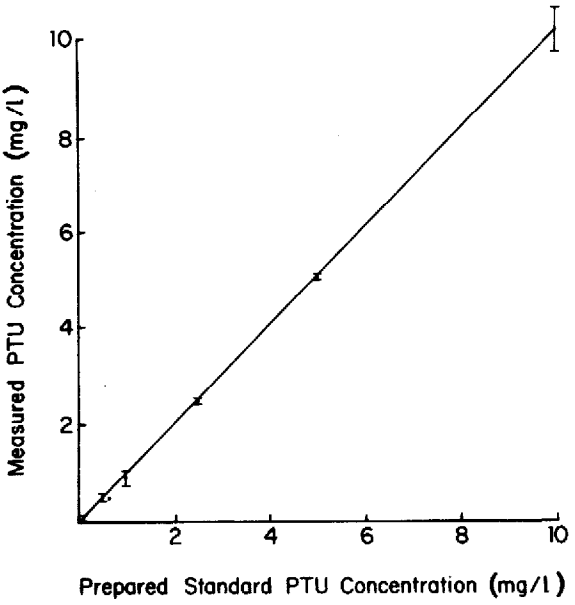

Fig. 2. PTU linearity. Mean (•) and range at each concentration (n = 5).

serum containing known concentrations of PTU. These samples were found to be stable for a period of at least eight weeks (Table I). Six randomly chosen patient samples were also re-analyzed six months later. The results indicated no loss of determined PTU (Table II).

Previously published HPLC methods [5, 7—9] have one or more of the following disadvantages. Three [5, 7, 8] require relatively large (1—5 ml)

TABLE I

STABILITY OF FROZEN PTU SERUM SAMPLES*

Week	Prepared concentrations	
	1 mg/l	5 mg/l
0	0.90	4.80
1	0.88	4.90
2	1.05	4.80
4	0.88	5.14
8	1.20	4.91
Mean ± S.D.	0.98 ± 0.14	4.91 ± 0.14

*All samples frozen at −20° C.

TABLE II

SIX-MONTH COMPARISON OF FROZEN PATIENT SAMPLES*

Patient No.	Determined concentration (mg/l)	
	Initial analysis	Re-analysis
1	1.00	1.24
2	2.40	2.78
3	0.66	0.82
4	0.70	0.71
5	2.87	2.80
6	1.04	0.95

*All samples frozen at −20° C.

volumes of serum or plasma. In addition, all of these procedures require extensive sample extraction, preparation or protein precipitation. Our method requires approximately 7 min for sample preparation, including 5 min for centrifugation. Two methods [5, 8] used chloroform as the extractant, although PTU is practically insoluble in chloroform. An internal standard was not incorporated in two methods [5, 7] and one [8] incorporated an internal standard after extraction, which may result in dilutional error. Two methods [7, 9] monitored PTU at 254 nm and 280 nm, respectively. PTU exhibits its maximum absorption at 272 nm. At this wavelength, the absorbance is more than twice that observed at 254 nm and greater than that observed at 280 nm.

A procedure for PTU determination utilizing a radioimmunoassay technique [10] requires extensive sample pretreatment, is liable to specimen deterioration, and necessitates overnight incubation. Sample deterioration is not a problem with our procedure, and unlike a recent HPLC method [9] does not require a lengthy deproteinization step.

Theophylline, acetaminophen and salicylate, drugs which might be concurrently administered to children for other purposes, were found not to interfere with the quantification of PTU. The method presented here provides

an accurate, simple and fast means of quantifying serum levels of PTU. The small volume of serum required makes this methodology particularly well suited for pediatric patients and routine therapeutic monitoring in those facilities equipped with HPLC.

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