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RAPID GAS CHROMATOGRAPHIC DETERMINATION OF UNDERIVATIZED THEOPHYLLINE IN WHOLE BLOOD

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SUMMARY

A rapid gas chromatographic method for the determination of underivatized theophylline in whole blood is described. Theophylline is extracted from acidified blood and chromatographed directly using cyheptamide as an internal standard. Concentrations of the drug down to 2 μg/ml in blood could be determined with recoveries ranging from 90 to 110%.

INTRODUCTION

Theophylline (1,3-dimethylxanthine) is a potent smooth muscle relaxant and as such has found frequent and efficacious use as a bronchodilator in the treatment of obstructive lung disease. However, because it has a relatively narrow therapeutic index[1] and considerable variability of serum levels of this drug has been found between patients on the same oral dose[2], there is a substantial risk of toxicity attendant with its use as a bronchodilator and occasional deaths have been reported[3]. This risk is particularly significant in pediatric patients, in patients with concomitant liver disease, and when theophylline is administered intravenously (as its ethylenediamine salt aminophylline). It would therefore appear useful that blood level measurements of this drug be used routinely to avoid the possibility of toxic symptoms.

Various analytical methods for the quantitative determination of theophylline in blood and urine have been reported[4–15]. Earlier colorimetric methods[4,5] required large amounts of blood, were quite tedious, and have largely been abandoned. The ultraviolet (UV) spectrophotometric method of Schack and Waxler[6] has found considerable use, but it suffers from interference by xanthines and other drugs. Recently Gupta and Lundberg[7] developed a differential UV procedure unaffected by other drugs, but the lengthy extraction procedure used makes it somewhat impractical for routine use. Also several specific gas chromatographic (GC) procedures have recently been reported[2,8–10]. However, they involve tedious extraction procedures, require derivatization prior to chromatographic analysis, or both. High pressure liquid chromatography[11] and high pressure cation-exchange chromatography[12] have also been used to quantitate theophylline, but these techniques are still not practical for most clinical laboratories.

This report describes a fast, simple, sensitive, and specific GC method of analysis for theophylline in whole blood that does not require derivative formation.

EXPERIMENTAL

Apparatus

A Varian 2100 flame ionization gas chromatograph was used in these studies. The instrument was fitted with U-shaped glass columns (1.83 m × 2 mm I.D.) packed with Chromosorb W HP (80–100 mesh) coated with 3% OV-1. The column was conditioned before use by heating to 300° for 24 h with 5 ml/min carrier gas flow-rate. Operating conditions were as follows: nitrogen (carrier gas) was set at a flow-rate of 20 ml/min, hydrogen at 30 ml/min, air at 300 ml/min; injection post and detector temperatures were 280°; the initial column temperature was 180°, the program rate was 6°/min up to 280° with 10 min isothermal at 280°.

Materials

Anhydrous sodium sulfate was reagent grade and all solvents were chromatoquality. Theophylline was obtained from Matheson, Coleman and Bell (Norwood, Ohio, U.S.A.) and cyheptamide from Ayerst Labs. (New York, N.Y., U.S.A.).

Standard solutions

A stock solution of 400 μg/ml theophylline was prepared by dissolving 40 mg free theophylline in 100 ml of de-ionized water. The 500-μg/ml cyheptamide standard was prepared by dissolving 50 mg of cyheptamide in 100 ml of absolute methanol. Stock solutions were stored at 4° when not in use.

Extraction procedure

In a 25-ml screw-capped vial are mixed 2.0 ml of whole blood (EDTA as anticoagulant), 10 μl of the 500 μg/ml cyheptamide standard, and 10 drops of glacial acetic acid. After swirling the contents of the vial briefly, 10 ml of chloroform are added and the vial is shaken vigorously for 5 min on a mechanical shaker. The solution is allowed to settle for 30 sec, then the chloroform layer is separated (the congealed blood cells remain adhered to the sides of the vial) and evaporated to dryness under reduced pressure at 60° (when serum was used in place of whole blood the chloroform layer was filtered through 1 g of anhydrous sodium sulfate prior to evaporation). The residue is reconstituted with 25 μl of chloroform and 3 μl of this solution injected directly onto the gas chromatograph.

Quantitation

Theophylline levels in patient blood specimens were quantitated using a daily response factor calculated from a 10-μg/ml theophylline blood standard treated as above according to the formula:

$$\text{concn. of theophylline} = \text{response factor} \times \frac{\text{peak height theophylline}}{\text{peak height cyheptamide}}$$

UV spectrophotometric measurement in whole blood

The UV spectrophotometric method of Schack and Waxler[6] was used without modification.

RESULTS

Fig. 1 shows a typical chromatogram from an extract of a blood standard containing 20 μg/ml of theophylline to which 5 μg of cyheptamide (10 μl of the 500 μg/ml cyheptamide standard) had been added. The quantity of theophylline present in a patient blood specimen was estimated using the peak height ratio of theophylline to cyheptamide derived from the chromatogram of the specimen and the plot of the peak height ratio of theophylline to cyheptamide *versus* concentration of theophylline (Fig. 2) obtained from blood standards to which known amounts of theophylline had been added. For day-to-day operation a single standard of 10 μg/ml of theophylline was used to calculate a response factor rather than reproducing the entire standard curve. To help compensate for the somewhat variable response of the standard from run to run, the GC column was overloaded with a saturated solution of theophylline in chloroform just prior to the analysis of each specimen. Under these conditions a standard deviation of 1 μg/ml ($n = 20$) with a coefficient of variation of 10% was calculated for a 10 μg/ml patient specimen. Fig. 2 shows that the method as described above is linear only up to 40 μg/ml. Specimens with values greater than 40 μg/ml were re-run starting with one half the original amount of blood. For practical purposes, the lower limit of sensitivity was found to be 2 μg/ml. Percent recovery was determined by adding known amounts of theophylline to known standards and patient specimens and was found to range between 90 and 110%. To check for possible GC interference, several drugs frequently administered in conjunction with theophylline were added to blood and analyzed in the same manner as the theophylline standards. Results of this study are shown in Table I: no interference was encountered

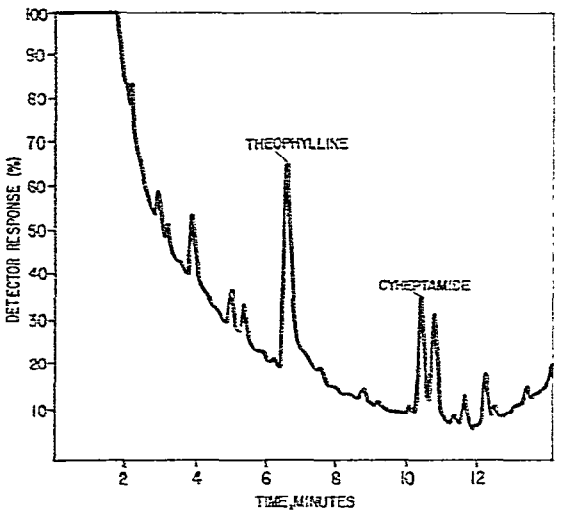

Fig. 1. Chromatogram of an extract from 2 ml of blood containing 40 μg of theophylline.

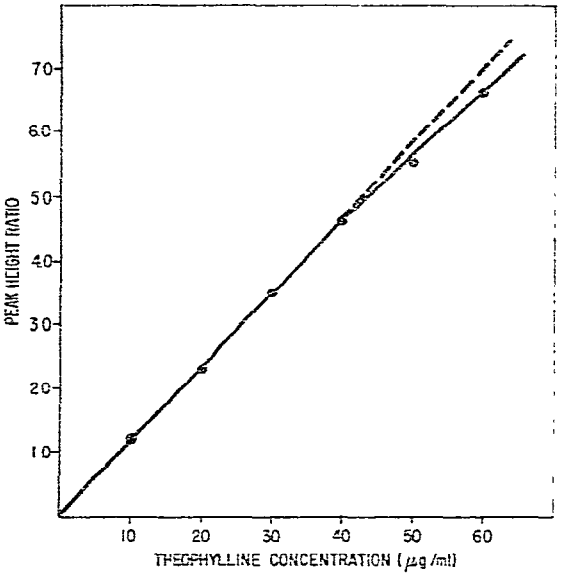

Fig. 2. Standard curve for theophylline in blood.

TABLE I

RELATIVE RETENTION TIMES OF DRUGS FOUND IN COMBINATION WITH THEOPHYLLINE

For GC conditions see Experimental.

Drug	*Relative retention time*
Butabarbital	0.24
Pentobarbital	0.30
Secobarbital	0.34
Caffeine	0.44
Theobromine	0.49
Phenobarbital	0.57
Theophylline	0.63
Cyheptamide (internal standard)	1.00
Diphenylhydantoin	1.11

with any of these drugs. Of all the common acidic and neutral drugs, furosemide was the only drug found to interfere with this procedure (it co-elutes with theophylline). Since it would be rare that this drug be used in conjunction with theophylline, this interference was not considered to be of major significance.

DISCUSSION

Arbin and Edlund[8] and also Dusci *et al.*[9] implied that due to poor peak shape and lack of sensitivity, theophylline could not be analyzed as the free compound by GC. Although we found the peak shape of theophylline when chromatographed as the free compound to be less symmetrical than when chromatographed as the alkylated derivative, adequate reproducibility and sensitivity were still achieved. In all

cases we could easily and reproducibly distinguish between therapeutic and toxic levels of the drug. As a consequence of these findings the necessity of complicated extraction procedures and/or derivatization could be avoided, thereby significantly improving the turn-around-time per specimen as compared with other GC procedures[2,8–10]. With a turn-around-time of 30 min per specimen, this procedure compares quite favorably in terms of speed with the high pressure cation-exchange method of Weinberger and Chidsey[12]. When patient specimens were split and analyzed by our method and by the UV method of Schack and Waxler[6], good correlation was obtained (the values were always within two standard deviations of each other). Mitenko and Ogilvie[13] recently reported that theophylline is only partially absorbed or bound by erythrocytes, and they calculated a mean whole blood–plasma concentration ratio of 0.82 ± 0.10 based on the UV method of Schack and Waxler. To determine whether this difference between blood and serum samples would be found when more specific GC methods were used, blood and serum samples obtained at the same time from patients on varying doses of theophylline were analyzed by this procedure. The results of this study are shown in Table II. These results are inconclusive because of the magnitude of the experimental error in lower regions of the therapeutic range, and further studies at higher levels of theophylline (greater than 20 μg/ml) are now in progress to help answer this question. At present no distinction is being made between blood and serum levels of this drug.

TABLE II

COMPARISON OF BLOOD AND SERUM LEVELS OF THEOPHYLLINE

Patient	*Theophylline level (μg/ml)*	
	Blood	*Serum*
I.D.	5	6
I.A.	10	11
H.D.	9	8
K.Q.	6	5
C.T.	8	8

For sixty patients on oral doses of theophylline and its admixtures who were free from toxic symptoms and for whom airway obstruction was apparently lessened after administration of the drug, theophylline blood levels were found to be 6–17 μg/ml with a mean of 11 μg/ml. This range agrees well with the optimal therapeutic range of 10–20 μg/ml for plasma recently reported by Piafsky and Ogilvie[1].

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