Journal of Chromatography, 182 (1980) 232—236
Biomedical Applications

CHROMBIO. 515

Note

Micro estimation of plasma theophylline by gas—liquid chromatography with on-column butylation and nitrogen-specific detection

GARY MARTIN SCHIER and IGNATIUS ENG THO GAN

Department of Biochemistry, The Wollongong Hospital, Crown Street, Wollongong, N.S.W. 2500 (Australia)

(First received September 26th, 1979; revised manuscript received December 11th, 1979)

Theophylline (1,3-dimethylxanthine) is well-established as a bronchodilator in the management of patients with asthma [1—3], and has proved effective in the treatment of apnea and bradycardia in premature and low birth-weight infants [4—7]. Measurement of plasma theophylline levels is useful, because of pronounced intersubject differences in the biological half-life of theophylline in adults [8, 9], children [10], and infants. Because plasma theophylline has a relatively narrow therapeutic range [5, 11] and considerable plasma variability among patients on the same oral dose [5, 12], safe and effective use of theophylline depends on information obtained by plasma monitoring. Although estimation of plasma theophylline has been provided by various analytical techniques [10, 13—19], gas—liquid chromatographic (GLC) methods have proved to be some of the more popular procedures [12, 20—30]. Most of these procedures have relied upon sample volumes of 1 ml to achieve sufficient analytical precision with the commonly used flame ionisation detectors (FID). The need for a micro analysis for paediatric use was apparent and a number of methods using nitrogen—phosphorus-specific detectors [26, 29], FID [27] and electron-capture detectors [24], were developed to fulfil this requirement. For routine use these methods have a number of disadvantages, since they require double solvent extraction [26], temperature programming of the GLC for resolution of the components [26], the use of aqueous tetrabutylammonium hydroxide [27], and have low extraction efficiencies [29], or are unduly complicated [24]. In view of these limitations, we developed a method involving the extraction of theophylline from 20 μl of plasma with 100 μl of extracting solvent. This is then followed by evaporation and on-column butylation [31], with detection by means of a nitrogen-specific detector.

MATERIALS AND METHODS

Apparatus

A Packard 427 gas chromatograph equipped with a Model 905 nitrogen—phosphorus detector was used. The instrument was fitted with a coiled glass column (1.8 m × 2 mm I.D.) packed with Gas-Chrom Q (100—120 mesh) coated with 3% OV-17. Operating conditions were: helium (carrier gas) flow-rate 30 ml/min, hydrogen 4.6 ml/min, air 100 ml/min; injection port and detector temperatures 280°; column temperature was isothermal at 230°.

Reagents

Theophylline and 7-β-hydroxypropyltheophylline were obtained from Sigma (St. Louis, Mo., U.S.A.) Chloroform (Spectrosol®) and propan-2-ol (Spectrosol®) were obtained from Ajax Chemicals (Sydney, Australia). The column packing (3% OV-17 on Gas-Chrom Q 100—120 mesh) was obtained from Applied Science Labs., State College, Pa., U.S.A. Tetrabutylammonium hydroxide (0.02 *M* in methanol) was prepared from 25% tetrabutylammonium hydroxide obtained from Matheson, Coleman and Bell (Norwood, Ohio, U.S.A.). The internal standard (7-β-hydroxypropyltheophylline, 11.91 mg) was dissolved in 100 ml of propan-2-ol. A 10-ml aliquot was diluted to 500 ml with 50% chloroform—propan-2-ol to give a combined extracting solution and working internal standard.

Extraction and on-column derivatisation of plasma theophylline

To 20 μl of plasma were added 100 μl of internal standard—extracting solution, followed by vortex mixing for 30 sec. Following centrifugation at 800 *g* for 2 min, the lower layer was transferred by pasteur pipette to a conical tube and evaporated to dryness with a stream of nitrogen at 40°. The residue was reconstituted with vortex mixing in 20 μl of 0.02 *M* tetrabutylammonium hydroxide solution; 1 μl was then used for injection into the gas chromatograph.

Standard solutions

A series of plasma standards with concentrations of 125, 100, 75, 50 and 25 μmol/l were prepared by adding pooled sera to 1 ml of a stock solution of theophylline in water (45, 36, 27, 18 and 9 mg per 100 ml) in 10-ml volumetric flasks. Aliquots of these solutions were kept frozen at −20°, and then thawed at 37° before use.

Quantitation

Theophylline levels in plasma samples were quantitated by reference to the standards. These were plotted as the ratio of the peak height of theophylline to that of the internal standard, versus the plasma theophylline concentration (μmol/l).

Spectrophotometric method for theophylline

The method of Koysooko et al. [32] was used to determine theophylline in plasma. Two millilitres of plasma were extracted with 10 ml of chloroform—

isopropanol (20:1, v/v). Eight millilitres of the organic phase were removed and extracted with 2 ml of NaOH (0.1 mol/l). Absorbance of the aqueous phase was measured in a quartz cuvette at 277 nm with a Model SP 1800 spectrophotometer (Pye-Unicam, Cambridge, Great Britain).

RESULTS AND DISCUSSION

Chromatography

Fig. 1 shows a typical chromatogram from an extract of plasma spiked with theophylline (50 μmol/l), with retention times calculated from the time of injection as follows: solvent front, 0.4 min; theophylline, 3.3 min; internal standard, 5.0 min.

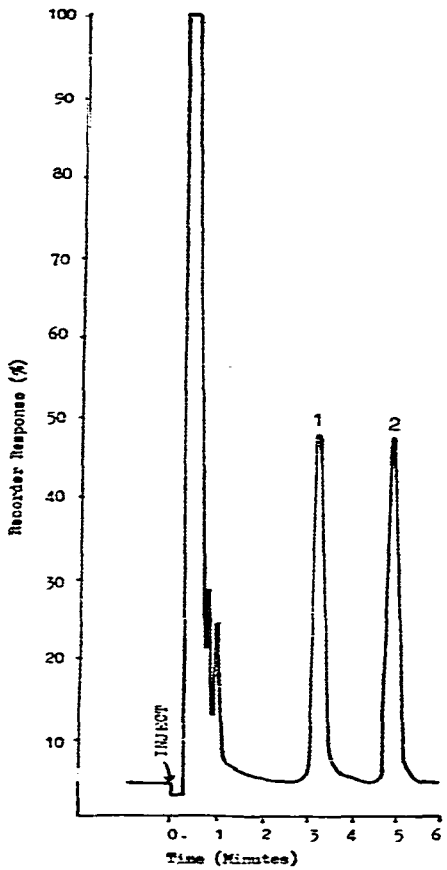

Fig. 1. Gas chromatogram of an extract from 20 μl of plasma containing 50 μmol/l of theophylline. Column, 3% OV-17 on Gas-Chrom Q 100—120 mesh, 1.8 m × 2 mm I.D. glass; attenuation 32×; column temperature 260°. Peaks: 1 = theophylline, 2 = 7-β-hydroxypropyltheophylline.

Linearity

The linearity of the assay method was established by analysing duplicate aliquots of the plasma standards. Analysis of the results by the least-squares method resulted in a straight line with a correlation coefficient of 0.999.

Precision and detection limit

The within-day precision was determined by analysing ten 20-μl aliquots of pooled serum with a theophylline concentration of 100 μmol/l. The result was 101 ± 2.0 (S.D.) μmol/l with a coefficient of variation of 2.0%. In a similar manner, aliquots of the same pooled sera were analysed daily for ten days and the between-run precision was 98 ± 3.8 (S.D.) μmol/l, with a coefficient of variation of 3.9%.

For practical use, the lower limit of theophylline estimation was 5 μmol/l.

Extraction efficiency and recovery

The extraction efficiency and recovery of theophylline were determined by carrying out the above procedure on twenty samples of plasma to which theophylline had been added to the 100 μmol/l level. The peak heights were compared to the peak heights of an equivalent amount of standard which had been dried and subjected to the same derivatisation procedure. The mean recovery following extraction was 76% with an S.D. of 3.5%.

Interference

To check for possible interference, several drugs (phenobarbital, caffeine, theobromine, phenytoin, primidone, carbamazepine, salicylic acid, acetylsalicylic acid and uric acid) were added to plasma at the 100 μmol/l level and analysed in the same manner as the theophylline standards. No interference could be detected, although the following drugs eluted on the same chromatogram: caffeine, 2.3 min; theobromine, 3.7 min; and phenobarbital, 4.1 min. To check for possible non-specific interference, several drug-free sera were subjected to the new procedure. Again, no interference was detected, although a peak with a retention time of 2.3 min (corresponding to caffeine) was observed in some sera.

Accuracy

Ten specimens of plasma obtained from patients taking theophylline medication were analysed for theophylline by the spectrophotometric method [32] and the method described here. The mean value obtained with the spectrophotometric method was 66 μmol/l and with this method 62 μmol/l. When values from this assay were compared to those obtained with the spectrophotometric assay, the correlation coefficient was 0.952.

Using the above method it is possible to determine ten plasma theophylline levels within 90 min. The analytical procedures of extraction, evaporation and chromatography can be completed in less than 10 min per specimen. The excellent precision, accuracy and small sample requirement make this procedure ideally suited to the analysis of theophylline in plasma from neonates and infants.

REFERENCES

1 M.M. Weinberger and E.A. Bronsky, J. Pediatr., 84 (1974) 421.
2 M.M. Weinberger and E.A. Bronsky, Clin. Pharmacol. Ther., 17 (1975) 585.
3 G. Hambleton, M. Weinberger, J. Taylor, M. Cavanaugh, E. Ginchansky, S. Godfrey, M. Tooley, T. Bell and S. Grinberg, Lancet, i (1977) 381.
4 J.A. Kuzemko and J. Paala, Arch. Dis. Child., 48 (1973) 404.
5 D.C. Shannon, F. Gotay, I.M. Stein, M.C. Rogers, I.D. Todres and P.M.B. Moylan, Pediatrics, 55 (1975) 589.
6 R. Uauy, D.L. Shapiro, B. Smith and J.B. Warshaw, Pediatrics, 55 (1975) 595.
7 J.F. Lucey, Pediatrics, 55 (1975) 584.
8 J.W. Jenne, E. Wyze, F.S. Rood and F.M. MacDonald, Clin. Pharmacol. Ther., 13 (1972) 349.
9 P.A. Mitenko and R.I. Ogilvie, Clin. Pharmacol. Ther., 14 (1973) 509.
10 E.B. Truitt, Jr., C.J. Carr, H.M. Bubert and J.C. Krantz, Jr., J. Pharmacol. Exp. Ther., 91 (1947) 185.
11 P.A. Mitenko and R.I. Ogilvie, New Engl. J. Med., 289 (1973) 600.
12 G.F. Johnson, W.A. Dechtiaruk and H.M. Solomon, Clin. Chem., 21 (1975) 144.
13 A.J. Plummer, J. Pharmacol. Exp. Ther., 93 (1948) 142.
14 J.A. Schack and S.H. Waxler, J. Pharmacol. Exp. Ther., 97 (1949) 283.
15 R.C. Gupta and G.D. Lundberg, Anal. Chem., 45 (1973) 2403.
16 R.D. Thompson, H.T. Nugasawa and J.W. Jenne, J. Lab. Clin. Med., 84 (1974) 584.
17 C.V. Manion, D.W. Shoeman and D.L. Azarnoff, J. Chromatogr., 101 (1974) 169.
18 M. Weinberger and C. Chidsey, Clin. Chem., 21 (1975) 834.
19 M. Riechert, J. Chromatogr., 146 (1978) 175.
20 A. Arbin and P.O. Edlund, Acta Pharm. Suecica, 11 (1974) 249.
21 B. Wesley-Hadzija, Brit. J. Pharmacol., 1 (1974) 515.
22 V.P. Shah and S. Riegelman, J. Pharm. Sci., 63 (1974) 1283.
23 W. Dechtiaruk, G.F. Johnson and H.M. Solomon, Clin. Chem., 21 (1975) 1038.
24 A. Arbin and P.O. Edlund, Acta Pharm. Suecica, 12 (1975) 119.
25 J.D. Lowry, L.J. Williamson and V.A. Raisys, J. Chromatogr., 143 (1977) 83.
26 C.J. Least, Jr., G.E. Johson and H.M. Solomon, Clin. Chem., 22 (1976) 765.
27 D. Perrier and E. Lear, Clin. Chem., 22 (1976) 898.
28 M. Sheehan and P. Haythorn, J. Chromatogr., 117 (1976) 393.
29 B. Vinet and L. Zizian, Clin. Chem., 25 (1979) 156.
30 L.J. Dusci, L.P. Hackett and I.A. McDonald, J. Chromatogr., 104 (1975) 147.
31 M. Kowblansky, B.M. Scheinthal, G.D. Cravello and L. Chafetz, J. Chromatogr., 76 (1973) 467.
32 R. Koysooko, E.F. Ellis and G. Levy, Clin. Pharmacol. Ther., 15 (1974) 454.