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Note

Measurement of plasma theophylline by gas—liquid chromatography on the stationary phase SP-2510

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Theophylline (1,3-dimethylxanthine) is well established as a bronchodilator in the management of patients with asthma [1], and has proved effective in the treatment of apnea and bradycardia in premature and low birth weight infants [2, 3]. However, safe and effective use of theophylline depends on information obtained by plasma monitoring, because of pronounced intersubject differences in the biological half-life and the resultant variability in patients on the same oral dose [4, 5].

Plasma theophylline levels are routinely estimated by a variety of analytical techniques [6—24], however we were restricted to a gas—liquid chromatographic (GLC) method as this used equipment available in our laboratory. The GLC method we describe uses the support SP-2510 DA and a pre-column of SP-2250 DA. This column was developed for the estimation of the anticonvulsant drugs [25, 26]. It was desirable and convenient to extend its use for the estimation of plasma theophylline, thereby allowing routine laboratories the flexibility of using the one column for both types of analysis. A number of previously published methods had achieved this on the support 3% OV-17 [14, 21, 23], but here theophylline requires derivatisation to the butyl or pentyl derivatives, whereas the method we describe eliminates this. As the procedure utilises a nitrogen—phosphorus detector, good sensitivity is achieved on microsamples of plasma (50 μl).

EXPERIMENTAL

Materials

Theophylline and 7-(β-hydroxypropyl)theophylline were obtained from

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Sigma (St. Louis, MO, U.S.A.). Chloroform (spectrosol) and propan-2-ol (spectrosol) were obtained from Ajax Chemicals (Sydney, Australia). The column packing 2% SP-2510 DA on 100—120 mesh Supelcoport and 3% SP-2250 DA on 100—120 mesh Supelcoport were obtained from Supelco (Bellefonte, PA, U.S.A.). The internal standard 7-(β-hydroxypropyl)-theophylline (11.91 mg) was dissolved in 100 ml of propan-2-ol. A 10-ml aliquot was diluted to 500 ml with chloroform—propan-2-ol (4:1, v/v) to give a combined extraction and internal standard solution.

Pooled plasma was prepared from expired blood packs supplied by the Blood Bank at The Wollongong Hospital.

Chromatography

A Packard 427 gas chromatograph equipped with a Model 905 NP detector was used. The instrument was fitted with a coiled glass column 0.9 m × 2 mm I.D. packed with 2% SP-2510 DA on 100—120 mesh Supelcoport, with an 8-cm pre-column of 3% SP-2250 on 100—120 mesh Supelcoport as previously described [26]. The oven temperature was maintained at 205°C with detector and injector block temperatures set at 280°C. The nitrogen detector was adjusted to a background current of 40 pA with hydrogen set at the flow-rate of 4.6 ml/min, air at 100 ml/min and the carrier gas helium at 40 ml/min. The attenuator was set at range 10 × 32 with the recorder set on 1 mV full scale deflection.

Extraction of plasma samples

A 250-μl amount of the internal standard/extracting solution was added to 50 μl of plasma, followed by vortex mixing for 30 sec. After centrifugation at 1000 *g* for 2 min, the lower layer was transferred by pasteur pipette to a conical shaped tube and evaporated to dryness with a stream of nitrogen at 40°C. The residue was reconstituted with vortex mixing in 20 μl of ethyl acetate, and 1 μl was then used for injection into the chromatograph. The recovery of plasma theophylline at the 100 μmol/l level by this method was previously found to be 76% [23].

Standard solutions

A series of plasma standards with concentrations of 25, 50, 75, 100, 125 and 150 μmol/l were prepared by adding pooled plasma to 1 ml of a stock solution of theophylline in water (4.5, 9, 13.5, 18, 22.5 and 27 mg per 100 ml) in 10-ml volumetric flasks. Aliquots of these solutions were kept frozen at —20°C and thawed at 37°C prior to use.

RESULTS AND DISCUSSION

Using the above GLC conditions, a theophylline assay involving extraction and GLC of the standards and sample from one patient would take approximately 50 min. Each additional assay would only add an extra 6 min. The internal standard, 7-(β-hydroxypropyl)theophylline proved to be a suitable internal standard, having a similar GLC response to theophylline, but having

TABLE I

RETENTION TIMES OF DRUGS AND OTHER POTENTIALLY INTERFERING COMPOUNDS IN THE GLC MEASUREMENT OF THEOPHYLLINE

Compound	Retention time (min)	Compound	Retention time (min)
Caffeine	1.0	Diphylline	16.7
Methylphenobarbital	1.4	Salicylic acid	Nil
Theobromine	1.7	Acetylsalicylic acid	Nil
Paracetamol	2.0	Uric acid	Nil
7-(β-hydroxypropyl)-theophylline	3.6	Cholesterol	Nil
Phenobarbital	4.1	Plasma peak	0.8
Theophylline	4.6	Plasma peak	1.0
Diazepam	5.9	Plasma peak	1.9
Butabarbital	Nil	Plasma peak	2.6
Amobarbital	Nil	Ethosuximide	Nil
Pentabarbitone	Nil	Valproic acid	Nil
Quinalbarbitone	Nil		

good separation from it. The separation is influenced by the length of the pre-column and we found that a length of 8 cm allowed separation of theophylline, the internal standard and all compounds listed in Table I. No interference could be detected from the anticonvulsant drugs, other xanthines and a number of commonly prescribed drugs with this method (Table I).

A number of serum peaks (Table I and Fig. 1) are characteristic of most plasma samples run by this method, even for those patients not on any medication (Fig. 2). However, these peaks do not interfere with the assay of theophylline. The standard curve is plotted as the ratio of the peak height of theophylline to that of the internal standard, versus the plasma theophylline concentration in μmol/l. This ratio is reproducible between analyses. The method is linear to 150 μmol/l, the correlation coefficient when analysed by the least-squares method was 0.9991 and the limit of detection was 5 μmol/l. The regression line for the standard curve is used to calculate the theophylline concentration in patient samples.

The within-run precision of this method was 1.75% at the 110 μmol/l level and 3.3% at the 20 μmol/l level of theophylline. This was determined by analysing the plasma of a patient fifteen times within the same assay. The between-run precision was 5.2% at the 110 μmol/l level and 8.7% at the 20 μmol/l level of theophylline. This was determined by assaying the plasma of one patient on ten separate occasions.

The theophylline standards prepared as above were stable at −20°C for at least twelve weeks. A solution of the internal standard was stored at room temperature in a sealed container and was also stable for at least twelve weeks.

We have demonstrated that theophylline in plasma can be conveniently and precisely measured utilising the commonly used anticonvulsant drug column 2% SP-2510 DA with an 8-cm pre-column of 3% SP-2250 DA. The assay requires very little plasma and is simple to perform with only a single extrac-

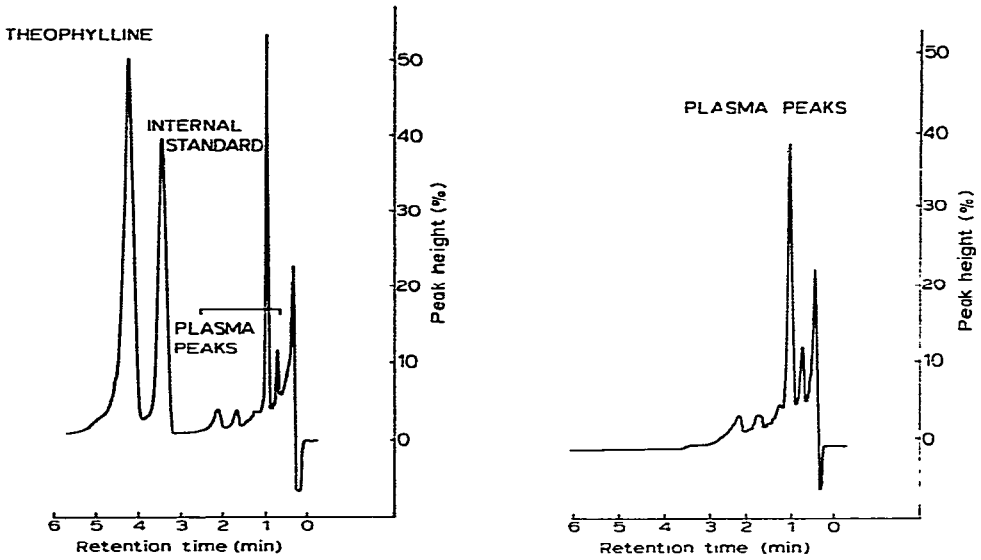

Fig. 1. GC separation of theophylline (62 μmol/l) and the internal standard, 7-(β-hydroxypropyl)-theophylline after extraction of a plasma sample obtained from an adult patient taking theophylline. Stationary phase is 2% SP-2510 DA with an 8-cm pre-column of 3% SP-2250 DA. Attenuation 320×; column temperature, 205°C; detector and injector temperature, 280°C.

Fig. 2. Chromatogram of a chloroform—propan-2-ol extract of a plasma sample from an adult patient not on theophylline medication. Stationary phase is 2% SP-2510 DA with an 8-cm pre-column of 3% SP-2250 DA. Attenuation, 320×; column temperature, 205°C; detector and injector temperature, 280°C.

tion and no derivatisation steps. Therefore this method is ideally suited for the routine estimation of theophylline in plasma. Since the development of this procedure, our laboratory has provided theophylline measurements on patients receiving theophylline medication, without encountering any difficulties.

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