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SPECIFIC THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF THEOPHYLLINE IN PLASMA IN THE PRESENCE OF SOME OTHER DRUGS

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SUMMARY

A simple spectrodensitometric method for the direct determination of theophylline was developed from measurement of the absorbance of the compound on silica gel thin layers irradiated at 275 nm. Quantities as low as 0.010 μg can be detected and a linear relationship was obtained between peak area and the amount of the drug in the spots from 0.025–0.200 μg. The recovery over the usual range of plasma concentration (2.5–20 μg/ml) was 95–107%. The method is sufficiently sensitive and specific for clinical purposes and the time for the assay is about 2 h. Caffeine, frequently present in human plasma, was well separated from theophylline at all concentration levels as were several other drugs commonly used in respiratory problems.

INTRODUCTION

Theophylline (1,3-dimethylxanthine) is closely related to caffeine (1,3,7-trimethylxanthine) and its pharmacological actions include stimulation of respiration, relaxation of smooth muscle of the bronchial and blood vessels other than cerebral vessels and diuresis. Its main use is in the treatment of reversible airway obstruction, as in asthma, and as an adjunct to the therapy of acute left ventricular failure[1].

There is a frequent need for measurement of plasma levels of theophylline to ensure that appropriate amounts of drug are given. The variability of absorption and elimination of this drug makes it important to adjust dosage to the individual patient.

Several methods have been described for the determination of theophylline in body fluids. These methods are based upon spectrophotometry[2–4], gas–liquid chromatography[5–7], and high-pressure liquid chromatography[8,9]. A previous method determining the levels of theophylline, 2,3-dihydroxypropyltheophylline and 2-hydroxypropyltheophylline in plasma using direct spectrophotometry on thin layers was described[10]. However, in that method the extraction procedure was quite time consuming and it required a large quantity of plasma (5 ml), making it inappropriate for clinical use.

In this paper a method is described based upon separation of theophylline on

a silica gel thin-layer chromatography (TLC) plate followed by direct determination of its absorbance. The procedure may be used to measure very low levels of theophylline in biological fluids and to measure the drug using relatively small volumes of plasma. Evidence is also given which indicates that the method may be satisfactorily applied when caffeine and some other drugs are present in the blood.

EXPERIMENTAL

Material and reagents

The pre-scored TLC plates were silica gel 60 of dimensions 20 × 20 cm (E. Merck, Darmstadt, G.F.R.). Hamilton microliter syringes were used for spotting.

All solvents were reagent grade and the ether was distilled before use. The buffer for the theophylline extraction was 0.5 *M* acetate buffer (pH 5.0).

The developing liquid consisted of 90 ml of chloroform and 10 ml of methanol.

The stock solution was prepared by dissolving the drug in methanol in a concentration of 1 mg/ml. A standard solution (100 μg/ml) was prepared by diluting 5 ml of the stock solution to 50 ml with methanol. Appropriate volumes were applied to the TLC plate to give a series of spots of theophylline in a range of 25–400 μg for the estimation of the calibration curve.

Apparatus

Absorbance was measured by scanning TLC plates with a Schoeffel SD-3000-1 spectrodensitometer using the reflectance mode. Absorbance intensity resulting from irradiation at 275 nm was recorded as a peak on a strip chart recorder SDC 300 density computer. Peak areas were obtained simultaneously by an electronic integrator Autolab System IV integrator (Mountain View, Calif., U.S.A.) and the standard curves relating areas to drug amount were calculated using the method of least squares.

Extraction

To 1 ml of plasma in a 20-ml centrifuge tube was added 0.5 ml of acetate buffer and the mixture was shaken for 10 min with 4 ml of extracting solvent consisting of diethyl ether and methylene chloride (7:4). The mixture was then centrifuged for 10 min at 3000 rpm. The supernatant organic layer was transferred to a second tube and the aqueous layer was again extracted with 4 ml of the solvent mixture. The combined organic layers were evaporated to dryness at 40° under a stream of nitrogen. With a syringe 100 μl of methanol were added and the tube was rotated to dissolve the residue. Three spots of 1 μl each of the methanol solution were applied to a TLC plate together with a series of six spots of theophylline (0.025–0.200 μg) by spotting appropriate volumes of the standard solution. The centers of the spots were 1 cm apart and 2 cm from the lower edge of the plate. The plate was developed in chloroform–methanol (9:1) in a saturated tank, allowing the solvent to migrate about 15 cm (elution time about 20 min). The plate was air-dried and the spots were scanned at 275 nm. The theophylline peak (R_F 0.40) appeared on the recorder chart as a symmetrical peak well resolved from caffeine (R_F 0.50) and some other drugs.

Recovery

The methanolic solution of theophylline was added to blank plasma in a

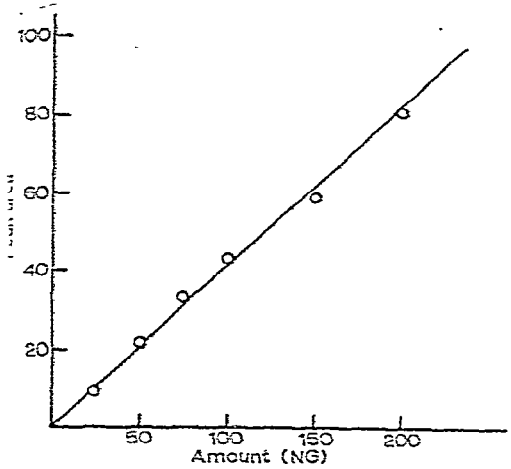

Fig. 1. Theophylline standard curve in methanol.

concentration range of 2.5–20.0 μg/ml and measurement was carried out as described above.

RESULTS AND DISCUSSION

The sensitivity of the method is illustrated in Fig. 1 with the calibration curve of theophylline. A linear relationship between the integrated area under the peak and the amount on the plate was observed between 25 and 200 ng. Typical peaks observed on the recorder chart after scanning theophylline standards are shown in Fig. 2.

Data illustrating reproducibility and recovery from plasma are shown in Table I. As can be seen, the per cent recovery from plasma varied from 95–107%. An application of the method is summarized in Table II, which shows theophylline plasma concentrations in patients who received oral doses of 200 mg aminophylline in tablet form.

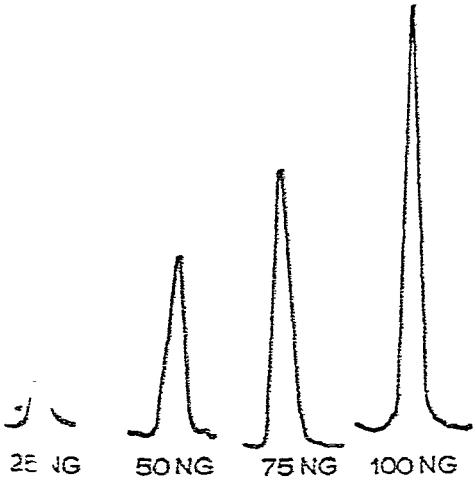

Fig. 2. Peaks observed when scanning the absorbance of theophylline standards on silica gel 60.

TABLE I

RECOVERY OF THEOPHYLLINE FROM HUMAN PLASMA

Amount added to plasma (μg/ml)	*Number of samples*	*Mean recovery*		*Standard error of mean (%)*
		μg	*%*	
2.5	6	2.69	107	3.05
5.0	6	4.74	95	4.71
7.5	6	7.15	95	1.17
10.0	6	9.74	97	3.47
15.0	6	14.53	97	1.91
20.0	6	20.19	101	2.52

TABLE II

PLASMA CONCENTRATION OF THEOPHYLLINE IN PATIENTS FOLLOWING ORAL DOSE OF 200 mg AMINOPHYLLINE TABLETS

Patient	*Time after dose (h)*	*Theophylline concentration (μg/ml)*
G.K.	5	0
V.M.	2	10
V.M.	2	11.6
G.L.	3	6.7
L.F.	3	3.9
S.K.	3	5.0

The outlined method is specific, accurate, and rapid. The samples can be measured readily in 2 h. As theophylline is often combined with antihistamines, tranquilizers and hypnotics in a dosage regimen, a method which is specific is of vital importance. In order to evaluate the specificity of this procedure, several drugs which are often given concurrently with theophylline were subjected to the same procedure of extraction and chromatography after being added in the usual amounts to human plasma containing theophylline. None of those tested interfered, since they were well separated on the TLC plates (Table III). With other drugs it may be necessary to modify extraction and/or chromatography procedures to achieve separation, but it seems unlikely that this will be difficult.

This method allows one to obtain reasonably accurate results with as little as

TABLE III

R_F VALUES OF SEVERAL DRUGS SUBJECTED TO THE THEOPHYLLINE PROCEDURE

Compound	R_F
Theophylline	0.40
Caffeine	0.50
Ephedrine	0.32
Diazepam	0.72
Phenobarbital	0.66
Codeine	0.10
Diphenhydramine	0.20

75 ng of theophylline. This suggests that a much smaller plasma sample might be used, so small perhaps that one might avoid puncturing the vein and use blood from the finger or ear lobes. The development of a micro procedure for this purpose is now being studied by the authors.

The only aspect of the described method that might be considered as a limitation is the cost of a good scanning spectrodensitometer. However, this technique can be used in quantitation of a wide range of different drugs in biological fluids which will certainly increase the application and usefulness of the instrument to assays in the clinical laboratories and research institutes.

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