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# A liquid chromatographic method for determination of theophylline in serum and capillary blood — a comparison*

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**Abstract**: A simple, fast and reliable liquid chromatographic method for the determination of theophylline in serum and capillary blood after a solid phase extraction is described for therapeutic drug monitoring. The employment of capillary blood permits the determination of an individual drug profile and other pharmacokinetic studies in neonates and infants. There were no differences in venous- and capillary-blood levels but these values compared poorly with those in serum. An adjustment of the results by correction of the different volumes of serum and blood by haematocrit was unsuccessful.

Differences in the binding of theophylline to erythrocytes could be an explanation for the differences in serum at blood levels of theophylline.

**Keywords**: *Theophylline therapeutic drug monitoring; capillary blood; serum; HPLC; solid phase extraction.*

## Introduction

The bronchodilating agent theophylline is often not used to full benefit because of its narrow therapeutic index [1]. It is generally necessary to individualize oral theophylline therapy because of differences in metabolic rate [2]. These individual differences in the pharmacokinetics and pharmacodynamics of theophylline are caused by a variety of factors including genetic polymorphism [3], other drugs [4–7], ethanol, cigarette smoke [5, 8] and other environmental factors [8]. Furthermore, theophylline clearance depends on age being higher during childhood [9, 10]. Several techniques have been described for the quantitative determination of theophylline in serum, saliva and blood [11–15], including photometry [12, 13], TLC [16], GC [15], HPLC [17] respectively, dry reagent strip [18] and immunological methods [19]. One problem in the determination of theophylline in blood and serum in infants or neonates is the need for venous puncture. This paper describes an HPLC method for determination of theophylline in capillary blood after solid phase extraction.

## Experimental

### *Materials and reagents*

Theophylline, theobromine and caffeine (Bergakademie Freiberg, Germany) were used as analytical standards without purification. 8-Bromotheophylline was used as internal standard and, being synthesized by bromination of theophylline, was found to contain 3% theophylline. All other chemicals were analytical reagent grade and all solvents were HPLC grade. The exception to the latter was solvents for the liquid–liquid extraction which were analytical reagent grade and purified by filtration through basic $Al_2O_3$, Activity I and then distilled.

The C18 (60 μm) solid-phase extraction columns and the HPLC Column Separon SGX-C18-glass cartridges (150 × 3 mm, 5 μm), fitted with a Separon SGX-C18 (30 × 3 mm, 7 μm) guard column, were purchased from Tessek Ltd, Czechoslovakia.

### *Equipment*

The liquid chromatography comprised an isocratic Shimadzu HPLC-pump LC6A (Shimadzu Europa GmbH, Germany) fitted

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with a sample loop LCI 30, a fixed wavelength detector LCD 2563 and a computing integrator CI-100 (Laboratorni pristroje, Czechoslovakia). A vacuum manifold apparatus (Dorcus, Tessek Ltd, Czechoslovakia) was used for the solid phase extraction.

*Standards and solutions*

Stock solutions of the standards were prepared by dissolving the substances in a small volume of methanol and then adjusting to a concentration of 100 mg $l^{-1}$ with mobile phase.

The stock solutions were diluted with water to give calibration-curve solutions in the range 5–50 mg $l^{-1}$. The mobile phase was methanol–potassium phosphate buffer (10 mM, pH 3.0, 40:60 w/w).

*Sample preparation*

The sample preparation was carried out, either by precipitating and centrifuging 1 part serum with 2 parts acetonitrile (containing internal standard), by liquid–liquid extraction with a methylenechloride (or chloroform) isopropranol (95:5, v/v) or by liquid–solid extraction.

For the liquid–liquid extraction 200 µl of serum was mixed with 200 µl 1 M HCl and extracted with 2 ml of the extraction solvent (containing internal standard). After centrifugation the organic layer was evaporated and dissolved in 100 µl of mobile phase. After centrifugation 20 µl of the sample were injected. For the liquid–solid extraction, 100 µl serum or blood was diluted with water or phosphate buffer pH 3.0 (containing internal standard) and passed through a C18 cartridge which was preconditioned with methanol. The sample was washed three times with 2 ml water and dried with vacuum for 2 min. The last water wash contained 100 µl *n*-hexane. The analytes were eluted with 1.0 ml methanol. The last procedure was the most reliable and reproducible method of sample preparation.

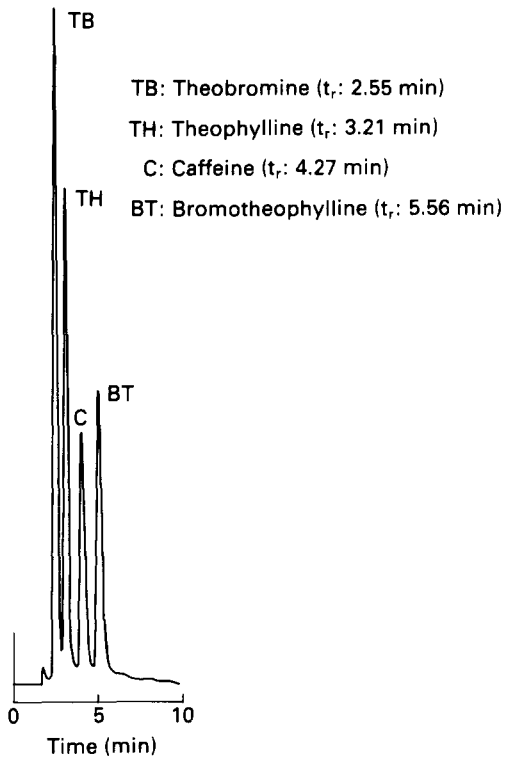


**Figure 1**
HPLC-separation of a standard mixture. HPLC-conditions: Column Separon SGX-C18, 5 µm 150 × 3 mm fitted with a guard column Separon SGX-C18, 7 µm, 30 × 3 mm, mobile phase methanol–potassium phosphate buffer pH 3.0 (40:60, w/w), flow rate 0.5 ml $min^{-1}$, pressure 18 bar, detection at 254 nm.

*Liquid chromatography*

Twenty microlitres of the theophylline solutions or extracts were injected onto a Separon SGX-C18 (5 µm) glass cartridge column fitted with a guard column (Separon SGX-C18, 7 µm). The separation was carried out isocratically at a flow rate of 0.5 ml $min^{-1}$ (Fig. 1). Detection was either at 254 or 270 nm.

## Results and Discussion

The described HPLC method for the determination of theophylline as well as for caffeine and some other purines is fast, practicable and reliable. By application of solid-phase extraction a better clean up of biological fluids is obtained than by liquid–liquid extraction. Furthermore, by solid phase extraction it was possible to isolate theophylline from capillary blood (CB) (Fig. 2). The theophylline values of adults in serum (S) and capillary blood (CB) were significantly different by calculation with the *t*-test for paired data ($t$: 6.02, $t_{0.05}$: 2.05 two-tailed probability). In contrast the linear regression analysis gave good correlation:

$$y_{VB} = 0.98 + 0.72\, x_S;\ r = 0.957;\ n = 54$$

(Fig. 3a).

The values for venous (VB) and capillary blood (CB) were not significantly different by *t*-test ($t$: 1.41; $t_{0.05}$: 2.07) and gave good correlation.

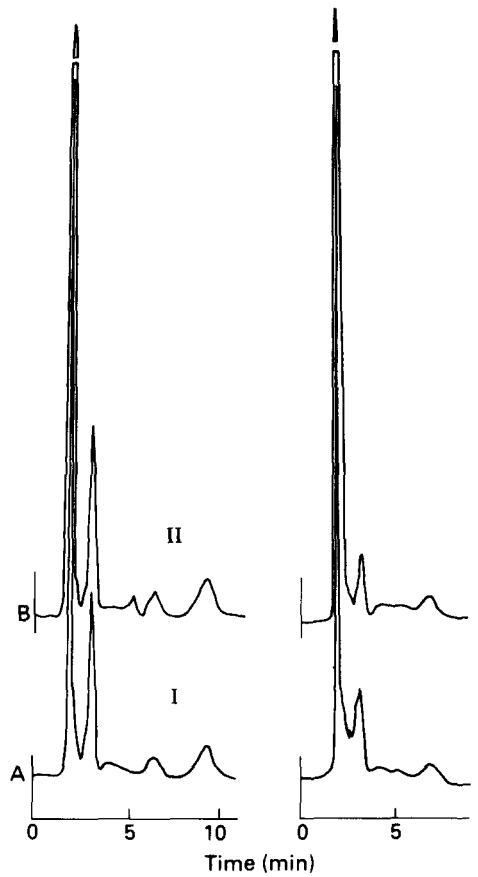


**Figure 2**
HPLC-separation of theophylline in capillary blood after solid phase extraction (A) before, (B) 4 h after theophylline administration. I: patient with a good compliance to theophylline. II: patient with bad compliance to theophylline.

$$y_{CB} = 0.88 + 0.92\, x_{VB};\ r = 0.977;\ n = 23$$

(Fig. 3b).

The relative standard deviations after solid phase extraction of replicate theophylline samples (5 mg $l^{-1}$) were 2.32% for serum, 4.78% for venous blood and 6.71% for capillary blood ($n$ = 10). The detection limit for a pooled serum at a signal-to-noise ratio of six times the standard deviation was 1 ng per 20 µl injection, corresponding to 0.5 mg $l^{-1}$ (detection at 254 nm).

Since the detection limit depended on the integrator-parameters, such as peak width, minimal peak area, and integration time, an additional safety factor ($F$ = 30) for the quantitative determination was used.

The therapeutic range for theophylline in serum is 10–20 mg $l^{-1}$. No such value for blood has been reported. A correction of estimated

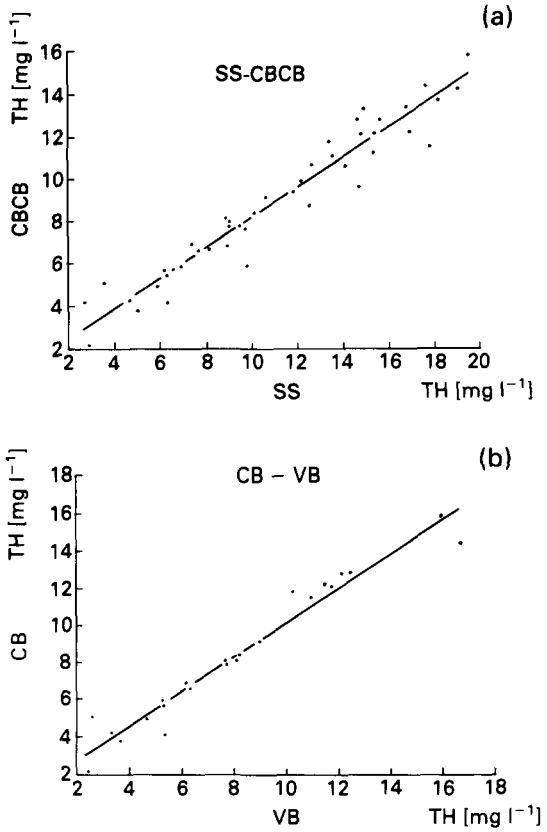


**Figure 3**
Correlations of theophylline values in venous blood ($y$) and in serum ($x$) (a), and between the values in capillary ($y$) and venous ($x$) blood (b).

values in blood by factorizing (e.g. with the help of haematocrit) to those in serum is not possible.

Up to a borderline value of 2.5 mg $l^{-1}$ (below the therapeutic range) the values in blood are higher than in serum and vice versa in the range upper 2.5 mg $l^{-1}$.

A new therapeutic range for blood needs to be defined, for adults, infants and neonates. A better evaluation of the theophylline compliance could be obtained from the theophylline ratio in serum and blood. The described, practicable determination of theophylline in capillary blood should enable drug monitoring in neonates, as well as infants and adults [20].

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