Journal of Chromatography, 104 (1975) 147–150

CHROM. 7945

GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF THEOPHYLLINE IN HUMAN PLASMA

L. J. DUSCI, L. P. HACKETT and I. A. McDONALD
State Health Laboratories, P.O. Box F.312, Perth, Western Australia 6001 (Australia)
(Received August 28th, 1974)

SUMMARY

A gas–liquid chromatographic method for the estimation of theophylline in human plasma is described. The method is based on the methylation of theophylline on-column and allows the determination of concentrations of the drug down to the level of 1.0 μg/ml in plasma.

INTRODUCTION

Theophylline is currently being used for the treatment of bronchial asthma and other cardiorespiratory disorders. There is now good evidence that both the therapeutic response and toxic side-effects are related to the concentration of theophylline in the plasma, rather than to its dosage[1].

Since the bronchodilatory effect of theophylline is directly related to the plasma concentration within the range 3.5 to 15 μg/ml (ref. 2), an accurate analytical method should make it possible to control treatment and reduce the risk of dangerous toxic symptoms.

There are few methods relating to the analysis of theophylline reported in the literature. The ultraviolet method of Schack and Waxler[3] suffers from the interference of caffeine and barbiturates and lacks specificity. Although theophylline can be analysed by gas–liquid chromatography (GLC) as the free compound, peak shape and sensitivity are poor. The method described in this paper is suitable for the analysis of theophylline in plasma down to levels of 0.1 mg%, theophylline being quantitatively converted to caffeine by flash-heater methylation with trimethylanilinium hydroxide[4].

EXPERIMENTAL AND RESULTS

Reagents

The following reagents were used: trimethylanilinium hydroxide (TMAH), 0.2 *M* in methanol (Supelco, Bellafonte, Pa., U.S.A.); 0.1 *N* sodium hydroxide; 1 *N* hydrochloric acid. The extraction solvent used was 5% isopropanol in dichloro-

methane (Nanograde; Mallinckrodt, St. Louis, Mo., U.S.A.). The internal standard was a 25 mg% ethanolic solution of medazepam.

Gas–liquid chromatography

A Packard Model 7400 Series gas chromatograph equipped with a flame ionization detector was used. The column was a 6-ft. × 4-mm-I.D. coiled glass tube which had been silanized with a solution of 5% dichlorodimethylsilane in benzene, then rinsed with methanol, and dried. The packing consisted of 3% OV-225 on HP Chromosorb W, 100–120 mesh (Johns-Manville, Denver, Colo., U.S.A.) and was prepared by the solvent evaporation technique[5]. After packing, the column was conditioned at 260° for 48 h with a nitrogen flow-rate of 25 ml/min. In the present work the instrument settings were as follows: column temperature, 235°; injection port temperature, 275°; detector temperature, 250°; carrier gas flow-rate, 40 ml/min; column inlet pressure, 20 p.s.i.; hydrogen flow-rate, 36 ml/min; and air flow-rate, 430 ml/min. Under these conditions the retention times of theophylline (methylated) and medazepam were 5.8 and 10.3 min, respectively.

Extraction procedure

A 2-ml sample of heparinized plasma was acidified with 0.5 ml of 1 *N* hydrochloric acid and extracted with 25 ml of the extraction solvent by shaking for 3 min in a separating funnel. After settling, the solvent was filtered through a Whatman No. 90 filter paper. A 20-ml aliquot of the filtrate was extracted with 5 ml of 0.1 *N* sodium hydroxide. The solvent was discarded and the aqueous phase acidified with 1.0 ml of 1 *N* hydrochloric acid, then extracted as before with 25 ml of the extraction solvent. The solvent was filtered through the No. 90 filter paper into a pointed glass tube, then carefully evaporated (50°) under a stream of dry nitrogen. The residue was dissolved in 100 μl of the internal standard solution. The on-column methylation was achieved as follows: 3.0 μl of the trimethylanilinium hydroxide solution and 5.0 μl of the sample were consecutively taken up in a 10-μl syringe; the mixture was then injected into the gas chromatograph.

Quantitation

To known amounts of theophylline in pointed glass tubes 100 μl of the internal standard solution were added; then each was examined by GLC as described above. Over a range of 0.3 to 2.4 μg of theophylline, the ratio of the peak height of theophylline (methylated) to the internal standard was linear (Fig. 1).

Recovery studies

Amounts of theophylline ranging from 6 to 48 μg were added to 2 ml of blank plasma in order to examine the efficiency of the extraction procedure. The mean recovery on fifteen spiked samples (between 0.3 and 2.4 mg%) was 88 $\pm$ 8%.

Specificity

In all of the plasma samples we have examined, the gas chromatograms have been free from interfering peaks. In addition, none of the common acidic drugs were found to interfere with the assay. When these drugs were examined by GLC as described above, the following retention times were observed: phenobarbitone, 1.0

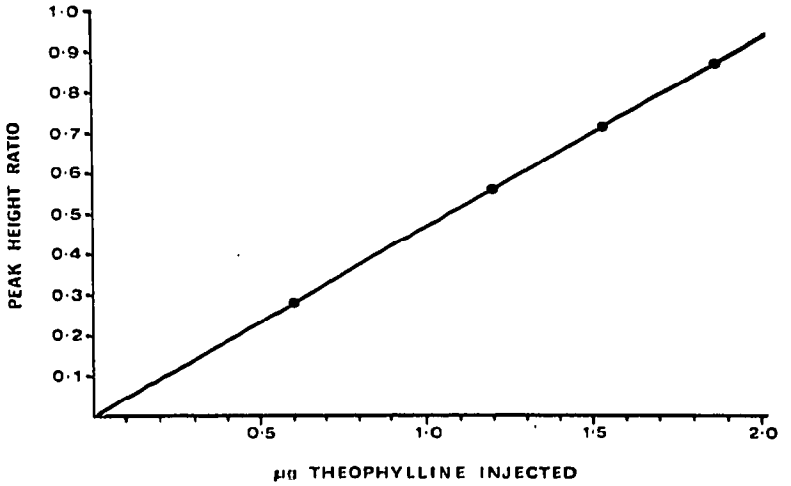

Fig. 1. Relationship of peak height ratios (methylated theophylline/internal standard) to the amounts of theophylline injected.

and 3.0 min; methylphenobarbitone, 3.0 min; phenytoin, 13.0 min; and phenylbutazone, 13.3 min.

Application

The above method was performed on plasma from asthmatic patients who were receiving treatment with various theophylline preparations. Some results from patients on long-term therapy are shown in Table I.

TABLE I

PLASMA LEVELS OF THEOPHYLLINE FROM PATIENTS ON THEOPHYLLINE PREPARATIONS

Patient	*Treatment*	*Time sample taken*	*Plasma level (μg/ml)*
1	250 mg t.d.s. of choline theophylline	before dose	5.0
		2 h after last dose	7.5
2	125 mg t.d.s. of choline theophylline	before dose	2.5
3	500 mg i.v. of aminophylline	1 h	17.0
		2 h	13.5
4	500 mg i.v. of aminophylline	1 h	16.3
		2 h	14.2
		3 h	11.5
5	125 mg q.i.d. of theophylline tablets	before dose	2.9
		2 h after last dose	7.2
6	50 mg q.i.d. of theophylline elixir	2 h after last dose	10.4

DISCUSSION

The results listed in Table I parallel those obtained in plasma by Mitenko and Ogilive[6], who studied theophylline levels, after single intravenous infusions of aminophylline, using the analytical method of Schack and Waxler[3].

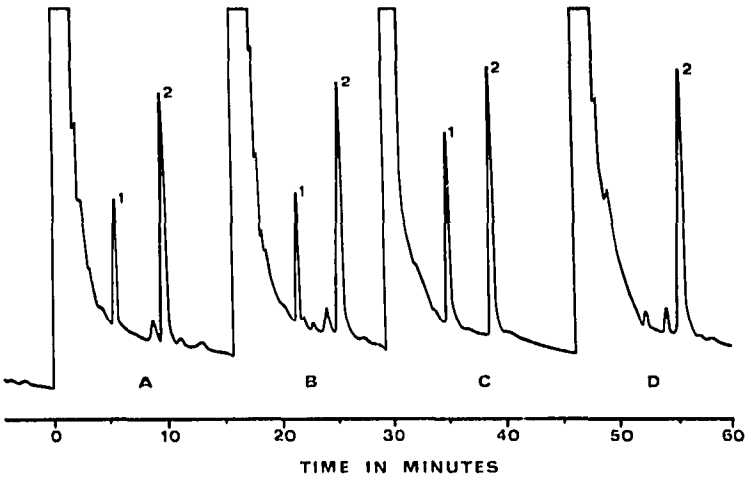

Fig. 2. (A) Chromatogram of a plasma extract containing added theophylline at a level of 1.2 mg%. (B) Chromatogram of a plasma extract from a subject receiving theophylline. (C) Chromatogram of a standard containing 1.2 μg of theophylline. (D) Chromatogram of a blank plasma extract. 1 = Theophylline (methylated); 2 = medazepam (internal standard).

The method outlined in this paper has proved to be satisfactory, reliable and specific and does not suffer from any interference (Fig. 2). Theophylline was quantitatively converted to caffeine by the method described above rather than the more conventional methylation technique of dissolving the sample in TMAH and injecting an aliquot into the gas chromatograph. In our hands this has proved to be an effective approach for the flash heater methylation of many compounds.

Since caffeine is the product of methylation of theophylline[4], it was important to have an extraction procedure which would separate theophylline from caffeine. The method described here satisfies this requirement. When plasma samples, spiked with caffeine (6 mg%), were treated as outlined above, no caffeine could be detected in the final residues. It was noted that the caffeine was not extracted into the 0.1 *N* sodium hydroxide solution, but could be recovered (98%) from the first isopropanol–dichloromethane extract.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. G. Stewart and Dr. J. Elder for providing the clinical samples and the Commissioner of Health for allowing the data to be published.

REFERENCES

1 J. W. Jenne, E. Wyze, F. S. Rood and F. M. MacDonald, *Clin. Pharmacol. Ther.*, 13 (1972) 349.
2 D. P. Nicholson and T. W. Chick, *Amer. Rev. Resp. Dis.*, 108 (1973) 241.
3 J. A. Schack and S. H. Waxler, *J. Pharmacol. Exp. Ther.*, 97 (1940) 283.
4 E. Brochmann-Hanssen and T. Olawuyi Oke, *J. Pharm. Sci.*, 58 (1969) 370.
5 L. J. Dusci and L. P. Hackett, *J. Chromatogr.*, 61 (1971) 231.
6 P. A. Mitenko and R. I. Ogilive, *Clin. Pharmacol. Ther.*, 14 (1973) 509.