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Simple and Rapid Method for the Determination of Propylthiouracil in Rabbit Serum by High Performance Liquid Chromatography

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Abstract

A simple, rapid method using an isocratic high performance liquid chromatography (HPLC) was developed and validated for the assay of propylthiouracil (PTU) in the serum of rabbits. Excellent linearity was observed between the PTU concentration and the peak area up to a concentration of 40μg/ml. Serum samples containing PTU were extracted with methanol to precipitate serum proteins. The absolute recovery ranged from 96.6 to 104.4%. The intra-day and inter-day accuracies ranged from 99.8 – 106.2% and from 95.3 – 103.9% respectively, at three different concentrations. The method will be used in the determination of the pharmacokinetic parameters of PTU after oral administration of microspheres containing PTU

Keywords: PTU, HPLC, rabbit serum

Introduction

6-n-propyl-2-thiouracil (propylthiouracil) is a potent inhibitor of thyroid peroxidase enzyme responsible for iodination of tyrosine residues of thyroglobulin and the coupling of iodotyrosine residues to form iodothyronine, so it has been used in the treatment of hyperthyrodism (Abdul-Fattah and Bhargava, 2001). Propylthiouracil has a half-life of 40-120 minutes and usually is administered three times daily in doses of 100 mg for the treatment of hyperthyroidism (Kampmann and Hansen, 1981).

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Various techniques have been applied for the determination of PTU in serum or plasma. These techniques include gas chromatography (Kampmann and Hansen, 1981, Zhang et al., 2005), radioimmunoassay (Cooper *et al.*, 1981), high performance liquid chromatography (HPLC) (Duarte *et al.*, 2000; Cannell *et al.*, 1991; Kim, 1983; Kenneth, 2005) and micro-HPLC (Mcarthur and Miceli, 1983).

In this study a simple methodology that allows the rapid, precise and accurate determination of PTU in rabbit serum is described. We present here an optimization of high performance liquid chromatography for determination of PTU levels in serum of rabbits. The mobile phase and the extraction solvent were modified leading to reduction in the PTU retention time when compared to data from literature (Giles *et al.*, 1979; Cannell *et al.*, 1991).

The method will be used in the determination of the pharmacokinetic parameters of PTU after oral administration of microspheres containing PTU (Obeidat and Price, 2005).

Materials and Methods

Materials: 6-n-propyl-2-thiouracil (lot. 11k2503, Sigma-Aldrich Co.), HPLC grade cetonitrile (AC0340, Scharlau Chemie, Spain), HPLC grade methanol (ME0310, Scharlau Chemie, Spain), potassium phosphate monobasic (PO0260, Scharlau Chemie, Spain). Water was deionized and triple distilled.

Preparation of standard solutions: Standard solution of PTU (0.5 mg/ml) was prepared by dissolving an accurately weighed amount of 50 mg of PTU in 5 ml of methanol, sonicated for 15 minutes and the volume was adjusted to 100 ml with filtered deionized distilled water. The standard solution was stored subsequently at 4 °C. The appropriate concentrations of standard solution were prepared by diluting the stock solution with water. The mean peak areas of all tested concentrations were used to construct a standard calibration curve.

Treatment of serum preparation: Blood samples of adult male Rabbits (3 kg ± 0.3 kg and supplied by the animal center of Jordan University of Science and Technology) were taken and left to clot at room temperature for 40 minutes. Serum was separated by centrifugation and kept at -70°C until analysis. A series of rabbit blood (0.4 ml) in polypropylene tubes was prepared by mixing 0.2 ml of the blood with 0.2 ml of water containing varying amounts

of PTU, ranging from 0.025 to 4.0 μg (corresponding to 0.25 – 40 μg/ml). The samples were deproteinized with 50 μl of methanol which also dissolved PTU that was bound to those proteins. The serum was then centrifuged for 15 minutes at 4000 rpm. Finally, the supernatant was injected into the HPLC column.

HPLC instrumentation and conditions: The HPLC system consisted of a pump and UV-VIS detector that is connected to a personal computer and a system controller (all Shimadzu Co., Japan). The column used was a reverse phase C18, 5 μm (Lichrocart 125-4).

The mobile phase consisted of a mixture of methanol and 0.025 M KH_2PO_4 at a ratio of 80:20 respectively. The mobile phase was degassed by passing through a 0.22 μm membrane filter (Millipore, Bedford, MA, USA) prior to use. The mobile phase was pumped isocratically at a flow rate of 1 ml/min. The injector was filled with an injector loop of 20 μl. The detection wavelength was 254 nm.

Results and Discussion

Development conditions for rapid extraction of PTU from rabbit serum: The extraction procedure developed for PTU from rabbit serum allowed samples to be available for HPLC analysis in approximately 10 minutes. Conditions for simple and rapid HPLC separation with UV detection were developed using an isocratic elution with a mobile phase composed of methanol and 0.025 M KH_2PO_4 at a ratio of 80:20. These conditions gave a well defined, sharp peak of PTU with a retention time of approximately 3 minutes. Under these conditions an amount of PTU as low as 0.025μg/ml could be detected. With these retention times, analysis could be completed in about 15 minutes.

Method validation

Linearity: The quantification of the chromatogram was performed using the peak area of PTU. Six standard solutions were prepared (0.25 μg/ml, 0.5 μg/ml, 2 μg/ml, 5 μg/ml, 12.5 μg/ml and 40 μg/ml) and subjected to triplicate analyses by HPLC. The peak area was determined and plotted versus the concentration of PTU. Statistical analysis using least square regression analysis indicated excellent linearity for PTU with the concentration range studied as shown in Table 1. In constructing the standard curve, samples of PTU in rabbit serum identical to those in the standard solutions were prepared and the PTU response ratios were plotted against the concentrations of PTU in μg/ml as shown in figure 1. The linearity

of the concentration and response relation was established over the range of 0.25 – 40 µg/ml serum (R^2 = 0.9994). Figure 2 shows the HPLC chromatograms of pure drug.

(PTU), drug-free rabbit serum and of standard serum sample containing the drug at a concentration of 12.5 µg/ml.

Table 1. Statistical analysis of lineer regression of PTU

Concentration µg/ml	Peak Area				
	1[a]	2[a]	3[a]	4[a]	5[a]
0.25	23,022	23,562	22,790	23,390	21,722
0.5	45,545	45,422	45,123	46, 423	47, 643
2	192,225	191,973	191,883	193,472	197,364
5	483, 457	483,863	483,492	484,986	473,223
12.5	1127,637	1127,899	1126,997	1130,122	1111,338
40	3887, 661	3886, 956	3887, 515	3912, 215	3856, 015
Slope	97179	97160	97179	97784	96339
Intercept	7790.1	7561.8	7956	8558	7924.2
R^2- value	0.9999	0.9999	0.9998	0.9998	0.9998

[a]Run No.

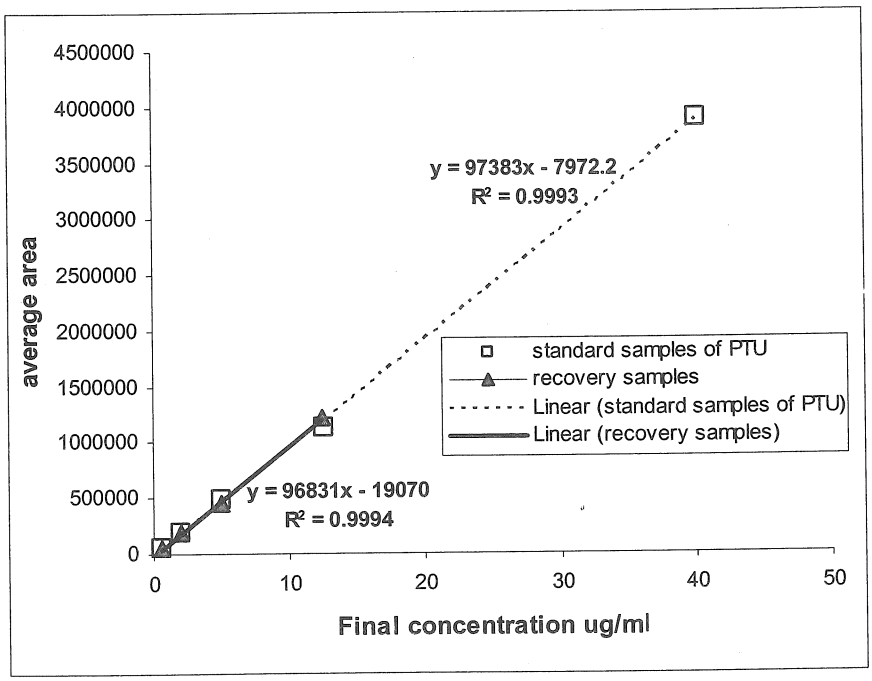

Figure 1. Linearity of standard samples of PTU and recovery samples with the slopes, intercepts and the linear regression coefficients

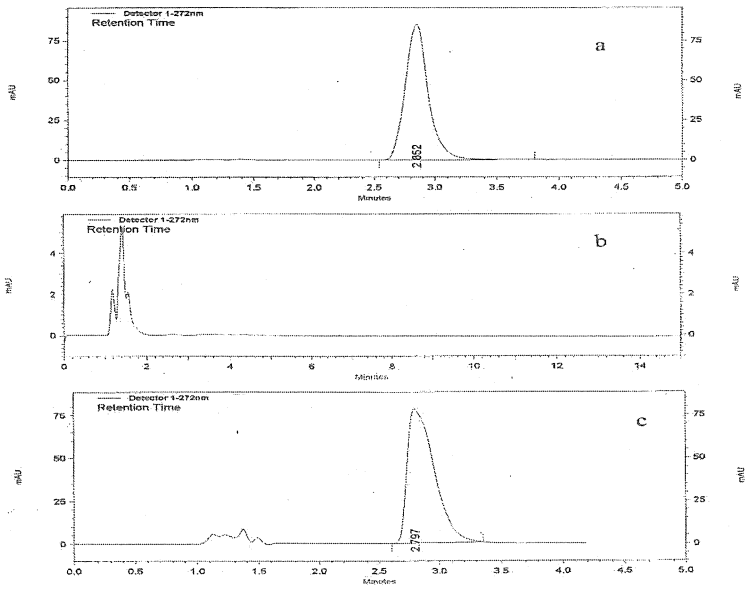

Figure 2. HPLC chromatograms of pure PTU (a), drug-free rabbit serum (b) and of standard serum sample containing the drug at a concentration of 12.5 µg/ml (c)

Accuracy and precision: The intra-day accuracy and precision of the assay was evaluated by analyzing three replicates of the serum containing PTU at three different concentrations. The intra-day precision of the analyzed samples as determined by R.S.D. (%) range from 0.56 to 1.32%, while the intra-day accuracy ranged from 99.8 – 106.2%. The inter-day precision of the assay was measured by analyzing three replicates of PTU serum samples for three consecutive days. Inter-day accuracy ranged from 95.3 – 103.9%, while the precision ranged from 0.56 to 1.45%.

Recovery: The absolute recovery was calculated by comparing the peak areas of PTU standards to those assessed by extraction of PTU in the concentration range of 0.1 – 40 µg/ml added to the rabbit serum. Results of absolute recovery of PTU ranged from 96.6 to 104.4% as shown in Table 2.

Table 2. Absolute recovery of PTU from rabbit serum.

	Concentration mg %	**Peak area**		**RSD %**	**Recovery %**
		methanol/water	**Serum**		
PTU	0.025	23,757	22,955	2.1	96.6
	0.05	45,545	47, 548	3.7	104.4
	0.20	192,023	194,868	0.4	101.5
Mean					100.8

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References

Abdul-Fattah, A.M. and Bhargava, H.N. (2001) Development and validation of high-performance liquid chromatographic method for the analysis of propylthiouracil in pharmaceuticals. Drug Dev. Industrial Pharm. 27:831-835

Cannell, G.R., Williams, J.P., Yap, A.S. and Mortimer, R.H. (1991) Selective liquid chromatographic assay for propylthiouracil in plasma. J. Chromatography 564:310-314.

Cooper, D.S., Saxe, V.C., Maloof, F. and Ridgway, E.C. (1981) Studies of propylthiouracil using a newly developed radioimmunoassay. J. Clin. Endocrin. Metabolism 52:204-213.

Duarte, C.G., Polizello, A.C.M., Assis-Pandochi, A.I. and Spadaro, A.C.C. (2000) Optimization of HPLC analysis for the determination of propylthiouracil levels in plasma and serum. J. Pharm. Biomed.Anal. 23:237-241.

Giles, H.G., Miller, R. and Sellers, E.M. (1979) High-performance liquid chromatographic determination of plasma propylthiouracil. J. Pharm. Sci. 68:1459-1460.

Kampmann, J.P. and Molholm-Hansen, J.E. (1981) Clinical pharmacokinetics of antithyroid drugs. Clin. Pharmacokinetics 6:401-428.

Kenneth, A.S. (2005) Stability of an extemporaneously compounded propylthiouracil suspension. Int. J. Pharm. Compound. Jan/Feb:93-101.

Kim, C. (1983) Simple and sensitive method for the determination of propylthiouracil in blood by high-performance liquid chromatography. J. Chromatography 272:376-379.

Mcarthur, B. and Miceli, J.N. (1983) Micro high performance liquid chromatography procedure for the quantitation of serum propylthiouracil. J. Chromatography 278:464-468.

Obeidat W.M. and Price, J.C. (2005) Preparation and in vitro evaluation of propylthiouracil microspheres made of Eudragit RL 100 and cellulose acetate butyrate polymers using the emulsion-solvent evaporation method. J. Microencapsulation 22:281-289.

Zhang, L., Liu, Y., Xie, M.X. and Oiu, Y.X. (2005) Simultaneous determination of thyreostatic residues in animal tissues by matrix solid-phase dispersion and gas chromatography-mass spectrometry. J. Chromatography. A. 1074(1-2):1-7.

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