BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **13**: 15–23 (1999)

ORIGINAL RESEARCH

Development and clinical application of high performance liquid chromatography for the simultaneous determination of plasma levels of theophylline and its metabolites without interference from caffeine

Junko Kizu,[1]* Shigekazu Watanabe,[1] Nobuhiro Yasuno,[1] Yoshihiro Arakawa,[1] Sonoko Uzu,[1]† Susumu Kanda,[2] Fusako Komoda,[3] Tsutomu Iwata,[3] Hiroshi Hayakawa,[3] Tetsuo Hayakawa[4] and Kazuhiro Imai[5]

[1]Department of Pharmacy, Branch Hospital, Faculty of Medicine, University of Tokyo, 3-28-6 Mejirodai, Bunkyo-ku, Tokyo 112, Japan
[2]Department of Pharmacy, Mitsui Memorial Hospital, 1, Kanda-Izumi, Chiyoda-ku, Tokyo 101, Japan
[3]Department of Pediatrics, Branch Hospital, Faculty of Medicine, University of Tokyo, 3-28-6 Mejirodai, Bunkyo-ku, Tokyo 112, Japan
[4]Department of Research Center for Rheumato-Allergology, National Sagamihara Hospital, 18-1 Sakuradai, Sagamihara-shi, Kanagawa 228, Japan
[5]Department of Bioanalytical Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

Received 17 October 1997; accepted 26 November 1997

ABSTRACT: A high performance liquid chromatography (HPLC) method has been developed for the simultaneous determination of plasma levels of theophylline and its metabolites without interference from caffeine or caffeine metabolites. The method is simple and of practical use because it is applicable even to plasma samples from patients who take caffeine-containing beverages. The method was also reproducible with a coefficient of variation of less than 5% for each analyte. The levels of theophylline, determined by HPLC, were validated by their high correlation to the levels obtained by fluorescence polarization immunoassay. HPLC was used to determine theophylline levels in patients with bronchial asthma. The data revealed that the ratio of 1,3-dimethyluric acid, the major metabolite of theophylline, to theophylline concentration in the plasma was within a narrow range in most patients (0.055 ± 0.01, $n = 66$), regardless of the method of theophylline administration or the time of blood sampling. Conversely, this ratio was as low as 0.027 ± 0.005 in the patient with a long plasma half-life of theophylline. These results suggest that it may be possible to predict the plasma half-life of theophylline for each patient from a single blood sample. This may be useful when planning theophylline administration, especially in patients with abnormal theophylline metabolism. Copyright © 1999 John Wiley & Sons, Ltd.

INTRODUCTION

Theophylline is a bronchodilator used in the treatment of acute and chronic asthma. The drug is effective within a narrow range of plasma concentrations (10–20 μg/mL) and side-effects occur when plasma levels exceed 20 μg/mL. The rate of metabolism of theophylline varies considerably from one individual to another and, therefore, effective and safe theophylline therapy requires dose optimization by measuring plasma theophylline levels (Hendeles *et al*., 1978).

Approximately 85%–90% of the administered dose of theophylline is metabolized by the hepatic microsomal enzyme cytochrome P450 and the rest is excreted in unchanged form in the urine (Tserng *et al*., 1981; Richer and Lam, 1993). The major step in the metabolic pathway is hydroxylation at position 8 of theophylline to generate 1,3-dimethyluric acid which accounts for 45%–55% of total theophylline clearance (Fig. 1). Other important steps in metabolism of theophylline are *N*-demethylation to form 1-methylxanthine (20%–25% of the total clearance) and 3-methylxanthine (13%–16% of the total clearance). 1-Methylxanthine is rapidly converted by xanthine oxidase to 1-methyluric acid and is thus normally undetectable.

Changes in theophylline metabolism have been shown to occur depending on the age of the patient. In neonates, for example, hepatic metabolism is not fully developed and, therefore, approximately 50% of the administered dose of theophylline is excreted in unmodified form in the urine and the rest is metabolized partly to caffeine by methylation (Tserng *et al*., 1981; Richer and Lam, 1993). Some of the theophylline metabolites are known to have bronchodilator activity. Among them 3-methylxanthine is as potent as theophylline (Williams *et al*., 1978). In a report on the use of theophylline in patients with renal failure, serious side-effects were observed following accumulation of high levels of 3-methylxanthine and 1,3-dimethyluric acid (Leakey *et al*., 1991). Therefore, in

*Correspondence to: J. Kizu, Department of Pharmacy, Branch Hospital, Faculty of Medicine, University of Tokyo, 3-28-6 Mejirodai, Bunkyo-ku, Tokyo 112, Japan.
†Present address: Department of Pharmacy, Faculty of Medicine, University of Shinshu, 311 Asahi, Matsumoto-shi, Nagano 390, Japan.

Copyright © 1999 John Wiley & Sons, Ltd.
CCC 0269–3879/99/010015–09 $17.50

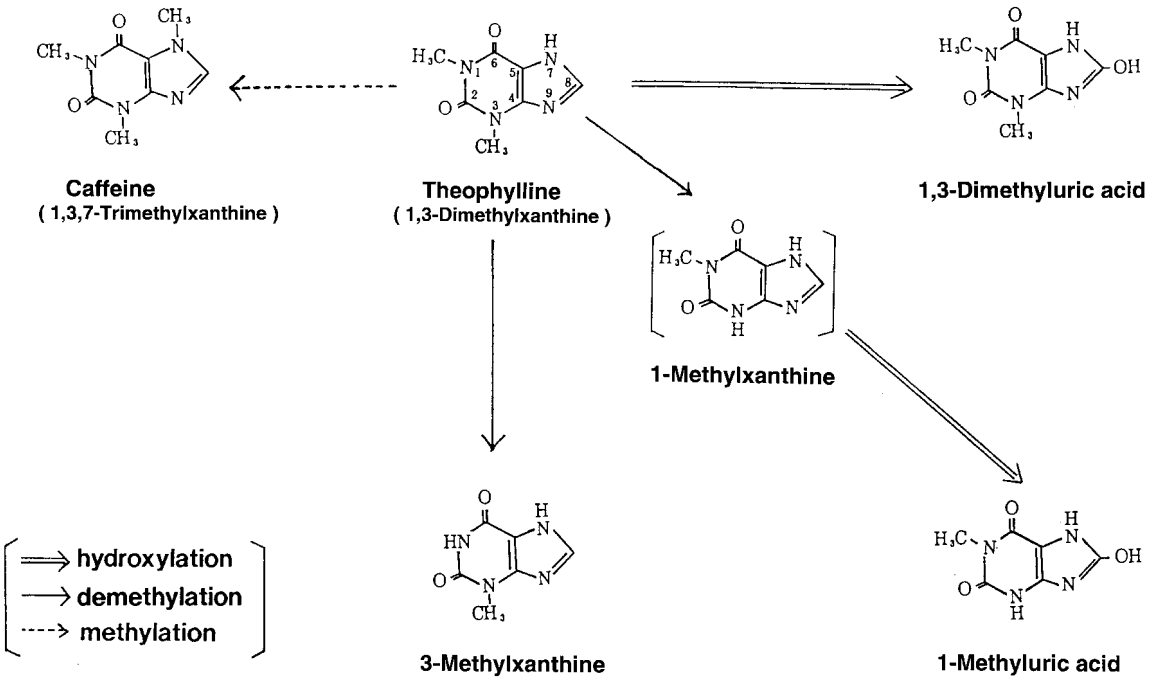

Figure 1. Proposed scheme for the metabolism of theophylline in humans. The major step in the metabolic pathway is the hydroxylation of theophylline at position 8 to generate 1,3-dimethyluric acid.

order that theophylline can be used safely, the simultaneous determination of plasma levels of theophylline and its metabolites is important.

A method for the determination of levels of theophylline and its three major metabolites by high performance liquid chromatography (HPLC) was reported by Kodera *et al*. (1985) and by Hamasaki *et al*. (1986). However, in these two studies the intake of caffeine-containing food and beverages such as coffee, tea, cola and chocolate was prohibited for 24 h prior to blood sampling. This is because caffeine is metabolized to paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine) and theophylline, and further to 1,7-dimethyluric acid (Tang-Liu *et al*., 1982a), and these metabolites, especially paraxanthine, interfere with the detection of theophylline and its metabolites. A gradient HPLC method (Sarkar *et al*., 1991) and an ion-pair reversed-phase HPLC method (Muir *et al*., 1982; Naline *et al*., 1987) were reported to solve this problem. However, these latter methods require relatively expensive gradient HPLC apparatus or a long stabilization time.

We have developed a simple method for the simultaneous determination of plasma levels of theophylline and its metabolites, together with caffeine and caffeine metabolites, by HPLC with isocratic elution. This method has been used successfully for the determination of plasma levels of theophylline and its metabolites in patients with bronchial asthma who were treated with theophylline. The clinical significance of the simultaneous determination of theophylline and theophylline metabolites is discussed.

EXPERIMENTAL

Reagents. The reagents used were: theophylline, 1,3-dimethyluric acid, 3-methylxanthine, 1-methylxanthine, 1-methyluric acid, caffeine, paraxanthine, theobromine, 1,7-dimethyluric acid, 1,3,7-trimethyluric acid and β-hydroxyethyltheophylline (Sigma Co. Ltd., St. Louis, MO, USA); L-tryptophan (Wako Pure Chemicals Co. Ltd., Osaka, Japan); and acetonitrile and methanol for HPLC (Wako Pure Chemicals Co.).

High performance liquid chromatography. The HPLC apparatus consisted of the following components: a LC-5A pump (Shimadzu Ltd., Kyoto, Japan), a KHP-U1-130A sample injector (Kyowa Seimitsu, Tokyo, Japan), a New Guard TM pre-column (15 × 3.2 mm i.d., 7 μm. Tosoh Co., Tokyo, Japan), a TSK ODS-80TM reversed-phase column (250 × 4.6 mm i.d., 5 μm. Tosoh Co.,), a L-4200 ultraviolet absorption detector (Hitachi Ltd., Tokyo, Japan), and a Chromatopac C-R2AX integrator (Shimadzu).

Three-hundred microlitres of acetonitrile, containing 0.3 μg of β-hydroxyethyltheophylline as an internal standard, were added to 100 μL of plasma sample. After vigorous mixing, the sample was centrifuged at 5 000 × ***g*** for 5 min, and 300 μL of supernatant was transferred into a new tube and evaporated to dryness at 40°C. The residue was dissolved in 30 μL of mobile phase and 5 μL of the solution was injected onto the HPLC column. The mobile phase

Copyright © 1999 John Wiley & Sons, Ltd.

was a mixture of 20 mM sodium acetate buffer, acetonitrile and methanol. The pH of the sodium acetate buffer and the percentages of the organic solvents were optimized for the HPLC separation. The flow-rate of the mobile phase was 1.0 mL/min and the HPLC column was operated at room temperature. The eluate was monitored at 275 nm. Plasma levels of theophylline and its metabolites were calculated from the ratio of their peak-heights relative to the internal standard, using the calibration curve.

Fluorescence polarization immunoassay (FPIA). To confirm the plasma theophylline levels determined by HPLC, approximately 50 μL of the plasma samples were applied to an automatic FPIA apparatus "TDx" (Abbott Co. Ltd., TX, USA), which utilizes a fluorescein-labeled theophylline tracer and an antibody to theophylline.

Blood samples from inpatients. Thirty-two blood samples were collected over a period of time from four patients with bronchial asthma who were treated with theophylline. Informed consent was given in all cases.

Patient A. A 60-year-old female weighing 49 kg. A slow-release 200 mg theophylline tablet (Theodur tablet, Nikken Kagaku Co. Ltd., Tokyo, Japan) was administered orally three times daily at 8:00 am, 15:00 pm and 20:00 pm until the day before the study. On the day of the study, an initial blood sample was collected at 8:00 am and then 375 mg of aminophylline (theophylline ethylenediamine; Neophyllin injection, Eisai Co. Ltd., Tokyo, Japan) was infused intravenously over a period of 3 h. Blood was again collected 30 min, 1 h, 2 h, 3 h, 4 h and 6 h after the start of the infusion.

Patient B. A 55-year-old male weighing 50 kg. A 200 mg Theodur tablet was administered orally four times daily at 8:00 am, 13:00 pm, 18:00 pm and 22:00 pm until the day before the study. On the day of the study, an initial blood sample was collected at 8:00 am and 250 mg of aminophylline was then infused intravenously over a period of 3 h. Blood was collected 30 min, 1 h, 2 h, 3 h and 4 h after the start of the infusion.

Patient C. A 49-year-old female weighing 63 kg. A 200 mg Theodur tablet was administered orally three times daily at 8:00 am, 15:00 pm and 20:00 pm until the day before the study. On the day of the study, an initial blood sample was collected at 8:00 am and a 200 mg Theodur tablet was then administered. Blood was collected 30 min, 1 h, 2 h, 3 h and 4 h later.

In these three inpatients, bronchial asthma attacks were prevented by the administration of theophylline and no side-effects were observed.

Patient D. A 14-year-old female weighing 57 kg. Because of a bronchial asthma attack, 170 mg of aminophylline was injected intravenously followed by the constant infusion of aminophylline at a rate of 0.8 mg/kg/h. After infusion for 10 h, the patient complained of nausea. As the plasma level of theophylline determined by FPIA was found to be as high as 35 μg/mL at this time, the infusion was stopped and blood samples were collected 0 h, 3 h, 6 h, 9 h, 13 h and 27 h later. After checking that the plasma level of theophylline had fallen to 10 μg/mL by FPIA, a 150 mg aminophylline granule (Neophyllin, Eisai Co.) was administered orally. Blood was collected 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 7 h and 24 h later.

These four patients had not been given any other medication known to affect theophylline clearance (Kawakatsu and Kawai, 1988), and had no clinical or biochemical evidence of liver disease or renal failure.

Blood samples from outpatients. Sixty-seven blood samples were collected from outpatients with bronchial asthma (40 males and 27 females, aged 6–80 years) who were being treated with theophylline. Informed consent was given by the patients. Among these patients, 17 were being treated with oral rapid-release preparations of theophylline [aminophylline (Neophyllin granules) or choline theophylline (choline theophyllinate; Theocolin tablets, Eisai Co.)]. The dose of aminophylline was 6–12 mg/kg/day for children and 300 mg/day for adults, and the dose of choline theophylline was 400–600 mg/day for adults. The other 50 patients were treated with oral slow-release preparations of theophylline (Theolong granules, Eisai Co., or Theodur tablets, Nikken Kagaku Co.). The dose of theophylline was 13–17 mg/kg/day for children and 400–600 mg/day for adults.

Since the blood from these patients was collected at the hospital, the time of collection after the theophylline dose was indeterminate for the 67 patients. These patients had not been given any medication known to affect theophylline clearance (Kawakatsu and Kawai, 1988).

Control blood samples before and after caffeine intake. To check whether caffeine and caffeine metabolites would interfere with the chromatogram, a healthy volunteer refrained from taking any caffeine-containing beverages for 3 days and blood was then sampled immediately before and after taking a caffeine-containing beverage.

All blood samples were collected into tubes containing 2 mg EDTA2Na as an anticoagulant and were centrifuged immediately. The plasma was stored at −20°C until analysis.

RESULTS

Optimization of HPLC

Initial studies on the optimum conditions for the separation of theophylline, caffeine and the metabolites of these two compounds on a reversed-phase column were done using a mixture of 20 mM sodium acetate buffer at different pHs and acetonitrile (10:1, v/v) as a mobile phase. At pH 4.0, insufficient separation of 1-methyluric acid from 3-methylxanthine was achieved while at pH 5.2, 1-methyluric acid was not separated from plasma components. At pH 4.8, 1-methyluric acid was separated from both 3-methylxanthine and plasma components but theophylline could not be separated from paraxanthine.

The effect of adding different organic solvents to the mobile phase was also investigated and it was found that the addition of methanol to the mobile phase improved the separation of theophylline and paraxanthine. The final composition of the mobile phase was 20 mM sodium acetate buffer (pH 4.8), acetonitrile and methanol (900:35:65, v/v). Under these conditions, theophylline, caffeine and the various metabolites of these two compounds were well separated from each other (Fig. 2).

As shown in Fig. 3 no interfering peaks were observed

Copyright © 1999 John Wiley & Sons, Ltd.

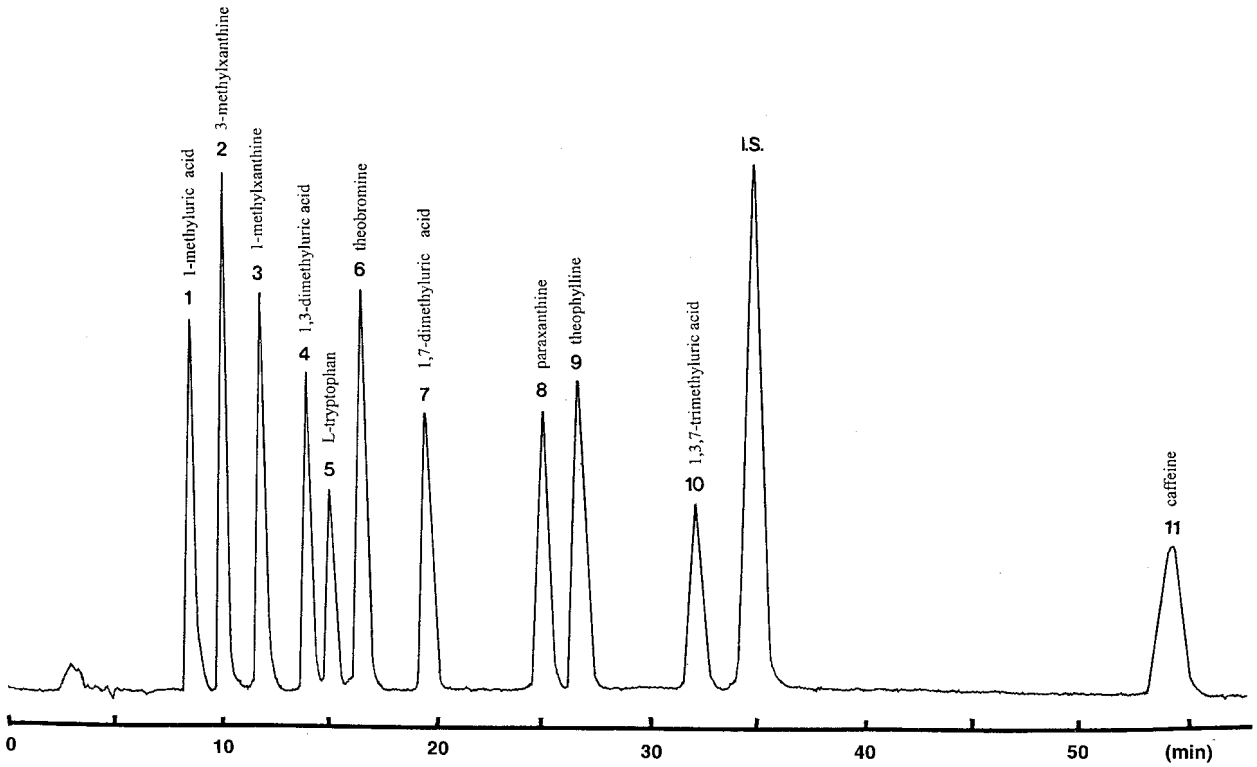

Figure 2. Chromatogram of theophylline, caffeine, their metabolites and L-tryptophan standards from reversed-phase HPLC. Peaks: (1) 1-methyluric acid; (2) 3-methylxanthine; (3) 1-methylxanthine; (4) 1,3-dimethyluric acid; (5) L-tryptophan; (6) theobromine; (7) 1,7-dimethyluric acid, (8) paraxanthine; (9) theophylline; (10) 1,3,7-trimethyluric acid; (11) caffeine, and (I.S.) β-hydroxyethyltheophylline. The amount of each standard loaded onto the HPLC column was 50 ng.

with the same retention times as the analytes on the chromatogram of the plasma sample taken from the healthy volunteer who had not taken theophylline or caffeine-containing beverages. However, after taking caffeine-containing beverages additional peaks corresponding to caffeine and its metabolites also appeared

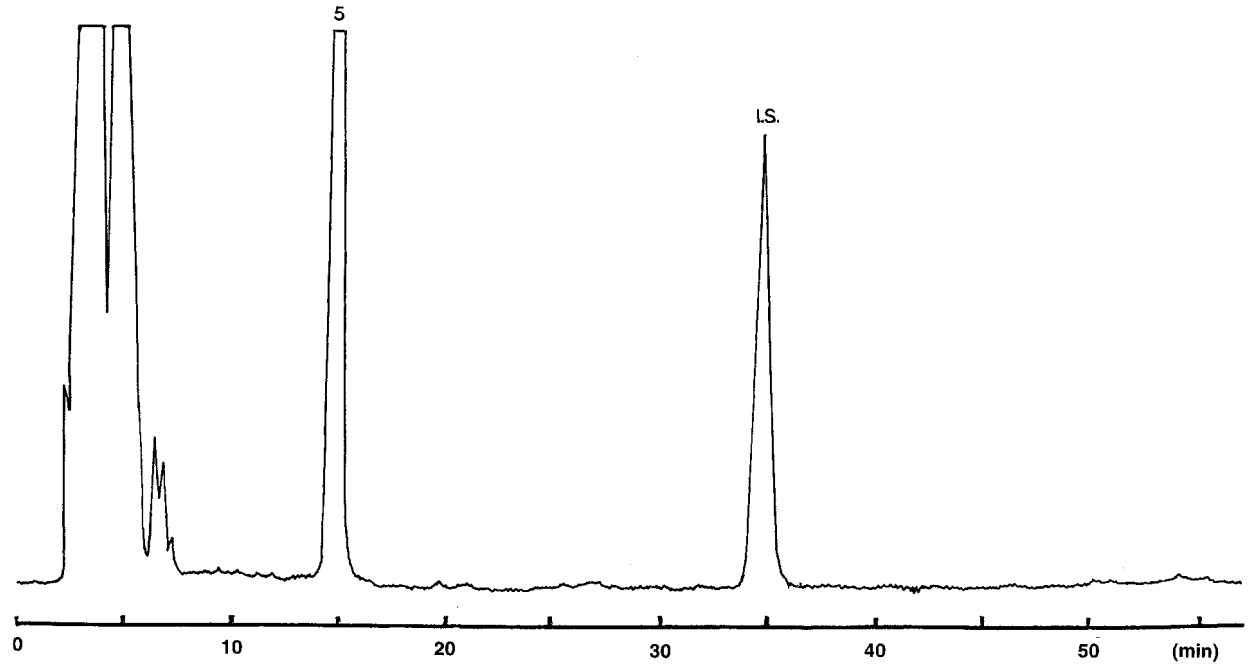

Figure 3. Chromatogram of a plasma sample from a healthy volunteer after refraining from taking caffeine-containing beverages for 3 days. Peaks: (5) L-tryptophan, (I.S.) β-hydroxyethyltheophylline.

Copyright © 1999 John Wiley & Sons, Ltd.

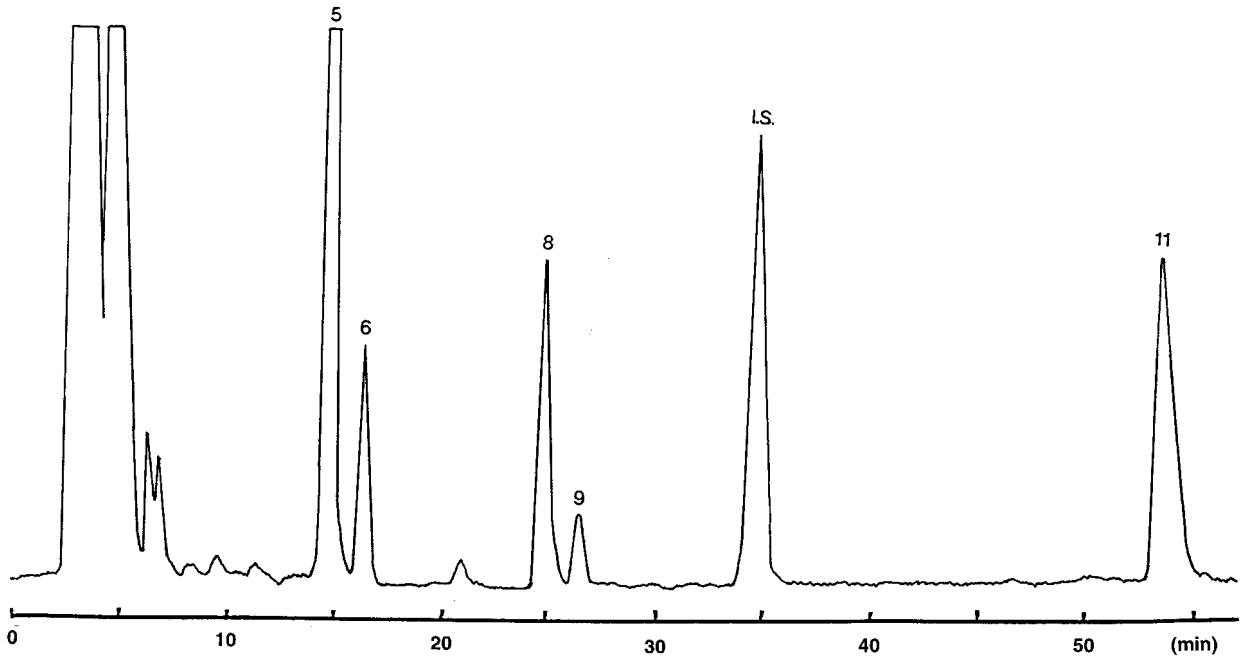

Figure 4. Chromatogram of a plasma sample from the same healthy volunteer as in Fig. 3 but after taking caffeine-containing beverages. Peaks: (5) L-tryptophan; (6) theobromine; (8) paraxanthine; (9) theophylline; (11) caffeine; and (I.S.) β-hydroxyethyltheophylline.

(Fig. 4). The chromatogram of a plasma sample taken from the patient given theophylline who also took caffeine-containing beverages shows satisfactory separation of theophylline, caffeine and their metabolites (Fig. 5). A large peak with a retention time of 16 min did not correlate with theophylline and was presumed, from its retention time, to be L-tryptophan. The concentration of the compound in the peak which was presumed to be L-

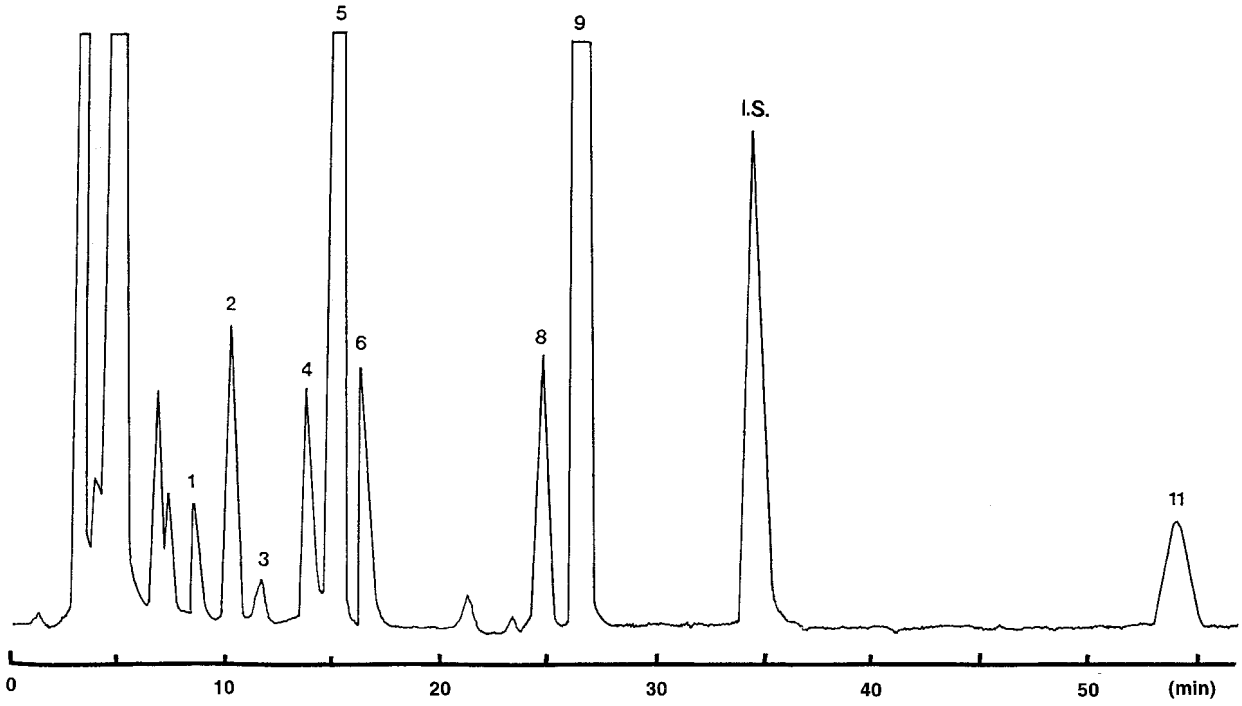

Figure 5. Chromatogram of a plasma sample from the patient with bronchial asthma undergoing theophylline therapy who took caffeine-containing beverages. Peaks: (1) 1-methyluric acid; (2) 3-methylxanthine; (3) 1-methylxanthine; (4) 1,3-dimethyluric acid; (5) L-tryptophan; (6) theobromine; (8) paraxanthine; (9) theophylline; (11) caffeine; and (I.S.) β-hydroxyethyltheophylline.

Copyright © 1999 John Wiley & Sons, Ltd.

Table 1. Within-run reproducibility and day-to-day precision of HPLC analysis

	Within-run ($n = 8$)		Day-to-day Precision ($n = 8$)	
	Level (μg/mL) (mean ± SD)	CV (%)	Level (μg/mL) (mean ± SD)	CV (%)
Theophylline	17.283 ± 0.194	1.12	12.038 ± 0.180	1.50
1-Methyluric acid	0.559 ± 0.025	4.56	0.597 ± 0.022	3.60
3-Methylxanthine	0.709 ± 0.005	0.73	0.297 ± 0.010	3.32
1,3-Dimethyluric acid	0.920 ± 0.019	2.06	0.602 ± 0.018	2.99

tryptophan ranged from 6.0–17.2 μg/mL (mean 9.6 μg/mL), which was consistent with the normal plasma L-tryptophan levels found in Japanese subjects (10.0 ± 2.7 μg/mL) (Saito and Muto, 1987).

The calibration curves for theophylline and its metabolites displayed good linearity in the range of 0.3–30 μg/mL for theophylline ($r = 1.0000$), and in the range of 0.3–2 μg/mL for the metabolites ($r = 0.9994$–1.0000).

The recoveries of theophylline and its metabolites by HPLC relative to the internal standard were checked by adding standards to plasma from the healthy volunteer at a concentration of 10.0 μg/mL for theophylline and 1.0 μg/mL for the metabolites. The results were 94.8 ± 1.2% [mean ± standard deviation (SD), $n = 5$] for theophylline, 99.8 ± 0.8% for 3-methylxanthine, 95.6 ± 0.6% for 1,3-dimethyluric acid and 79.4 ± 2.4% for 1-methyluric acid. These percentage recoveries were used to correct the plasma levels.

The within-run reproducibility was checked by the successive determination of levels in eight aliquots taken from the same individual plasma sample. The coefficient of variation (CV) for theophylline and its metabolites was less than 5%. The day-to-day precision of the method was determined by measuring the levels in the same pre-treated sample on eight different days. The CV for theophylline and its metabolites was less than 4% (Table 1).

Correlation of plasma theophylline levels obtained by HPLC and FPIA

Figure 6 shows good correlation between the plasma theophylline levels determined by HPLC and FPIA. The regression equation determined by the least squared method was $y = 1.006x - 0.103$ (y = HPLC, x = FPIA) and the correlation coefficient r was 0.997 ($p < 0.001$) ($n = 65$). The paired t-test revealed no significant differences between the two sets of determinations obtained by these methods ($p < 0.05$).

Clinical application of the HPLC method

Time-course analysis in inpatients. The plasma levels of theophylline and its metabolites were determined in blood samples which were obtained from patients A and B treated with aminophylline infusion, patient C treated orally with a slow-release theophylline tablet and patient D treated with infusion on the first day and oral administration of aminophylline on the second day. As shown in Figs 7–10, the plasma levels of 3-methylxanthine and 1-methyluric acid were fairly constant in all four patients and did not show time-dependent variance during the observed period, suggesting the presence of saturated metabolic pathways. However, the plasma levels of 1,3-dimethyluric acid and theophylline changed in similar time-dependent patterns. The ratio of the plasma level of 1,3-dimethyluric acid to theophylline (DMU/TP ratio) was 0.051 ± 0.007 (mean ± SD, $n = 6$) for patient A, 0.063 ± 0.011 ($n = 6$) for patient B and 0.047 ± 0.002 ($n = 6$) for patient C, while in patient D it was as low as 0.027 ± 0.005 ($n = 13$). The plasma half-life of theophylline in patient D was calculated to be 16 h by the non-linear least square fitting.

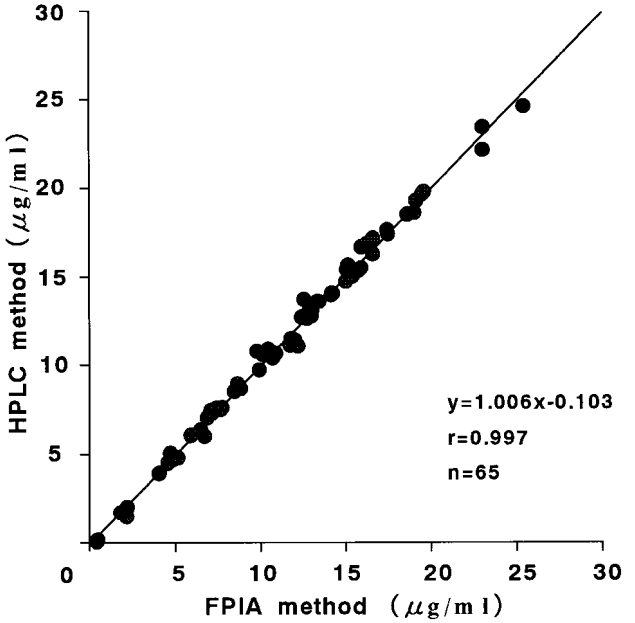

Figure 6. Correlation between plasma theophylline levels obtained by HPLC and FPIA.

Copyright © 1999 John Wiley & Sons, Ltd.

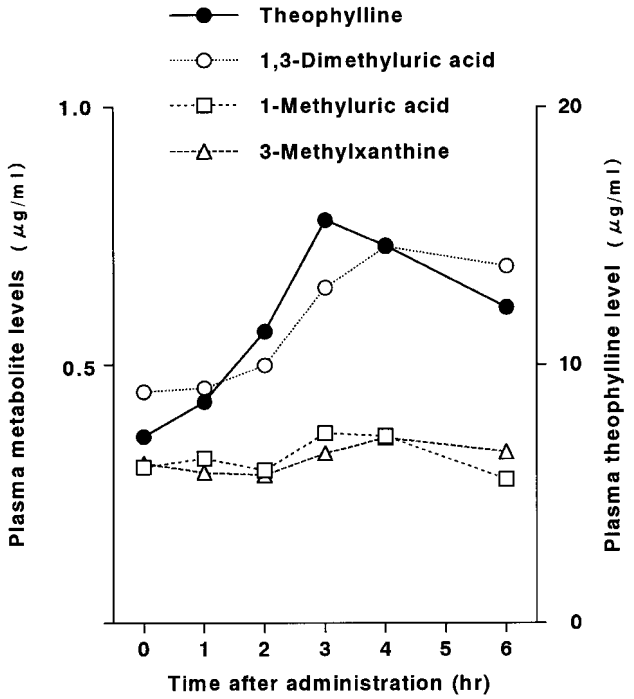

Figure 7. Time-course analysis of plasma levels of theophylline and its metabolites in patient A given aminophylline infusion treatment.

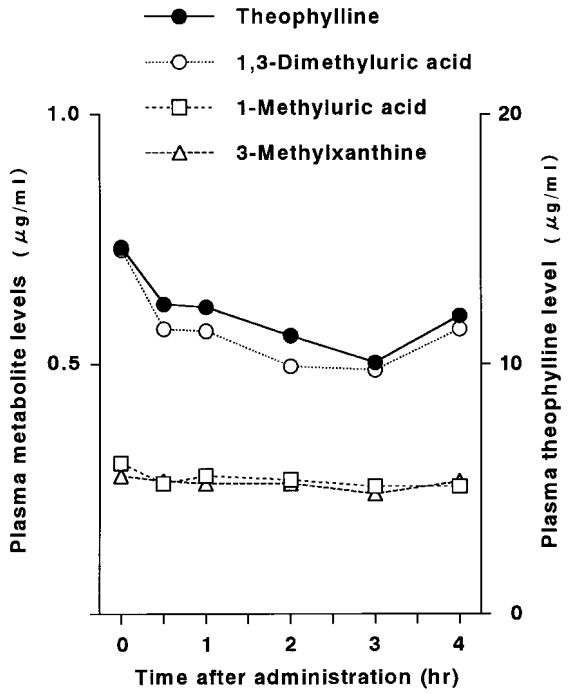

Figure 9. Time-course analysis of plasma levels of theophylline and its metabolites in patient C given oral slow-release theophylline tablets.

Plasma levels of theophylline and its metabolites in outpatients. The plasma levels of theophylline in the 67 outpatients were 0–21.761 μg/mL as determined by our HPLC method. In three outpatients, no theophylline was detected and in another patient only a very low level of theophylline (0.24 μg/mL) was detected in spite of the prescription of a slow-release preparation of the drug. Consultation with their doctor revealed that these outpatients had not taken the medicine as requested.

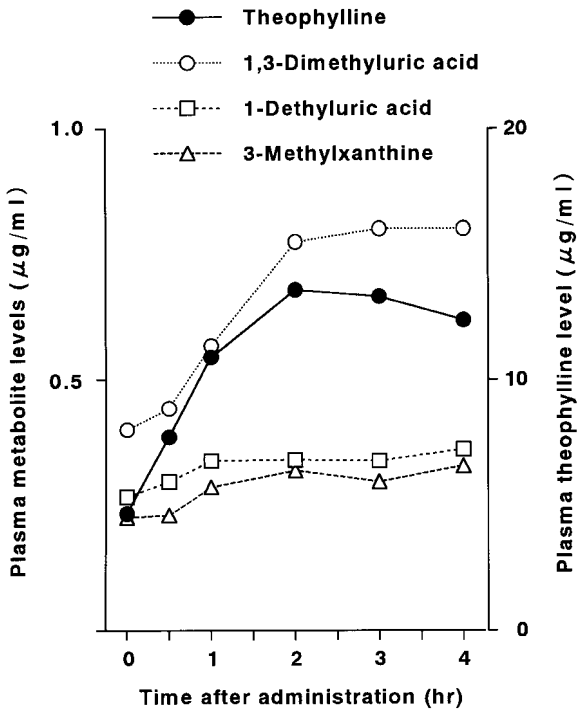

Figure 8. Time-course analysis of plasma levels of theophylline and its metabolites in patient B given aminophylline infusion treatment.

The plasma levels of 1,3-dimethyluric acid were 0–1.021 μg/mL. As shown in Fig. 11, there was a strong correlation between the plasma level of 1,3-dimethyluric acid and that of theophylline, correlation coefficient $r = 0.960$ ($p < 0.001$) for 17 outpatients who were administered with rapid-release preparations of theophylline. A similar result was also seen for the 50 outpatients who were administered slow-release preparations of theophylline, $r = 0.949$ ($p < 0.001$). DMU/TP ratios were calculated as 0.056 ± 0.009 ($n = 17$) for the outpatients administered with rapid-release preparations and 0.054 ± 0.011 ($n = 50$) for the outpatients with slow-release preparations, which agreed well with the ratios for patients A, B and C. The overall DMU/TP ratio was 0.055 ± 0.010 ($n = 66$) including the 63 outpatients and the three inpatients but omitting patient D.

The chromatograms for almost all outpatients showed peaks corresponding to caffeine and its metabolites, but there was no correlation between the peak-height and the plasma theophylline level.

DISCUSSION

The HPLC method developed in this study was capable of separating theophylline, caffeine, and their various

Copyright © 1999 John Wiley & Sons, Ltd.

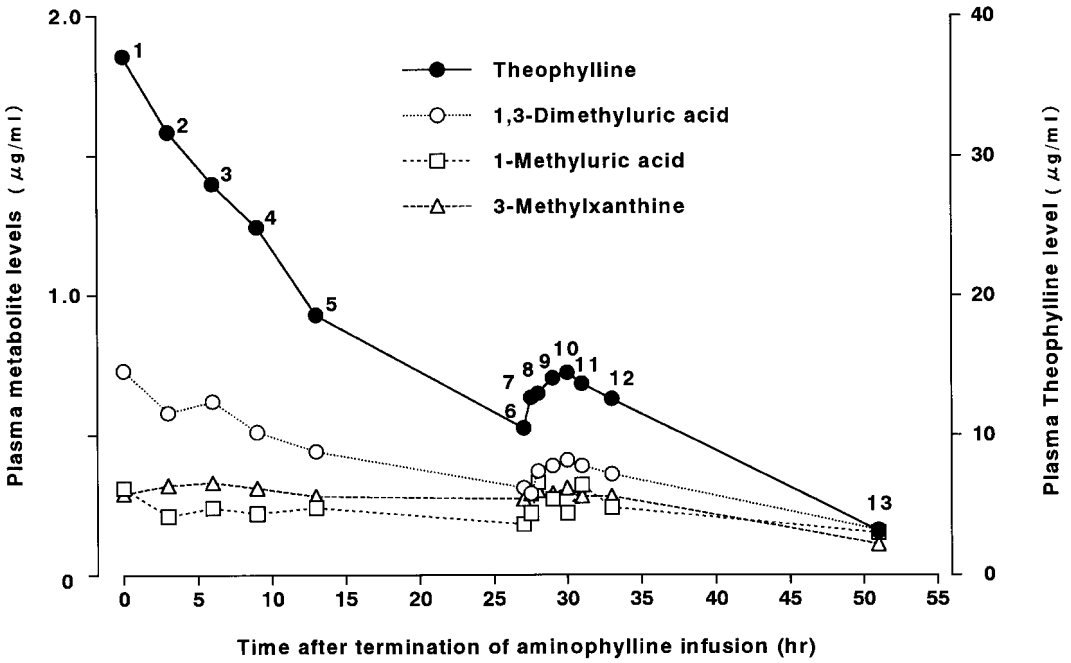

Figure 10. Time-course analysis of plasma levels of theophylline and its metabolites in patient D after stopping aminophylline infusion (No. 1–6) and starting oral aminophylline (No. 7–13).

metabolites, from each other by a simple procedure and, hence, it was not necessary to ban the intake of caffeine-containing beverages. Furthermore, the quantitative precision of the whole procedure was excellent (Table 1). The method was validated for theophylline determinations by its good correlation with FPIA, which is conventionally regarded as a highly reliable technique (Kawakatsu and Chikuma, 1985). Thus, the present method should be fully applicable to the simultaneous assay of theophylline and its metabolites in routine therapeutic drug monitoring.

Time-course analysis of plasma samples from inpatients with bronchial asthma revealed that the levels of theophylline and its major metabolite, 1,3-dimethyluric acid, changed in parallel with each other, while the other metabolites, 3-methylxanthine and 1-methyluric acid, did not change as much, regardless of way in which theophylline was administered. A high correlation was also observed between the level of theophylline and that of 1,3-dimethyluric acid in the outpatients and this was not dependent on the form of theophylline given (Fig. 11).

The metabolic pathway of theophylline is known to have several saturable steps. The maximal rate of formation of metabolites (V_{max}) and the concentration of theophylline at which metabolite formation rate is half of V_{max} (K_m), were reported to be 4.9 mg/h and 2.0 μg/mL, respectively, for 3-methylxanthine, 13.1 mg/h and 9.3 μg/mL for 1-methyluric acid, and 34.2 mg/h and 14.2 μg/mL, respectively, for 1,3-dimethyluric acid (Tang-Liu *et al.*, 1982b). Therefore, in the range of effective plasma theophylline levels (10–20 μg/mL), the

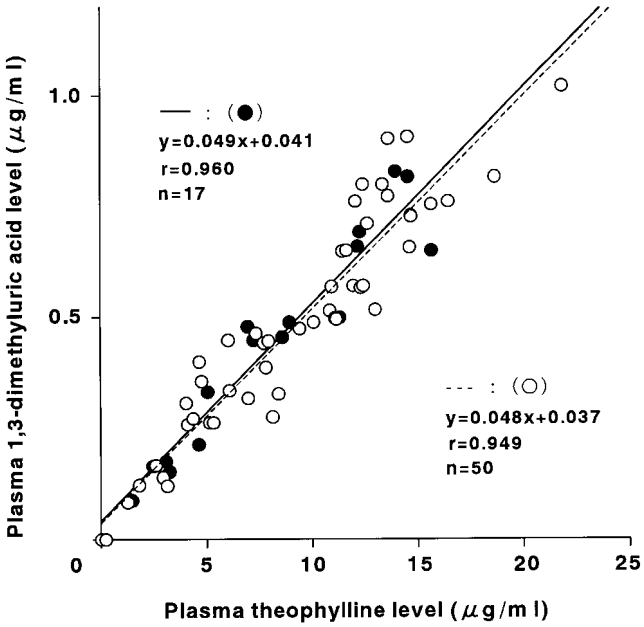

Figure 11. Correlation between plasma theophylline and plasma 1,3-dimethyluric acid levels in patients given rapid-release preparations (●) and slow-release preparations (○). ——: ●'s regression line ($r = 0.960$, $n = 17$) - - - - - - -: ○'s regression line ($r = 0.949$, $n = 50$).

former two steps are almost saturated, while the latter step has the largest capacity and is still in a range of concentration-dependent formation. This seems to explain the phenomena seen in the time-course analysis and may also be true for the high correlation between the

Copyright © 1999 John Wiley & Sons, Ltd.

plasma levels of theophylline and 1,3-dimethyluric acid observed among the outpatients (Fig. 11).

Since 1,3-dimethyluric acid is the major metabolite and its formation depends on theophylline concentration, the DMU/TP ratio would be a good indicator for the metabolic clearance of theophylline. The DMU/TP ratio was almost constant at 0.055 ± 0.01 for all patients except for patient D, regardless of the dose form of theophylline and the blood sampling time. This ratio is close to the ratios calculated for theophylline and its metabolites by Naline *et al*. (1987) (0.054, $n = 12$), by Beckmann *et al*. (1987) (0.055, $n = 5$) and by Hurwitz *et al*. (1991) (0.060, $n = 8$), suggesting that there is no difference in theophylline metabolism between different races.

For patient D, the DMU/TP ratio was as low as 0.027 ± 0.005 and the half-life of theophylline was as long as 16 h. The half-life of theophylline generally varies according to the age of the patient; 30 h for neonates, 4.4 h for 3 month–1-year-olds, 3–4 h for 1–16-year-olds, 6.6 h for 17–50-year-olds and 8.0 h for over 50-year-olds (Kawakatsu and Kawai, 1988). Considering that patient D was only 14-years-old, a half-life of 16 h is very long. Since 1,3-dimethyluric acid is a major metabolite of theophylline, the low DMU/TP ratio seen in patient D may reflect the slow metabolism of theophylline to 1,3-dimethyluric acid. In the report of Kodera *et al*. (1985), for the patient whose theophylline half-life was as short as 3.2 h, the plasma level of 1,3-dimethyluric acid was very high relative to that of theophylline (DMU/TP ratio = 0.117). These facts strongly support the idea that there might be a close relationship between the DMU/TP ratio and the half-life of theophylline. Thus, the single determination of the plasma DMU/TP ratio by the present HPLC method may predict the plasma half-life of theophylline. Further work on a larger variety of patients is required to confirm this.

REFERENCES

Beckmann J., Elsaßer W., Gundert-Remy U. and Hertrampf R. (1987). *Eur. J. Clin. Pharmacol.* **33**: 227.

Hamasaki Y., Ichimaru T. and Miyazaki S. (1986). *Jap. J. Clin. Chem.* **15**: 44.

Hendeles L., Weinberger M. and Johnson G. (1978). *Clin. Pharmacokinet.* **3**: 294.

Hurwitz A., Vacek J. L., Botteron G. W., Sztern M. I., Hughes E. M. and Jayaraj A. (1991). *Clin. Pharmacol. Ther.* **50**: 299.

Kawakatsu K. and Chikuma M. (1985). *Jap. Pharmacol. Ther.* **13**: 4509.

Kawakatsu K. and Kawai M. (1988). *Ther. Res.* **8**: 34.

Kodera H., Misawa M., Yanaura S., Iwata T., Kobayashi N., Ishimura K. and Hayakawa H. (1985). *Allergy* **34**: 33.

Leakey T. E. B., Elias-Jones A. C., Coates P. E. and Smith K. J. (1991). *Clin. Pharmacokinet.* **21**: 400.

Muir K. T., Kunitani M. and Riegelman S. (1982). *J. Chromatogr.* **231**: 73.

Naline E., Flouvat B., Advenier C. and Pays M. (1987). *J. Chromatogr.* **419**: 177.

Richer M. and Lam Y. W. F. (1993). *Clin. Pharmacokinet.* **25**: 283.

Saito K. and Muto Y. (1987). *Medical Practice 4* (suppl.), 252.

Sarkar M. A., Hunt C., Guzelian P. S. and Karnes H. T. (1991). *Drug Metab. Disp.* **20**: 31.

Tang-Liu D. D., Williams R. L. and Riegelman S. (1982a). *Clin. Pharmacol. Ther.* **31**: 358.

Tang-Liu D. D., Williams R. L. and Riegelman S. (1982b). *J. Pharmacol. Exper. Ther.* **224**: 180.

Tserng K. Y., King K. C. and Takieddine F. N. (1981). *Clin. Pharmacol. Ther.* **29**: 594.

Williams J. F., Lowitt S., Polson J. B. and Szentivanyi A. (1978). *Biochem. Pharmacol.* **27**: 1545.

Copyright © 1999 John Wiley & Sons, Ltd.