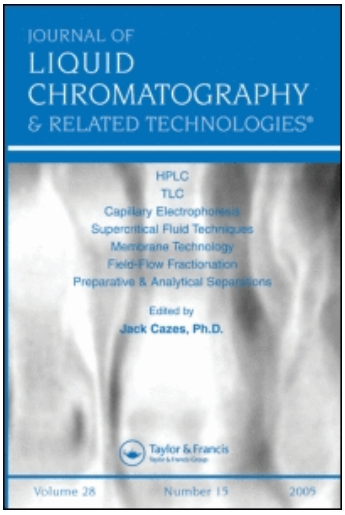

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Determination of Propylthiouracil in Human Breast Milk by Direct Injection High Performance Liquid Chromatography

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DETERMINATION OF PROPYLTHIOURACIL IN HUMAN BREAST MILK BY DIRECT INJECTION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A high-performance liquid chromatographic(HPLC) method was developed for the assay of propylthiouracil in human breast milk. After filtration with membran filter(Molcut Ⅱ), the eluent was injected into a liquid chromatogaph equipped with C_{18} precolumn and analytical column in series according to column switching techniques. This method is sufficiently sensitive for most pharmacokinetic studies in human breast milk. The concentration of propylthiouracil was linear over the 50 - 5000ng/ml range. The recovery and the coefficient of variation was 92.0 - 100.6 % and 1.6 - 2.9 %, respectivery. This assay has the advantages of specificity, simplicity and reproducibility for the measurement of propylthiouracil in human breast milk.

INTRODUCTION

Propylthiouracil has been used in the treatment of hyper thyroidism for many years. It has been reported that propylthiouracil prevents synthesis of thyroid hormone by disruption of thyroid peroxidase catalyzed reactions. The pharmacokinetic studies in patients treated with propylthiouracil have been described in some previous paper employed high-performance liquid chromatography[1]. Previous HPLC methods were involved to extraction step[2.3] and direct injection of deproteinized sample[1.4], those methods were time consuming and no good affected for column maintenance.

In the previous paper, it has been described that propylthiouracil was eliminated from plasma to milk in human[5.6]. Therefore, it appears the problem that the benefits of breast-feeding have to be balanced against the risk of interference with the infant's thyroid function and the possible haematological side-effects of this drug. Imamura et al.[7] reported the assay method by high-performance liquid chromatography of propylthiouracil in human breast milk, but this method was tedious and time consuming. The several previous HPLC methods did not include an internal standard[2,4,7]. We have tried to measurement of proplythiouracil in human breast milk by HPLC according to previous extraction method[2,3]. How ever the extraction yields are not satisfactry. In our previous paper, we described direct injection method by column switching techniques without extraction in serum mitomycin C[8]. The HPLC method with non extraction procedure is useful for the compound of low extraction efficiency.

In the present paper, we described on a simple and sensitive high-performance liquid chromatographic methods for the

determination of the propylthiouracyl in human breast milk by column switching techniques. The milk samples were filterated with membrane filter (Molcut Ⅱ) and the filtrate was directly injected to the high-performance liquid chromatographic system.

EXPERIMENTAL

Materials

Propulthiouracil was extracted from commercial available tablets and purified by repeated recrystallization in our laboratories. The structure and the purity were checked by TLC, IR and NMR spectra. Molcut Ⅱ membrane filter was obtained from Millipore Co. (Bedford, MA, U.S.A). All the solvents were used of HPLC grade(Wako Pure Chemicals, Indrstries Ltd. Tokyo, Japan). Salicylic acid was purchased from Nakalai Tesque Inc.(Kyoto, Japan). All other reagents and chemicals were supplied from Wako Pure Chemicals Industries Ltd. and purified by recrystallization or distillation prior to use.

Instruments

The liquid chromatographic system consisted of a Jasco PU-880 pump (Jasco Co. Ltd. Tokyo, Japan) combined with an UV-variable-wavelength detector, Jasco UVIDEC-Ⅱ (Jasco Co. Ltd., Tokyo, Japan) operating at 254nm and Reodyne Model 7125 injector (Rheodyne Inc., Cotati, CA, U.S.A) with an effective volume of 100 μl loop. HPLC was carried out using Develosil ODS-5 (50 × 4.6mm, I.D.) as a precolumn, and separation of propylthiouracil was performed on a Develosil ODS-5 column (150 × 4.6mm, I.D.) (Nomura Chemical Co., Seto, Japan) at a flow-rate of 1.0ml/min at ambient temperature. The mobile phase consisted of 0.2M potassium dihydrogen phosphate (pH4.6) : methanol (82:18, v/v). Before

mixing, the buffer was adjusted to pH4.6 and degassed ultrasonically.

Procedure for the determination of propylthiouracil in human breast milk

Salicylic acid (200ng) in methanol (10μl) was added to human breast milk sample(400μl) as an internal standaed, and then 400μl of water and 200μl of ethanol were added. The solution was sonicated for 5min and then filtrated through a Molcut Ⅱ membrane filter for deproteinization. Then the filtrate(50-100 μl) was loaded on the precolumn for elimination of interfering substances in human breast milk. After washing for 1.5min, propylthiouracil and salicylic acid were eluted from the precolumn and then led to the analytical column by a column switching technique using 0.2M potassium dihydrogem phophate(pH4.6) : methanol (82:18 v/v) as the isocratic mobile phase.

Calibration curves

Known amounts of propylthiouracil in the ranges 50 - 5000ng/ml were added to blank human breast milk. These milk samples were treated according to the method described above. Peak-hight ratios of propylthiouracil to salicylic acid were measured and plotted against, the concentration of propylthiouracil in human breast milk.

RESULTS AND DISCUSSION

There are several reports described on the techniques for the determination of propylthiouracil in biological fluids involving extraction and purification steps[2.3]. We tried to pursue the

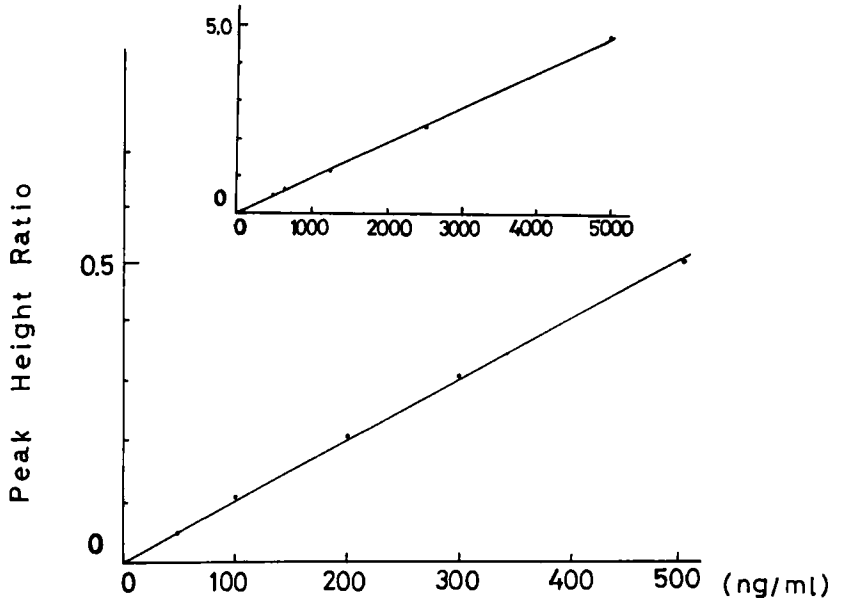

Fig.1. Chromatogram of 1) a milk blank, 2) a milk sample after 2hr oral administration of propylthiouracil and added with salicylic acid (I.S).

assay of propylthiouracil using these methods, but the satisfactory results were not obtained in the point of the recovery rate. Therefore, our effort was directed to the determination of propylthiouracil in human breast milk by the direct injection method employed the column switching technique[8]. A typical chromatogram obtained form the analysis of a human breast milk blank containing in internal standard was shown in Fig.1a. Fig.1b illustrated a chromatogram obtained from drug-free human breast milk to which 2500 ng/ml of propylthiouracil was added. Clearly separated peaks representiong propylthiouracil and its internal standard were seen on a

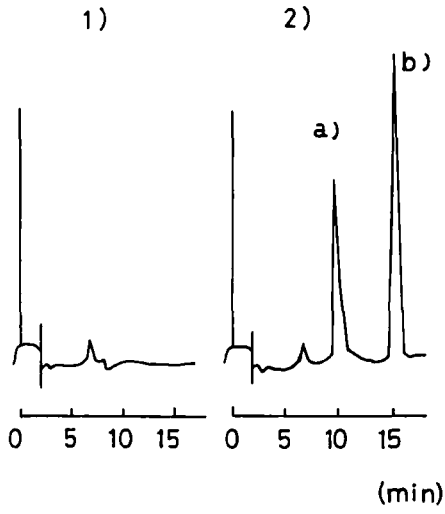

Fig.2. Calibration curves for propylthiouracil in human breast milk.

chromatogram. Under these conditions, the retention times of salicylic acid and propylthiouracil were 9.7 and 15.0 min, respectively. No interfering endogenous compounds were found on the chromatograms obtaoned with human breast milk. Propylthiouracil was added to drug-free human breast milk to yield concentrations of 50 to 5000 ng/ml. The concentration and the peak height ratio to internal standard were linearly related over this range as shown in Fig.2. It was proved that the sensitivity of this method was useful in the acqusition of pharmacokinetic data of human breast milk after several doses of propylthiouracil. Recovery studies were carried out by preparing identical propylthouracil concentration (250, 500, 1250 and 2500 ng/ml) in human milk sample(Table 1). The recovery of propylthiouracil was shown over the 92.0% (n=7). The mean

TABLE 1

Recovery of Propylthiouracil Added to Human Milk

Added (ng/ml)	Found (ng/ml) (Mean ± S.D)	Recovery (%) (Mean ± S.D)
250	251.7 ± 5.2	100.2 ± 1.9
500	478.4 ± 8.1	95.6 ± 1.6
1250	1173.1 ± 37.1	93.9 ± 2.9
2500	2291.8 ± 61.8	91.7 ± 2.5

between-run coefficient of variation recovered from human breast milk was not over 2.9%, at the level of 250, 500, 1250 and 2500 ng/ml. It was evident from the data that the proposed procedure was satisfactory with respect to the accuracy and the precision. The detection limit for propylthiouracil is sufficiently low (50ng/ml) to permit this procedure for the determination of propylthiouracil in human breast milk, above a concentration of 5000ng/ml.

The present method was applied to the determination of propylthiouracil level in human breast milk from four patients received oral administration of 50-300 ng/day of propylthiouracil.

TABELE 2
Human Milk Levels in Patients undergoing Propylthiouracil Treatment

Case	Dose (mg/day)	Milk (ng/ml)
1	50	117.9
2	150	48.2
3	150	78.1
4	300	206.0

The milk level of propylthiouracil were shown in Table 2. Kampman et al[6]. reported that the maximam concentration of propylthiouracil in milk was only about 10% of the maximam concentration in serum. Our data agreed with the results of Kampman et al[6]. The proposed method for the determination of propylthiouracil in clinical sample was proved to be satisfactory with respect to feasibility, simplicity and rapidity. We have developed a precise and an accurate high-performance liquid chromatographic method for the determination of propylthiouracil in human breast milk without extraction. This method is useful for pharmaceutical studies of propylthiouracil are being

conducted in our laboratories, and the details will be reported elsewhere.

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