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Short Communication

Determination of chlorpromazine in human breast milk and serum by high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic (HPLC) assay was developed for the determination of chlorpromazine in serum and human breast milk. Chlorpromazine in serum and human breast milk was extracted by a rapid and simple procedure based on C_{18} bonded-phase extraction, and a reversed-phase HPLC separation technique was developed. Chlorpromazine and levomepromazine as the internal standard were detected by ultraviolet absorbance at 254 nm. Determination was possible for chlorpromazine in the concentration range 10–300 ng/ml. The recoveries of chlorpromazine added to serum and human breast milk were 80.1–87.6 and 80.3–84.4%, respectively, with coefficients of variation of less than 10.2 and 7.8%. The method is applicable to drug level monitoring in the serum and human breast milk of patients treated with chlorpromazine.

INTRODUCTION

Chlorpromazine is widely used in the treatment of psychosis. In patients with puerperal psychosis treated with chlorpromazine, substantial amounts of the drug are secreted in the breast milk [1,2] and ingested by the infant. Adverse effects of chlorpromazine on the baby are reported to be drowsiness and lethargy [2]. Studies to determine chlorpromazine concentration in milk and the relationship between the serum concentration and amount secreted in milk should be able to predict the adverse effects of chlorpromazine on the breast-fed children of chlorpromazine-treated mothers. However, only a few previous reports have determined chlorpromazine concentration in milk and the relationship between the serum concentration and amount secreted in milk [1,2]. Several methods for the determination of plasma and serum levels of chlorpromazine involving high-performance liquid chromatography (HPLC) and ultraviolet detection [3,4] or electrochemical detection [5–7] have been described. However, none of these HPLC methods has been used to analyse chlorpromazine in human breast milk.

However, the previously described HPLC methods are time-consuming and require a tedious liquid–liquid extraction step to extract chlorpromazine from serum or plasma. Smith *et al.* [3] have described a simple solid-phase method for the extraction of chlorpromazine derivatives from plasma using a C_8 bonded-silica extraction column. Recently, we developed an ex-

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traction procedure using a C_{18} bonded phase (Sep-Pak C_{18} cartridge) for the efficient recoveries of several drugs from a clinical sample [8]. This method is satisfactory in terms of simplicity and rapidity. However, none of the Sep-Pak C_{18} extraction methods that have been described have been used for the determination of chlorpromazine in biological fluids. There are a few reports which describe HPLC methods for the determination of chlorpromazine using an ODS [4,5], CN [6,7] and C_8 [3] stationary phase.

In the present paper, we describe a rapid and simple method of extracting chlorpromazine from serum and human breast milk using Sep-Pak C_{18} cartridges coupled with a new mobile phase using C_8 stationary phase and a ultraviolet detector.

EXPERIMENTAL

Chemicals and materials

Chlorpromazine hydrochloride and methoxypromazine were kindly donated by Yoshitomi Pharmaceutical (Osaka, Japan), levomepromazine hydrochloride and prometazine by Shionogi Pharmaceutical (Osaka, Japan) and promazine hydrochloride by Hokuriku Pharmaceutical (Tokyo, Japan) (Fig. 1). Sep-Pak C_{18} (1 ml) cartridges were purchased from Millipore-Waters (Milford, MA, USA). All other solvents used were of HPLC grade (Wako Pure Chemical Industries, Tokyo, Japan). All other reagents and chemicals were purchased from Wako Pure Chemical Industries or Nakarai Tesque (Kyoto, Japan) and were analytical reagent grade.

Apparatus

The apparatus used for HPLC consisted of a Philips–Pye Unicam PU 4010 chromatography pump and a Pye Unicam PU 4020 ultraviolet detector (Philips–Pye Unicam, Cambridge, UK). Test samples were injected using a Model 7125 injector (Rheodyne, Cotati, CA, USA) with an effective volume of 100 μl. The HPLC sorbent was Develosil C_8-5 (5 μm, Nomura Chemical, Seto, Japan). Stainless-steel columns (150 mm × 4.6 mm I.D.) were packed in our laboratories by a conventional high-pressure slurry-packing procedure.

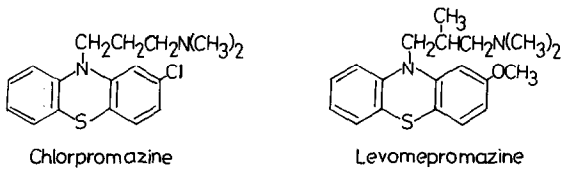

Fig. 1. Structures of chlorpromazine and levomepromazine.

Chromatographic conditions for serum and human breast milk

The mobile phase consisted of 0.5% potassium dihydrogenphosphate (pH 4.5)–acetonitrile (65:35, v/v). Before mixing, the pH of the mobile phase was adjusted with 50% phosphoric acid and it was degassed ultrasonically. The flow-rate was 1 ml/min and maintained absorbance was monitored at 254 nm with an attenuation of 0.02.

Extraction method

Serum extraction method. Levomepromazine (500 ng) in methanol (5 μl) was added to the serum sample (1 ml) as an internal standard, the serum sample was then diluted with 5 ml of 0.5 *M* hydrochloric acid and the solution was briefly mixed. The mixture was applied to a Sep-Pak C_{18} cartridge that had previously been activated with 5.0 ml of methanol and water. The cartridge was then washed with 5.0 ml of water and 20% methanol, and the desired fraction was eluted with 5 ml of 60% methanol. The eluate was evaporated to dryness in vacuum at 60°C. The residue was dissolved in 100 μl of mobile phase and injected into an HPLC apparatus.

Human breast milk extraction method. Levomepromazine (500 ng) in methanol (5 μl) was added to the human breast milk sample (1 ml) as an internal standard, the milk sample was then diluted with 5 ml of 1 *M* hydrochloric acid and mixed vigorously. The mixture was applied to previously activated Sep-Pak C_{18} cartridges. The subsequent protocol of washing and sample preparation was as described above for serum.

Calibration graphs

Known amounts of chlorpromazine in the

range 10–300 ng/ml were added to blank serum and blank human breast milk samples. These serum and breast milk samples were treated according to the extraction procedure described above. The peak-height ratios of chlorpromazine to levomepromazine were measured and plotted against the concentration of chlorpromazine in serum and human breast milk.

Recovery test for chlorpromazine

The spiked samples were prepared by adding 25, 100 and 200 ng/ml chlorpromazine to blank serum and blank human breast milk. Sample extraction and the subsequent HPLC technique were carried out as described above.

RESULTS AND DISCUSSION

Initially, our efforts were directed towards developing an efficient chromatographic method for the determination of chlorpromazine in serum and human breast milk. The effect of the pH of the mobile phase on the capacity factors (k') of chlorpromazine and promazine, methoxypromazine, levomepromazine and promethazine as an internal standard was studied using C_8 stationary phases. The k' values of all these compounds increased with increasing pH in the range 5.5–7.5 with 0.5% potassium dihydrogenphosphate–acetonitrile (65:35, v/v). However, sufficient resolution was not obtained at pH 7.5 as a result of peak broadening and tailing.

These phenothiazine derivatives were retained strongly on C_8 stationary phase and could be easily separated with this mobile phase over the entire pH range. The time of analysis at a mobile phase pH of 2.5–4.5 was shorter than that at higher pH, and since good resolution was obtained in the low pH range pH 4.5 was selected for the mobile phase. Adequate resolution and a suitable retention time were obtained for levomepromazine and promazine with these stationary phases. Levomepromazine was suitable as an internal standard because there were no interference peaks from biological fluid at its retention time.

In our preliminary experiments, chlorpromazine adsorbed on the Sep-Pak C_{18} in spiked serum, breast milk and water was not eluted by methanol, ethanol and ethyl acetate. It is considered that chlorpromazine is strongly adsorbed to octadecyl stationary phases because of its highly lipophilicity. Therefore, the applied acidic solution was used for the adsorption of chlorpromazine in serum and human breast milk. Fig. 2 shows the recovery profile of chlorpromazine in serum and human breast milk using Sep-Pak C_{18} cartridges and various concentrations of hydrochloric acid as an applied solution. The maximum extraction of chlorpromazine was obtained when 0.5–1 M hydrochloric acid in serum or >1 M hydrochloric acid in human breast milk was used as the applied solution. Therefore, 0.5 M hydrochloric acid in serum and 1 M hydrochloric

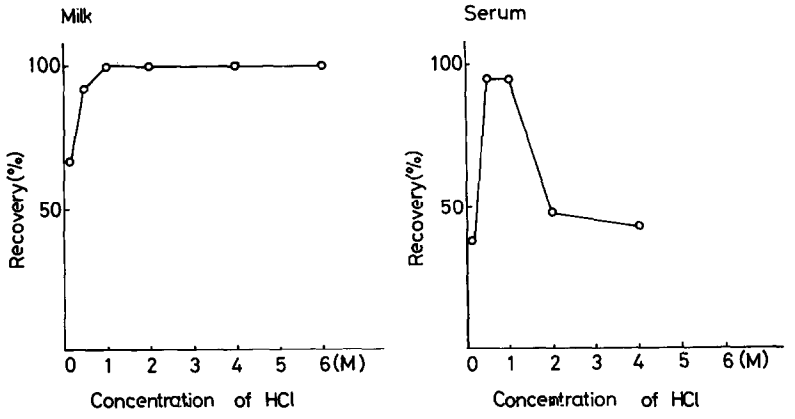

Fig. 2. Recovery profile of chlorpromazine in serum and human breast milk for Sep-Pak C_{18} cartridges using various concentration of hydrochloric acid as an applied solution.

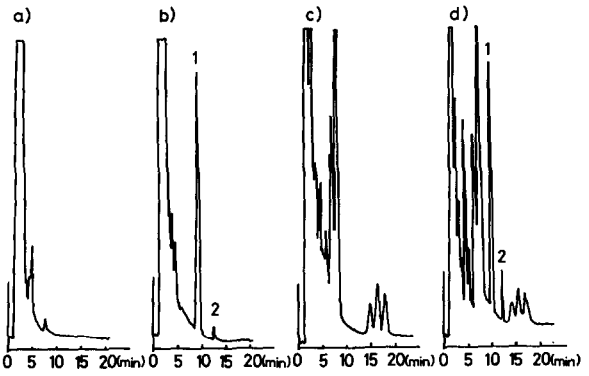

Fig. 3. Chromatograms of (a) a blank milk sample, (b) a milk sample after treatment (12 h) with chlorpromazine (40 mg/day dose) and with added levomepromazine (internal standard), (c) a blank serum sample and (d) a serum sample after treatment (12 h) with chlorpromazine (200 mg/day dose) and with added levomepromazine (internal standard). Chlorpromazine concentration found: (b) 5.5. ng/ml; (d) 12.0 ng/ml. Peaks: 1 = levomepromazine; 2 = chlorpromazine.

acid in human breast milk were chosen as the applied solutions in the Sep-Pak C_{18} extraction system. Fig. 3 shows representative chromatograms of blank samples and serum and human breast milk samples obtained from patients treated with chlorpromazine. The separation on the chromatogram of chlorpromazine or levomepromazine from interfering peaks derived from biological matrix was satisfactory.

TABLE I

RECOVERY OF CHLORPROMAZINE ADDED TO HUMAN BREAST MILK AND HUMAN SERUM

Added (ng/ml)	Found (mean ± S.D.) (ng/ml)	Recovery (mean ± S.D.) (%)	C.V. (%)
Serum (n = 6)			
25	20.0 ± 1.4	80.1 ± 5.6	7.0
100	87.6 ± 9.0	87.6 ± 9.0	10.2
200	172.6 ± 15.0	85.8 ± 7.5	8.7
Human breast milk (n = 6)			
25	21.1 ± 1.6	84.4 ± 6.4	7.6
100	80.5 ± 3.7	80.5 ± 3.7	4.6
200	160.2 ± 3.8	80.3 ± 1.9	2.3

TABLE II

CHLORPROMAZINE LEVEL IN HUMAN BREAST MILK AND SERUM OF PATIENTS UNDERGOING CHRONIC CHLORPROMAZINE TREATMENT

Patient	Dose (mg/day)	Milk level (ng/ml)	Serum level (ng/ml)
1	120	–	7.5
2	100	–	5.0
3	40	5.5	–
4	200	–	12.0

Calibration graphs for chlorpromazine in human serum and human breast milk were linear in the range 10–300 ng/ml. The limit of detection for chlorpromazine was 5 ng/ml (signal-to-noise ratio = 5). The results of recovery studies are shown in Table I. The recovery of chlorpromazine was determined by adding the three known concentrations of 25, 100 and 200 ng/ml to serum and blank human breast milk. The recovery values for chlorpromazine were 80.1–87.6% in serum and 80.3–84.4% in human breast milk at 25–200 ng/ml. Inter- and intra-assay variation were less than 10.2% in serum and less than 7.6% in human breast milk at 25–200 ng/ml (Table I). These results show that the proposed method is satisfactory with respect to accuracy and precision.

The concentrations of chlorpromazine in serum and human breast milk from patients receiving perorally various amounts of chlorpromazine were determined by the proposed method (Table II). The levels of chlorpromazine in serum were within the range measured by Cooper *et al.* [9], and those in human breast milk were within the range measured by Wiles *et al.* [2]. It is shown that the proposed method for the determination of chlorpromazine in clinical samples is satisfactory with respect to feasibility, simplicity and rapidity.

REFERENCES

1 K. H. Blacker, B. J. Weinstein and G. L. Ellman, *Am. J. Psychiatr.*, 119 (1962) 178–179.

2 D. H. Wiles, M. W. Orr and T. Kolakowska, *Br. J. Clin. Pharm.*, 5 (1978) 272–273.
3 C. S. Smith, S. L. Morgan and S. V. Greene, *J. Chromatogr.*, 423 (1987) 207–216.
4 G. Tamai, H. Yoshida and H. Imai, *J. Chromatogr.*, 423 (1987) 163–168.
5 K. Murakami, K. Murakami, T. Ueno, J. Hijikata, K. Shirasawa and T. Muto, *J. Chromatogr.*, 227 (1982) 103–113.
6 S. H. Curry, E. A. Brown, O. Y.-P., Hu and J. H. Perrin, *J. Chromatogr.*, 231 (1982) 361–376.
7 J. W. Hubbard, J. K. Cooper, E. M. Hawes, D. J. Jenden, P. R. A. May, M. Martin, G. McKay, T. Van Putten and K. K. Midha, *Ther. Drug Monit.*, 7 (1985) 222–228.
8 T. Ohkubo, R. Shimoyama and K. Sugawara, *J. Pharm. Sci.*, 81 (1992) 947–949.
9 J. K. Cooper, G. McKay and K. K. Midha, *J. Pharm. Sci.*, 72 (1983) 1259–1262.