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Note

Rapid method for the high-performance liquid-chromatographic determination of acephylline in human serum

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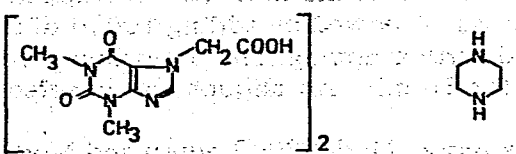
Acephyllinepiperazine (Acepiphylline, Etaphylline ®) has been used for the treatment of asthma. Acephylline is a derivative of theophylline and is chemically described as 1,3-dimethylxanthine-7-acetic acid (7-theophylline-acetic acid).



acephylline piperazine

Until now no specific method for the determination of acephylline has been described in the literature. Turner-Warwick [1] used the spectrophotometric assay of theophylline introduced by Schack and Waxler [2]. This method is not specific and involves solvent extraction of the drug. The author interpreted the results as theophylline levels. There is no evidence that acephylline is metabolized to theophylline and the results of this author therefore give a false picture of the real blood levels. The UV absorption curves are different, as can be seen from Fig. 1.

A new high-performance liquid-chromatography (HPLC) procedure for the determination of acephylline and study of its absorption and pharmacokinetics in serum has been developed. Proteins are removed by precipitation with per-

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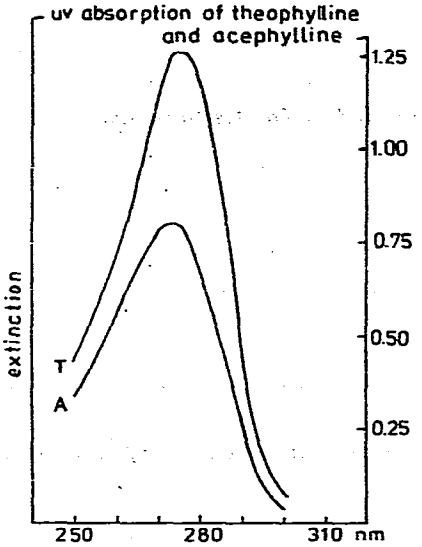

Fig. 1. The UV absorption curves of theophylline (T, 20 μg/ml) and acephylline (A, 26.4 μg/ml). The concentrations are molar equivalents.

chloric acid. The supernatant is neutralized by potassium carbonate. Excess perchloric acid is thus precipitated as potassium perchlorate.

PROCEDURE

A 0.5-ml serum sample was pipetted into a tube containing 50 μl of 70% perchloric acid. After mixing for 30 sec on a whirl-mixer, the tube was centrifuged to precipitate the denatured proteins. This was followed by adding 100 μl of a saturated potassium carbonate solution and further mixing. The potassium perchlorate was precipitated by centrifuging for 10 min. An aliquot was injected on to the column.

Analyses were performed using a Waters Assoc. Model 6000 pump and Modet 440 absorbance detector. A reversed-phase system was used, consisting of a Bondapak C_{18} column (30 cm × 4 mm I.D.) with a particle size of 10 μ (Waters Assoc.), and methanol 4% in 0.01 *M* sodium dihydrogen phosphate, at a flow-rate of 2.0 ml/min. Absorbance was monitored at 280 nm. The detector was operated at a sensitivity of 0.02 a.u.f.s. Peak heights were used for quantitation.

RESULTS AND DISCUSSION

Chromatograms of serum samples (Fig. 2) demonstrate that no contamination peaks occur. The retention time of acephylline is 10 min. Decreasing this time by increasing the methanol concentration in the eluent causes the serum and acephylline peaks to merge. Dietary xanthines, caffeine, theophylline and theobromine and metabolites did not interfere with the assay. The standard curve of acephylline added to serum was linear over the range 1–50 μg/ml and

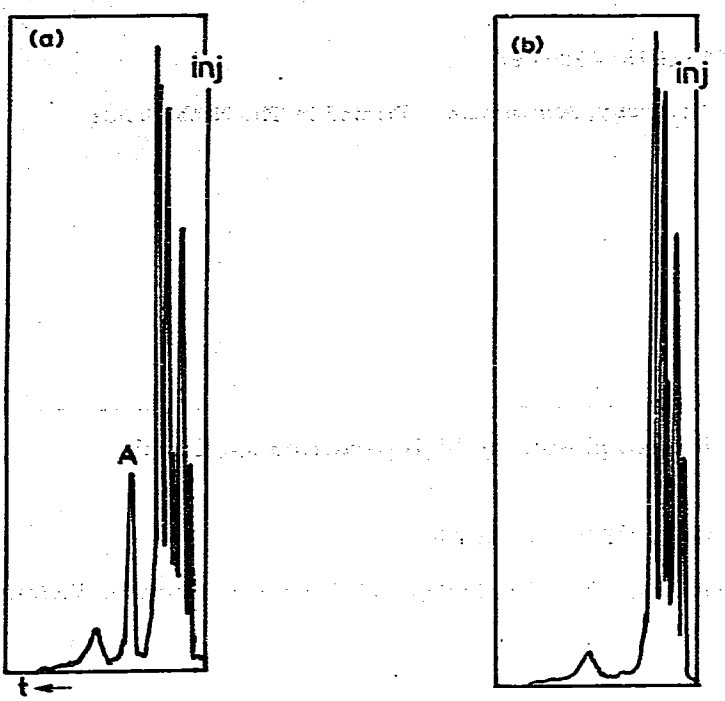

Fig. 2. a, representative chromatogram of the HPLC of acephylline in human serum. A, acephylline. b, chromatogram of a blank human serum.

passed through the origin. The correlation coefficient was $r = 0.997$, and the standard deviation 2.8% ($n = 6$ at 10 μg/ml.) The lower detection limit was 1 μg/ml serum.

This HPLC method is easy to perform, involves no extraction or derivatization procedures, and can be successfully performed with 50 μl of serum. The standards were made in serum, so the recovery was 100%.

A white Wener rabbit was administered acephyllinepiperazine 4 mg/kg as an intravenous bolus. Venous blood samples were taken every 5 min. From the decline of the concentration curve an elimination half-life of 15 min was found. After 1 h neither acephylline nor theophylline could be detected in the serum. These investigations will be continued with volunteers and patients.

ACKNOWLEDGEMENT

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REFERENCES

1 M. Turner-Warwick, Brit. Med. J., 2 (1957) 67.
2 S. Schack and J. Waxler, J. Pharmacol. Exp. Ther., 97 (1949) 283.