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Note

Simple and reliable gas–liquid chromatographic assay for underivatized theophylline in plasma using an organic-nitrogen specific detector

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Plasma levels of the bronchodilator theophylline (1,3-dimethylxanthine) are now measured routinely in many laboratories and many methods have been described. The original procedure[1] involved UV spectrophotometry, but this has largely been replaced by the more specific techniques of thin-layer chromatography (TLC)[2], gas–liquid chromatography (GLC)[3–14] and, more recently, high-performance liquid chromatography (HPLC)[15–26]. Many HPLC techniques appear to be ideally suited to the measurement of theophylline in that they are simple, rapid and require only a small sample volume, but unfortunately an HPLC system was not available in this laboratory. As quantitative TLC tends to be time consuming[2], GLC was chosen as the most suitable alternative.

The requirement of this laboratory was a GLC procedure that was rapid, reliable, suitable for paediatric samples and in which theophylline could be chromatographed underivatized. Most of the existing GLC methods[3–13] involve derivatization (alkylation) of theophylline and in many instances a lengthy extraction procedure in order to eliminate interference from endogenous dietary xanthines such as theobromine (3,7-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine), metabolites such as 3-methylxanthine and co-administered drugs such as phenobarbitone and paracetamol. Lengthy extraction procedures limit the number of samples that can readily be processed in one batch, whereas derivatization introduces an additional step that may be difficult to control. Sheehan and Haythorn[14] described a GLC system in which underivatized theophylline was chromatographed after a simple extraction, but a sample volume of 2 ml was required. When their system was applied in this laboratory to paediatric samples (volumes of 500 μl or less), satisfactory peaks for theophylline could not be obtained.

There is therefore a need for a reliable GLC procedure that possesses the advantages of speed, simplicity and sensitivity for paediatric samples and which also eliminates the potential problems associated with derivative formation. In this paper, a system that fulfils these criteria is described.

EXPERIMENTAL

Materials and equipment

Most drugs and related compounds were obtained from Sigma (London) (Kingston-upon-Thames, Great Britain). 1-Methyluric acid, 3-methyluric acid, 1,3-

dimethyluric acid and 1-methylxanthine were obtained from Adams (Round Lake, Ill., U.S.A.), and heptabarbitone was supplied by Geigy Pharmaceuticals (Macclesfield, Great Britain). All solvents were redistilled before use. GLC analyses were carried out with a Pye Unicam Series 104 chromatograph equipped with an alkali flame-ionization (organic-nitrogen specific) detector.

Method

Theophylline together with added internal standard (heptabarbitone, 0.08 μmole) was extracted from plasma (500 μl) acidified with 2 *M* sulphuric acid (50 μl) by shaking with chloroform (5 ml) for 5 min. Ammonium sulphate (1 g, chloroform-washed) was added and, after further shaking for 30 sec, the organic phase was decanted into a conical centrifuge tube and evaporated to dryness under nitrogen at 60°. The residue was dissolved in acetone (100 μl) and an aliquot (4 μl) injected into the chromatograph. Chromatograms were run at 255° with a glass column (1.0 m × 0.4 mm I.D.) packed with 3% poly(cyclohexyldimethanol succinate) on Diatomite CLQ (JJ's Chromatography, King's Lynn, Great Britain) and a carrier gas (argon) flow-rate of 45 ml/min. The injection port and detector temperatures were 270°. The concentration of theophylline was determined by calculating the peak-height ratio of theophylline with respect to the internal standard in each chromatogram and relating it to a calibration graph derived from plasma standards (50 and 100 μmole/l) analyzed at the same time.

RESULTS AND DISCUSSION

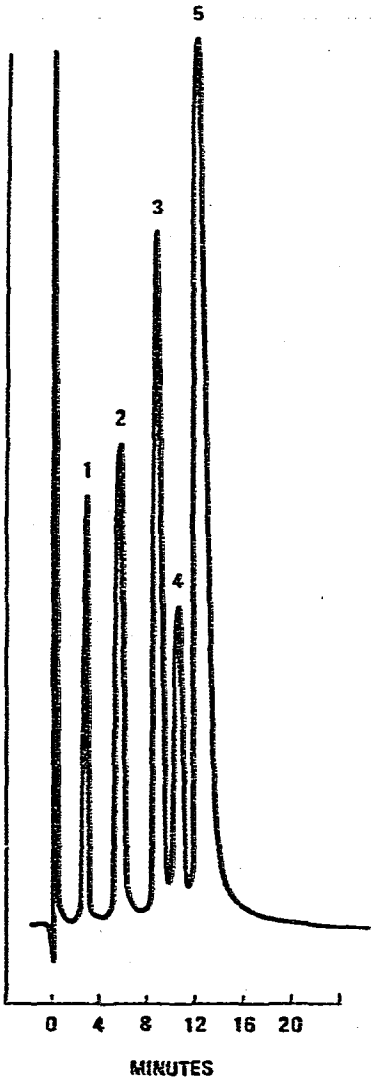
As is shown in Fig. 1, caffeine (peak 1), theobromine (peak 2), paracetamol (peak 2) and phenobarbitone (peak 4) are separated adequately from theophylline (peak 5), as is the internal standard heptabarbitone (peak 3). The retention times of these and other related compounds are given in Table I. None interferes in the analysis.

TABLE I

RETENTION TIMES AND RETENTIONS RELATIVE TO THEOPHYLLINE OF POTENTIALLY INTERFERING DIETARY XANTHINES, METABOLITES AND CO-ADMINISTERED DRUGS

Compound	*Retention time (min)*	*Relative retention*
Theophylline	12.0	1.00
Caffeine	2.6	0.22
Theobromine	5.4	0.45
Paracetamol	5.4	0.45
1,7-Dimethylxanthine	6.5	0.54
Heptabarbitone*	8.8	0.73
Phenobarbitone	10.4	0.87
3-Methylxanthine	29.5	2.46
1-Methylxanthine	34.0	2.83
Xanthine	No peak	—
Hypoxanthine	No peak	—
Uric acid	No peak	—
1-Methyluric acid	No peak	—
3-Methyluric acid	No peak	—
1,3-Dimethyluric acid	No peak	—

* Internal standard.



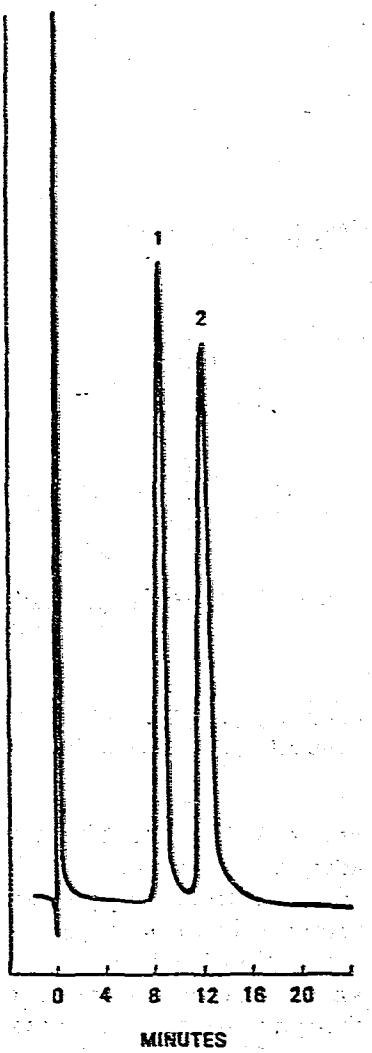


Fig. 1. Chromatogram illustrating the separation of theophylline (5) from caffeine (1), theobromine and paracetamol (2), internal standard (heptabarbitone) (3) and phenobarbitone (4).

Fig. 2. Chromatogram from an extract of plasma containing 160 μmole/l of internal standard (heptabarbitone) (1) and 95 μmole/l of theophylline (2).

Similarly, interference from chloroform-soluble plasma constituents such as cholesterol and lipids does not occur. Although probably present in the plasma extract, these compounds do not contain nitrogen and therefore do not produce a response in the nitrogen detector. In this GLC system, therefore, neither lengthy extraction to remove possible contaminants nor derivatization to improve separation, one or both of which procedures are required by most GLC techniques, are necessary.

Fig. 2 is a typical chromatogram from a patient receiving theophylline. Peak 1 is the internal standard, heptabarbitone (160 μmole/l), and peak 2 is theophylline (95

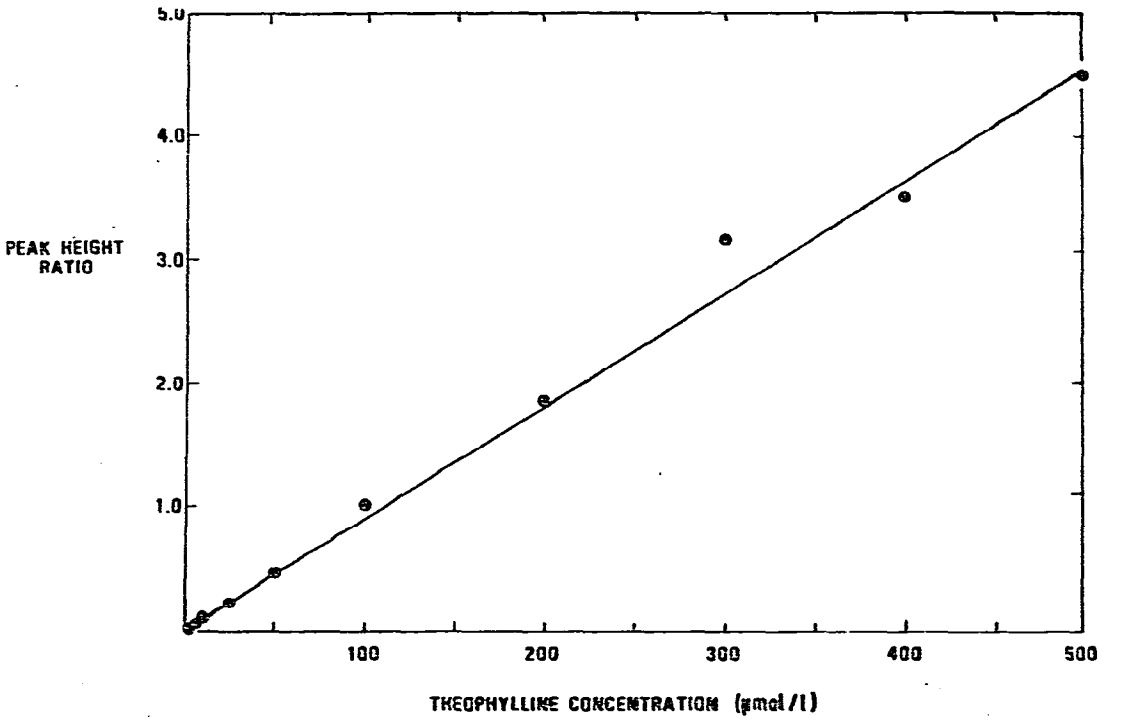


Fig. 3. Calibration graph obtained by plotting the peak-height ratio of theophylline to internal standard against theophylline concentration. Each point represents a single determination.

μmole/l). Heptabarbitone was chosen as the internal standard because it separates adequately from theophylline and the other potentially interfering compounds (Table I), it is extracted to the same extent (80%) as theophylline, it produces an adequate response in the nitrogen detector and it is not given to asthmatic patients.

Fig. 3, which is a calibration graph prepared from plasma samples containing known concentrations of theophylline, demonstrates the linearity of the assay for theophylline over the range 0–500 μmole/l. This more than covers the therapeutic range (55–110 μmole/l).

The accuracy and reproducibility of the procedure are satisfactory for clinical measurements. The within-batch precision, as determined by 20 simultaneous analyses of a plasma pool to which theophylline (90 μmole/l) had been added, was 91 ± 3.2 μmole/l (coefficient of variation = 3.5%). The between-batch precision, as determined from 20 serial analyses of the plasma pool, was 90 ± 5.0 μmole/l (coefficient of variation = 5.6%).

Hence, this GLC procedure is both reliable and simple to perform. It is sufficiently sensitive to measure theophylline in paediatric samples, a large number of determinations can readily be carried out in one batch and a derivatization step is not required. The system therefore is entirely suitable for the routine measurement of theophylline in plasma.

REFERENCES

1 J. A. Schack and S. H. Waxler, *J. Pharmacol. Exp. Ther.*, 97 (1949) 283.
2 B. Wesley-Hadzija and A. M. Mattocks, *J. Chromatogr.*, 115 (1975) 501.
3 V. P. Shah and S. Riegelman, *J. Pharm. Sci.*, 63 (1974) 1283.
4 A. Arbin and P. O. Edlund, *Acta Pharm. Suecica*, 11 (1974) 249.
5 G. F. Johnson, W. A. Dechtiaruk and H. M. Solomon, *Clin. Chem.*, 21 (1975) 144.
6 W. Dechtiaruk, G. F. Johnson and H. M. Solomon, *Clin. Chem.*, 21 (1975) 1038.
7 L. J. Dusci, L. P. Hackett and I. A. McDonald, *J. Chromatogr.*, 104 (1975) 147.
8 C. J. Least, G. F. Johnson and H. M. Solomon, *Clin. Chem.*, 22 (1976) 765.
9 D. Perrier and E. Lear, *Clin. Chem.*, 22 (1976) 898.
10 J. D. Lowry, L. J. Williamson and V. A. Raisys, *J. Chromatogr.*, 143 (1977) 83.
11 M. Sheehan, R. H. Hertel and C. T. Kelly, *Clin. Chem.*, 23 (1977) 64.
12 H. Kinsun, M. A. Moulin, R Venezia, D. Laloum and M. C. Bigot, *Clin. Chim. Acta*, 84 (1978) 315.
13 D. G. Bailey, H. L. Davis and G. E. Johnson, *J. Chromatogr.*, 121 (1976) 263.
14 M. Sheehan and P. Haythorn, *J. Chromatogr.*, 117 (1976) 393.
15 C. V. Manion, D. W. Shoeman and D. L. Azarnoff, *J. Chromatogr.*, 101 (1974) 169.
16 R. D. Thompson, H. T. Nagasawa and J. J. Jenne, *J. Lab. Clin. Med.*, 84 (1974) 584.
17 D. S. Sitar, K. M. Piafsky, R. E. Rangno and R. I. Ogilvie, *Clin. Chem.*, 21 (1975) 1774.
18 A. G. Maijub, D. T. Stafford and R. T. Chamberlain, *J. Chromatogr. Sci.*, 14 (1976) 521.
19 M. A. Evenson and B. L. Warren, *Clin. Chem.*, 22 (1976) 851.
20 R. F. Adams, F. L. Vandemark and G. J. Schmidt, *Clin. Chem.*, 22 (1976) 1903.
21 L. C. Franconi, G. L. Hawk, B. J. Sandmann and W. G. Haney, *Anal. Chem.*, 48 (1976) 372.
22 J. J. Orcutt, P. P. Kozak, S. A. Gillman and L. H. Cummins, *Clin. Chem.*, 23 (1977) 599.
23 R. E. Hill, *J. Chromatogr.*, 135 (1977) 419.
24 R. K. Desiraju, E. T. Sugita and R. L. Mayock, *J. Chromatogr. Sci.*, 15 (1977) 563.
25 M. J. Cooper, B. L. Mirkin and M. W. Anders, *J. Chromatogr.*, 143 (1977) 324.
26 S. J. Soldin and J. G. Hill, *Clin. Biochem.*, 10 (1977) 74.