MEASUREMENT OF THEOPHYLLINE IN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Preparations containing theophylline are widely used in the management of airways obstruction. In 1957, Turner-Warwick recommended that the plasma level of theophylline for optimum effect should be greater than 10 μg/ml. More recently Jenne, Wyze, Rood & MacDonald (1972) suggested that the practical minimum serum level for maximum bronchodilatation lies in the range 10-20 μg/ml. Jenne *et al.* (1972) considered that, in 25% of their patients with bronchospasm, a knowledge of serum theophylline levels had contributed to their management. Mitenko & Ogilvie (1973) showed a correlation between plasma levels of theophylline and its effect in asthmatic subjects. In their study a continuous improvement in vital capacity and first-second forced expiratory volume was observed with increasing plasma theophylline levels of 5-20 μg/ml.

Measurement of plasma theophylline levels was described by Schack & Waxler in 1949 and their ultraviolet spectrophotometric method is still widely used. This method is subject, however, to interferences by compounds showing similar ultraviolet absorption characteristics to theophylline, such as caffeine or barbiturates. Gas chromatography of theophylline using flash-heater-N-butylation has been described (Kowblansky, Scheinthal, Cravello & Chafetz, 1973) and recently a method for estimating unchanged theophylline in plasma by gas chromatography has been published (Wesley-Hadzija, 1974). We find high performance liquid chromatography to offer a convenient method for determination of theophylline in plasma.

To plasma (2 ml) were added 0.1 ml of an aqueous solution of phenobarbitone (500 μg/ml) as internal standard and 0.2 ml of 0.1 M HCl. This mixture was extracted by adding 10 ml of 5% isopropyl alcohol in chloroform and shaking gently for 10 min. The mixture was centrifuged and 5 ml of the solvent removed and filtered. The organic phase was evaporated to dryness and the residue dissolved in 30 μl of chromatography solvent mixture. Aliquots (5 μl) were injected into the chromatograph. Standards were prepared for each set of samples by adding known amounts of drug to blank plasma and carrying these through the above procedure.

A Jobling series 1 modular liquid chromatograph was used, incorporating a UV absorbance detector operated at 280 nm. (254 nm operation was also satisfactory). The column was glass, 450 mm x 2 mm i.d., packed with silica gel 10 μm average particle diameter (Partisil 10, Reeve Angel Scientific Limited). The chromatography solvent mixture was methylene chloride/methanol/28% ammonium hydroxide (92 : 7 : 1 v/v/v). Pressure in the system was 250 p.s.i. providing a flow rate of 18 ml/hour.

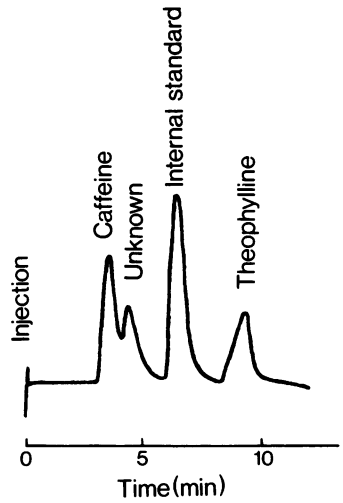

Figure 1 Chromatogram of plasma extract from a subject who had received theophylline. The plasma concentration was 2.2 μg/ml.

Under the above conditions, phenobarbitone and theophylline gave well resolved peaks with retention time of 3.0 min. The ratio of the (Figure 1). Caffeine appeared earlier with a retention time of 3.0 minutes. The ratio of the peak heights of theophylline to internal standard plotted against theophylline concentration gave a straight line passing through the origin. Sensitivity of the method was 0.1 μg/ml theophylline in plasma.

We feel that the sensitivity and technical simplicity of this procedure offers advantages over other methods.

J.G. KELLY & W.J. LEAHEY

Department of Therapeutics & Pharmacology, The Queen's University of Belfast, Whitla Medical Building, 97 Lisburn Road, Belfast BT9 7BL

Received March 3, 1976

References

JENNE, J.W., WYZE, E., ROOD, F.S. & MacDONALD, F.M. (1972). Pharmacokinetics of theophylline. Application to adjustment of the clinical dose of aminophylline. *Clin. Pharmac. Ther.*, **13**, 349-360.

KOWBLANSKY, M., SCHEINTHAL, B.M., CRAVELLO, G.D. & CHAFETZ, L. (1973). Specific gas chromatographic determination of xanthines and barbiturates by flash-heater N-butylation. *J. Chromatog.*, **76**, 467-470.

MITENKO, P.A. & OGILVIE, R.I. (1973). Rational intravenous doses of theophylline. *New Eng. J. Med.*, **289**, 600-603.

SHACK, J.A. & WAXLER, S.H. (1949). An ultraviole spectrophotometric method for the determination of theophylline and theobromine in blood and tissues. *J. Pharmac. exp. Ther.*, **97**, 283-291

TURNER-WARWICK, M. (1957). Study of theophylline plasma levels after oral administration of new theophylline compounds. *Br. med. J.*, **2**, 67-69.

WESLEY-HADZIJA, B. (1974). A simple method for the estimation of theophylline in plasma by gas liquid chromatography. *Br. J. clin. Pharmac.*, **1**, 515-516.