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Note

A rapid micromethod for the high-performance liquid chromatographic determination of theophylline in human serum

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Theophylline (1,3-dimethylxanthine) has been used extensively for the treatment of asthma and more recently for the management of apnea and bradycardic spells in premature infants. The absorption of theophylline varies considerably from subject to subject so that blood levels are not predictable even when the same unit dosage (mg/kg) is administered. Since adverse drug reactions have been associated with serum theophylline concentrations in excess of therapeutic levels, the rational clinical use of this agent may be facilitated by careful monitoring of serum levels.

Several methods have been described for the determination of theophylline in biological fluids. The spectrophotometric assay [1] has been largely replaced by gas chromatographic (GC) [2—9] and high-performance liquid chromatographic (HPLC) [10—15] techniques. Most of the GC procedures involve solvent extraction of the drug followed by derivatization and are often time consuming. The HPLC methods, on the other hand, do not require derivatization, but most of the reported procedures require solvent extraction.

A new HPLC procedure for the determination of theophylline in serum has been developed in which proteins are removed by perchloric acid precipitation and, after neutralization of the supernatant, an aliquot is injected directly into the chromatographic system. Serum volumes as low as 50 μl can be analyzed successfully; this makes the method particularly attractive for pediatric work.

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EXPERIMENTAL

All chemicals used were reagent grade with the exception of methanol, which was glass distilled (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.).

Procedure

A 100-μl serum specimen was pipetted into a tube containing 6 μl of 70% perchloric acid. After mixing for 30 sec on a Vortex mixer, the tube was kept in ice for 20 min; this was followed by centrifugation at room temperature to remove the denatured proteins. The supernatant was transferred to another tube and 10 mg of potassium carbonate were added. The contents were mixed on a Vortex mixer and centrifuged briefly to precipitate the potassium perchlorate formed. An aliquot was then injected on the column.

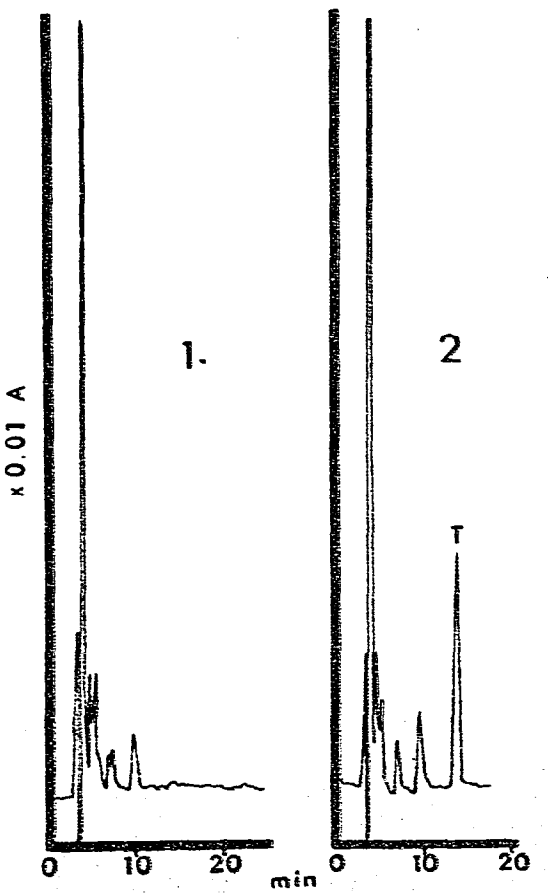

Fig. 1. HPLC of human serum samples. 1, Control serum; 2, serum containing 10 μg/ml theophylline (T). Conditions: 30 cm × 4 mm I.D. μBondapak C_{18} column, with methanol—10 m*M* sodium dihydrogen phosphate (1:4) as eluent; flow-rate 0.8 ml/min; detection at 280 nm.

Liquid chromatography

Analyses were performed on a Waters Assoc. Model 6000 pumping system coupled to a Spectroflow SF 770 multiwavelength detector (Schoeffel Instrument Corp.). A reversed-phase system consisting of a 30 cm × 4 mm μBondapak C_{18} column (Waters Assoc.) and methanol—10 m*M* sodium dihydrogen phosphate (1:4) as eluent at a flow-rate of 0.8 ml/min were used. Absorbance was monitored at 280 nm. The detector was operated at a sensitivity of 0.01 a.u.f.s. Peak heights were used for quantitation. All standard curves were linear and passed through the origin.

RESULTS AND DISCUSSION

Representative chromatograms of serum samples shown in Fig.1 demonstrate that control samples are free from contaminating peaks. Dietary xanthines, caffeine and theobromine and theophylline metabolites did not interfere with the assay. The average recovery of theophylline added to serum over the range of 1 to 25 μg/ml was 88.2 ± 4.9% (mean ± S.E., n = 16). The lower limit of detection was 1 μg/ml serum.

This technique is very simple and involves no lengthy solvent extraction or derivatization procedures. When sample size is limited, as in the case of premature infants being treated for apneic spells, the whole procedure can be successfully performed with 50 μl of serum. Most earlier chromatographic procedures require larger sample volumes. Very recently Least et al. [9] have reported a GC method that can be performed on 20-μl samples; however, their method involves extraction and derivatization. The method described in this paper is currently being used for pharmacokinetic studies of theophylline in premature and full-term infants and the results will be published at a later date.

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