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Note

Simultaneous determination of dyphylline and theophylline in human plasma by high-performance liquid chromatography

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Dyphylline [7-(2,3-dihydroxypropyl)theophylline] and theophylline are used clinically as bronchodilators in the treatment of asthma. At present, their therapeutic effect appears to be dependent upon their concentration in the blood, with the effective range 10—20 μg/ml. In order to compare and correlate efficacy with plasma levels of dyphylline with those of theophylline in clinical studies, a fast and accurate analytical method for both drugs is needed in which other dietary xanthines do not interfere.

A number of procedures using high-performance liquid chromatography (HPLC) have been published for theophylline [1—12] and dyphylline [13—17]. But only the procedure of Maijub and Stafford [13] can determine both drugs simultaneously. However, this procedure requires silica column deactivation and extraction from plasma with 65% recovery for dyphylline. We have developed a rapid, precise and accurate method for the simultaneous determination of dyphylline and theophylline in the presence of caffeine and theobromine which is suitable for automated HPLC analysis of human plasma samples. The results of our experiments are described in this report.

EXPERIMENTAL

Materials

All reagents were analytical reagent grade. Aqueous solutions were prepared using deionized water (Mill-Q-Water System, Millipore, Bedford, MA, U.S.A.).

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Glass-distilled acetonitrile (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) was used for HPLC. Theophylline was obtained from Pfaltz and Bauer (Stanford, CT, U.S.A.) and dyphylline (Lot No. CRM 1062) from Mallinckrodt Pharmaceutical Division (Mallinckrodt, St. Louis, MO, U.S.A.). Theobromine, caffeine and β-hydroxyethyltheophylline (β-HET) were obtained from Sigma (St. Louis, MO, U.S.A.).

High-performance liquid chromatography

The chromatograph was a Hewlett-Packard Model 1084B, equipped with a variable-wavelength spectrophotometric detector, automatic sampler and LC terminal. A 10-μm μBondapak C_{18} column (Waters Assoc., Milford, MA, U.S.A.) was used; the mobile phase was acetate buffer—acetonitrile (94:6). The buffer was prepared by adjusting the pH of a 0.01 *M* solution of sodium acetate in deionized water to 4.0 with reagent grade glacial acetic acid. The column oven temperature and solvent temperature were 40°C. A flow-rate of 2.0 ml/min was used yielding an operating pressure of 85 bar (approximately 1300 p.s.i.). The spectrophotometric detector had a 12-μl cell volume and was operated at a wavelength of 274 nm.

Standards

Standard solutions of dyphylline and theophylline (5 mg/ml) were prepared in buffer and stored at 4°C. These solutions were then diluted as necessary to prepare the appropriate plasma standards for each drug and each assay run. The internal standard of β-hydroxyethyltheophylline was also prepared in buffer (0.2 mg/ml) and stored at 4°C. Peak area ratios of dyphylline and theophylline to β-hydroxyethyltheophylline were determined for plasma standards.

Sample preparation procedure

To 1 ml of plasma (or standard) were added 100 μl of an internal standard solution and 100 μl of a 40% aqueous trichloroacetic acid solution. The mixture was vigorously stirred for 30 sec on a Vortex Genie Mixer (Scientific Products, Evanston, IL, U.S.A.), allowed to stand for 5 min, and then centrifuged for 15 min at 2000 *g*. The supernatant was transferred to a 2-ml vial, sealed and placed in the automatic sampler. A blank plasma sample (1.0 ml) was treated in an identical manner. The sampler injected a 25-μl volume on to the column of the high-performance liquid chromatograph.

Recovery and reproducibility

Drug recovery from plasma after protein precipitation was determined at concentrations of 2.5, 5.0, 10.0, 25.0 and 50.0 μg/ml in plasma by comparing the peak areas with those obtained for aqueous solutions containing known concentrations of dyphylline and theophylline. Reproducibility was determined for the same concentration range by quadruplicate analysis of samples at each concentration.

RESULTS AND DISCUSSION

A linear relationship between the peak area ratio and plasma concentration

of theophylline and dyphylline exists in the range of 2.5—50 μg/ml. The correlation coefficient is 1.0000.

The precision (reproducibility) of this method was determined by quadruplicate analyses of standard samples at each concentration. The results (Table I)

TABLE I

PRECISION (C.V.) AND ACCURACY (M.E.) OF THE SIMULTANEOUS DETERMINATION OF DYPHYLLINE AND THEOPHYLLINE IN HUMAN PLASMA IN THE RANGE 50.0—2.5 μg/ml

Theoretical (μg/ml)	Dyphylline			Theophylline		
	Calculated*	C.V. (%)	M.E. (%)	Calculated*	C.V. (%)	M.E. (%)
50	49.67 ± 1.14	2.3	0.7	47.94 ± 2.53	5.3	4.3
25	24.96 ± 0.70	2.8	0.1	24.09 ± 1.50	6.2	3.8
10	9.90 ± 0.18	1.8	1.0	9.94 ± 0.56	5.7	0.6
5	4.91 ± 0.14	2.9	1.8	5.33 ± 0.33	6.2	6.1
2.5	2.37 ± 0.11	4.8	5.7	2.89 ± 0.16	5.6	13.5

*As mean concentration ± S.D. (μg/ml).

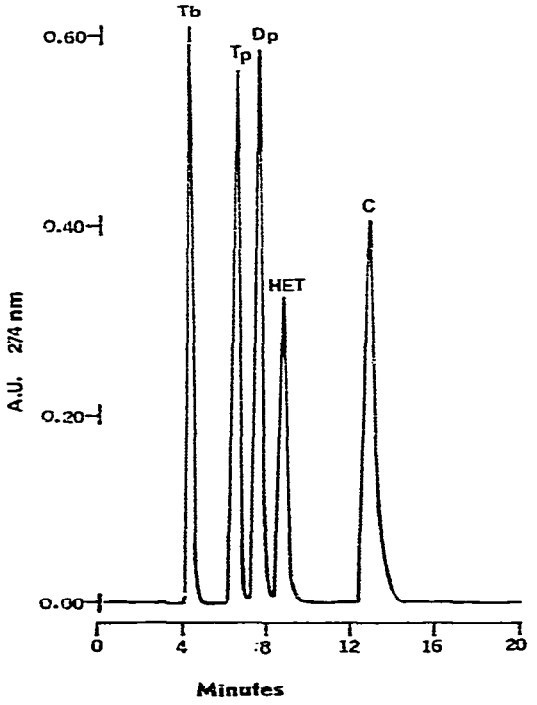

Fig. 1. Separation of theobromine (Tb), theophylline (Tp), dyphylline (Dp) β-hydroxyethyltheophylline (HET) and caffeine (C). Column, μBondapak C_{18}; mobile phase, 0.01 *M* sodium acetate (pH 4.0)—acetonitrile (94:6).

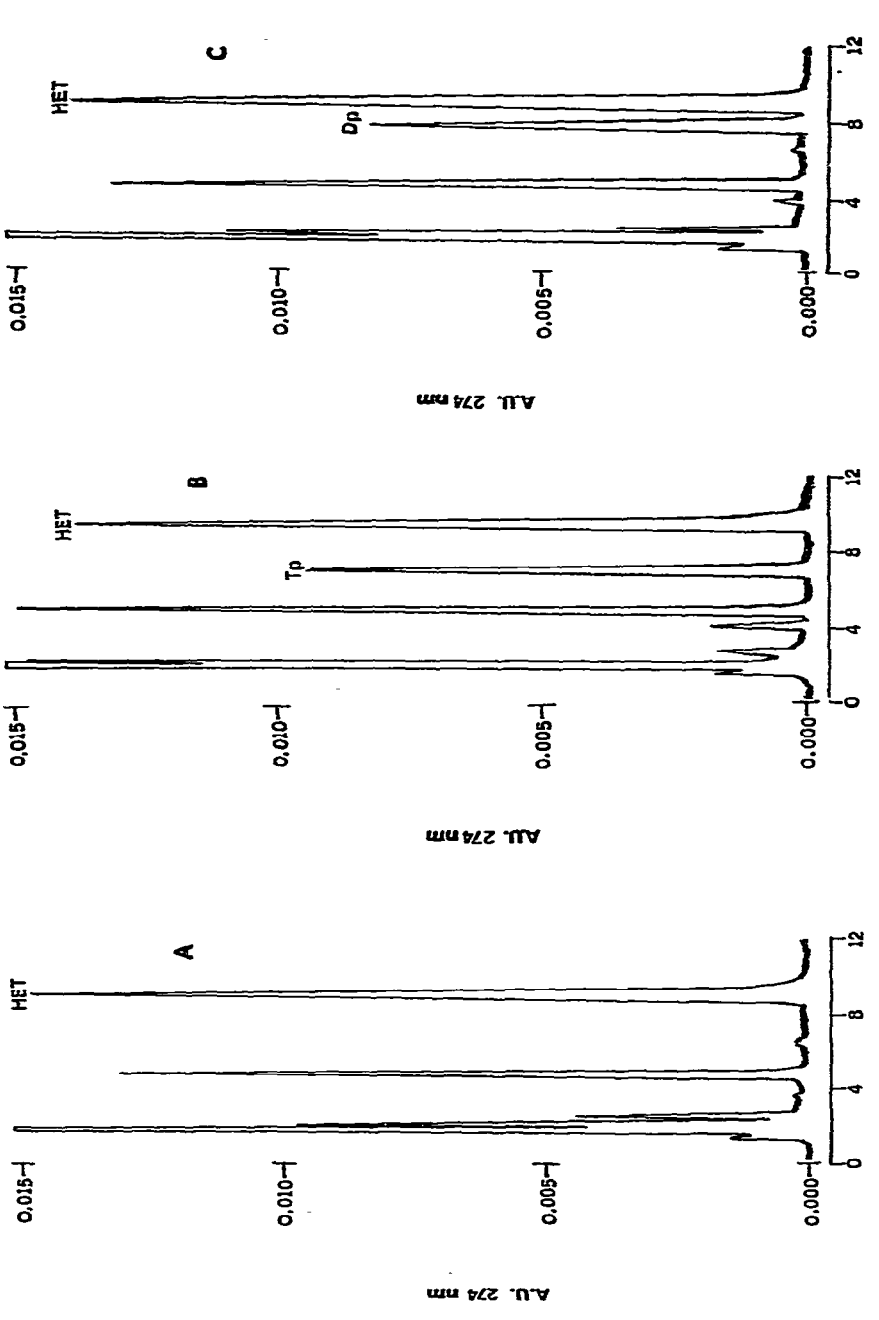

Fig. 2. Chromatogram of plasma from a patient before (A) and after a single oral dose of 6 mg/kg theophylline (B) or 10 mg/kg dyphylline (C). Column, μBondapak C_{18}; mobile phase, 0.01 *M* sodium acetate (pH 4.0)—acetonitrile (94:6).

show that the precision, expressed as the coefficient of variation (CV), was 4.8% or better for dyphylline and 6.2% or better for theophylline.

The accuracy, calculated as relative mean error (M.E.*) was 5.7 and 13.5% or better for dyphylline and theophylline, respectively. The accuracy is more commonly expressed as recovery, which for our method was 94.6—99.9% for dyphylline and 95.9—115.6% for theophylline.

In order to obtain a realistic estimate of the sensitivity of the assay, the limit of detection [18] was calculated based on the peak area ratio value for zero concentration as estimated from linear regression and the standard deviation for the lowest plasma concentration used. The limit of detection was found to be 0.4 μg/ml for dyphylline and 1.0 μg/ml for theophylline.

Complete (baseline) resolution of both drugs from each other and from caffeine and theobromine was considered a prerequisite for a good assay and was achieved by the use of the described mobile phase composition (see Fig. 1). An example of an analysis of plasma obtained 1 h after dosing from the same patient taking 6 mg/kg theophylline (Theolair tablet) or 10 mg/kg dyphylline (Lufyllin tablet) is shown in Fig. 2. The concentrations determined were 8.25 and 10.54 μg/ml, respectively.

The time needed for analysis was 30 min for sample preparation and 12 min for chromatographic analysis. In the automated mode many samples can be prepared for analysis within an hour, and with the automatic sampler capacity of 60 samples, all samples can be analyzed in a 12-h overnight run.

CONCLUSION

An automated HPLC assay has been developed that is sufficiently sensitive, accurate and precise for the routine clinical monitoring of dyphylline and theophylline. Caffeine and theobromine do not interfere.

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*Relative mean error = $\frac{\text{absolute value of theoretical value} - \text{determined value}}{\text{determined value}} \times 100.$

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