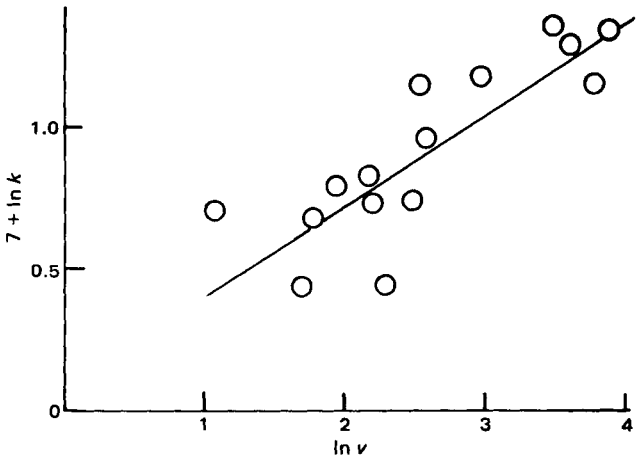

Figure 8—*Dissolution data from dissolution apparatus reported here plotted according to Eq. 4a.*

The effect of liquid velocity on the dissolution of nitrofurantoin from two dosage forms (capsules and tablets) was checked by conducting dissolution tests with all mentioned methods. All (except one) sets of data adhered to a σ^{-}-type plot (17) of the type:

$$\ln(m/m_0) = -Kt + Q \qquad \text{(Eq. 7)}$$

where m is mass undissolved, the zero subscript implies the initial condition, K is a dissolution constant (seconds^{-1}), and Q is a constant. The value of K depends both on dissolution and on how well the dissolution apparatus disintegrates the dosage unit, and Q depends on dissolution only (16). Table V lists K values from the data. There is a correlation between K and v (correlation coefficient of 0.432, which is significant for $n = 29$ at $p = 0.95$), but it is not sufficiently exacting to allow correlation from method to method. The different methods undoubtedly act differently regarding the disintegration of the dosage unit. The fact that the correlation is real, however, makes it a sound basis for calibration or correlation between different pieces of the same apparatus.

The heterogeneity of the dissolution medium reported earlier for the USP basket method (8) is not nearly as pronounced in the other apparatus studied. The paddle apparatus in particular gives good homogeneity (Table VI).

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Quantitative TLC Determination of Propranolol in Human Plasma

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Abstract □ A spectrodensitometric assay was developed for propranolol based on measurement of the absorbance of the drug on silica gel plates irradiated at 288 nm. Quantities as low as 0.010 μg can be detected, and a linear relationship was obtained between 0.010 and 0.400 μg. The percent recovery from plasma spiked with known amounts of the drug was 90.0–102.0. This procedure was used to determine propranolol in the plasma of patients receiving therapeutic doses of the drug.

Keyphrases □ Propranolol—TLC analysis in plasma □ TLC—analysis, propranolol in plasma □ Cardiac depressants—propranolol, TLC analysis in plasma

Propranolol produces a specific blockade of β-adrenergic receptors with minimal intrinsic activity, resulting in an effect on the cardiovascular system. It is capable of suppressing heart rate and contractility and of increasing vasoconstriction in those beds where there may be some degree of masked β-adrenergic vasodilator effect. It is used for the treatment of angina pectoris and in cardiac arrhythmias, hypertrophic subaortic stenosis, pheochromocytoma, and digitalis-induced ventricular tachycardia.

Propranolol is presently receiving much attention in the treatment of essential hypertension associated with a high cardiac output or high plasma angiotensin levels (1, 2).

BACKGROUND

To follow the pharmacokinetics of this drug and to understand the mechanism of its action, plasma propranolol levels are determined. The low levels and the wide variability of plasma propranolol concentrations

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Table I—Reproducibility of Recovery of Propranolol from Spiked Human Plasma (1 ml)

Amount Added, μg[a]	Mean Recovery	Recovery ± CV, %
0.010	0.0094	94.0 ± 2.4
0.020	0.019	95.0 ± 4.9
0.040	0.036	90.0 ± 1.9
0.080	0.073	91.3 ± 1.9
0.120	0.114	95.0 ± 2.7
0.160	0.154	96.3 ± 1.8
0.200	0.204	102.0 ± 1.6

[a] Six samples were determined in each case.

(0.010–0.500 μg/ml) in different subjects after administration necessitated the development of rapid, specific, and sensitive methods for its analysis in biological specimens so that dosage adjustments for the individual patient could be made.

In previous studies, fluorometric methods were applied to the determination of propranolol (3–5), but they had high and variable blank values which, when subtracted from plasma readings, caused considerable variation in results (6). GLC (7, 8) and GLC–mass spectrometric (9, 10) analyses also were reported. Although these methods may be useful for drug quantitation, they require derivatization techniques and often are difficult as well as time consuming because of the slow elution of contaminant peaks, which limits the frequency of sample injection. The most recent GLC method (11) increased the speed of elution of contaminant peaks by using a different derivative and internal standard.

A TLC method, published recently (12), consists of extraction of propranolol and one of its metabolites from biological fluids, separation, and direct measurement of the fluorescence of the spots on the plate. The extraction of propranolol from plasma was only 70%. Fluorescence was achieved by spraying the developed plates with citric acid solution. The plates had to be scanned while still wet from the spraying, a fact that may result in unreliable readings if there is any delay in scanning.

This paper reports a highly sensitive and specific TLC method for the rapid estimation of propranolol in human plasma. The drug is extracted from plasma, separated on silica gel plates, and measured directly by UV absorption. With this method, propranolol concentrations as low as 0.010 μg may be measured accurately, and as many as six samples and three standards can be chromatographed simultaneously on a single plate.

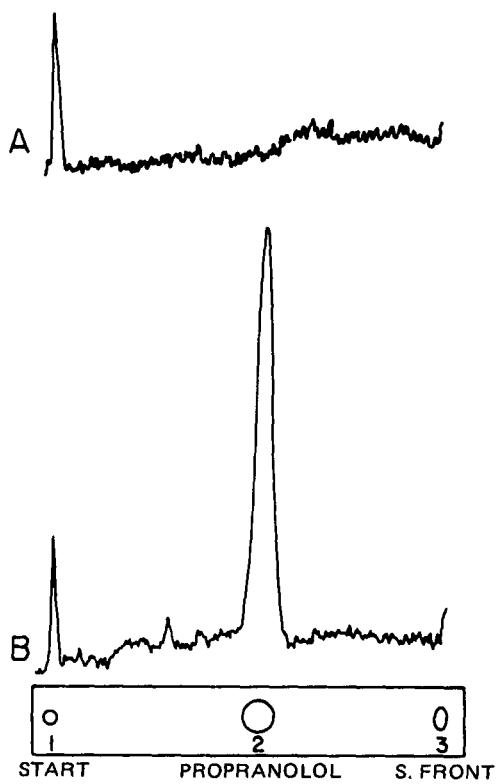

Figure 1—*Chromatograms of plasma samples from a patient before (A) and after (B) an oral dose of propranolol.*

EXPERIMENTAL

Reagents—Propranolol hydrochloride was used for method development[1]. The compound was dissolved in and diluted with methanol to appropriate concentrations (expressed as the free base). All solvents were spectroanalyzed grade, which is especially important to this method. Other chemicals were analytical grade. The developing solvent was methanol–28% aqueous ammonium hydroxide (100:1.5).

Instrumentation—A spectrodensitometer[2] in combination with an integrator[3] and strip-chart recorder[4] was used.

Standard Solutions—The stock solution was prepared as a 1-mg/ml solution in methanol. A standard solution (100 μg/ml) was prepared by diluting 5 ml of the stock solution to 50 ml with methanol. Appropriate volumes were used on the TLC plates to give a series of spots of propranolol in the 0.010–0.400-μg range.

Plasma Extraction—A volume of 1.0 ml of plasma was pipetted into a 20-ml glass centrifuge tube with a polytef-lined screw cap. A 0.5-ml aliquot of 0.5 *N* NaOH and 4 ml of dichloromethane were added, and the tube was shaken for 10 min mechanically. The tube was then centrifuged briefly to separate the layers, the organic phase was transferred to another tube, and extraction of the aqueous layer was repeated with an additional 4 ml of the solvent. The tube with the combined organic phases was immersed in a water bath at 45°, and the solvent was evaporated to dryness under a nitrogen stream.

The solid residue was dissolved in 50 μl of methanol, and suitable aliquots, 5–10 μl, were spotted on a TLC plate[5] along with a series of extracts from plasma spiked with known amounts of propranolol covering at least three concentrations in the 0.010–0.400-μg/ml range. The samples were applied with an automatic spotter[6] supplied with temperature and speed control or by hand using 10-μl syringes[7]. Prior to use, the plates were activated at 105° for 20 min or were used directly from a freshly opened package.

The chromatograms were developed at room temperature in a glass tank by the ascending technique in the developing solvent and were removed from the chamber after the solvent reached a height of 15 cm (~40 min). The solvent system was freshly prepared each day and allowed to equilibrate for 30 min prior to use. After development, the plate was air dried prior to scanning.

The densitometric measurements were performed using the reflectance mode on the scanner, and the plates were scanned at 25 mm/min at the absorption maximum of propranolol (288 nm).

A series of spiked plasma samples in the 0.010–0.400-μg range was run to establish the relation between peak areas (square millimeters) and concentration for the method. The assay result for each sample was calculated by dividing the propranolol peak area of the sample by that of the standard with the closest area and multiplying the ratio by a known concentration of the standard.

Recovery Experiments—The recovery of propranolol from spiked plasma was determined by comparison of the absorbance peak areas from spiked plasma with those of the standard solutions spotted directly on the same TLC plate.

Patient Plasma Samples—Eight hospitalized patients were treated with 120 mg of propranolol hydrochloride[8] daily given orally in three 40-mg doses. Blood samples were drawn 1 week after the start of the treatment at 2 hr after the morning dose. Blood (3–4 ml) was collected in heparinized tubes and centrifuged to separate the plasma. Plasma samples were stored in a refrigerator for not more than 24 hr before analysis.

RESULTS AND DISCUSSION

Plasma Propranolol Determination—Chromatograms of plasma samples from a patient before and after an oral dose of propranolol are shown in Fig. 1. In the chromatogram of the blank plasma, there were no interfering peaks in the region of propranolol. The R_f value of propranolol on the plates was 0.53 in the solvent used. Chromatogram B shows that none of the metabolites of propranolol usually formed after oral administration (13) interfered. Either the extraction procedure used does not

[1] Gift of Ayerst Laboratories, New York, N.Y.
[2] UV-VIS-2 chromatograph scanner, Farrand.
[3] CDS 101 chromatography data system, Varian.
[4] Model 100, Farrand.
[5] Silica gel 60 glass plates, 20 × 20 cm, 0.25 mm, E. Merck Laboratories.
[6] Multi-Spotter, Analytical Instrument Specialties.
[7] Hamilton.
[8] Inderal tablets, 40 mg, Ayerst Laboratories.

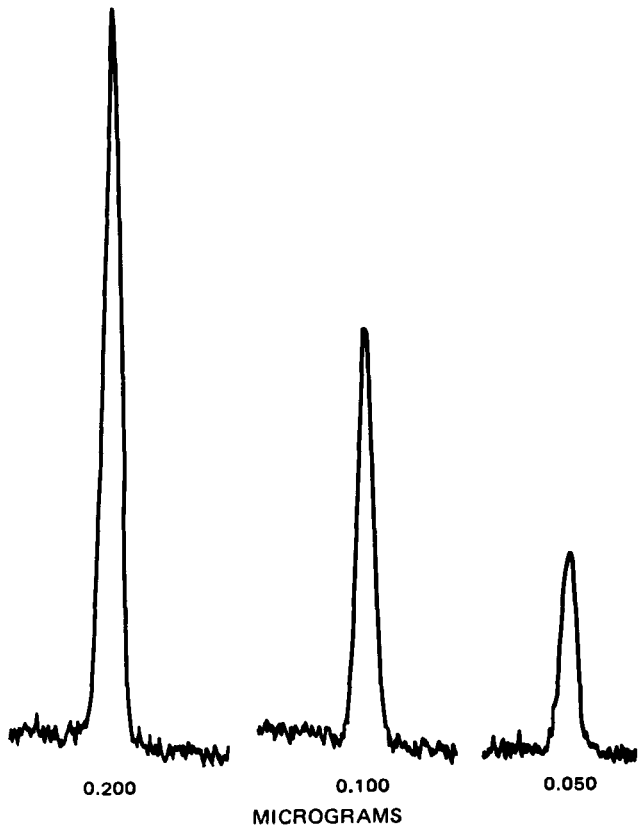

Figure 2—*Typical TLC chromatogram scans obtained with spiked plasma samples.*

extract the metabolites from the plasma or the metabolites do not absorb at 288 nm.

The curve obtained from determinations of known propranolol concentrations in spiked plasma and measurement of the areas under the peaks was linear within the range of 0.010–0.400 μg of drug/ml. The minimum measurable concentration was about 0.010 μg/ml.

Typical TLC chromatogram scans obtained with spiked plasma samples are shown in Fig. 2.

The reproducibility and recovery results from a normal range of plasma concentrations are given in Table I. The recovery varied from 90 to 102%. It is recommended that both the methanol standards, spotted directly on the plate, and the extracts from spiked plasma specimens be determined routinely and concurrently with plasma samples from patients containing unknown quantities of propranolol. This practice enables the detection of incomplete extraction or possible contamination of the observed spots with unknown plasma components.

Application to Clinical Samples—This method is applicable to plasma samples from patients (Table II). Large variations in plasma levels between individuals receiving the same dosage of the drug were reconfirmed.

In all of these assays, 1-ml plasma samples were used. Lower volumes can be used successfully by spotting larger aliquots of the redissolved residue.

Table II—Plasma Propranolol Concentration in Patients following Oral Dose of 40-mg Propranolol Tablets

Patient	Propranolol, μg/ml[a]
AG	0.085
PS	0.017
MD	0.107
MM	0.039
BH	0.149
FS	0.103
CS	0.128
PJ	0.045

[a] At 2 hr after dosing in all cases.

The described TLC method has several advantages over previously reported assays; it is specific, highly sensitive, and fast. About 30 samples can be determined within 8 hr by one person. Its specificity is of particular importance for plasma level studies since the drug is separated from possible interfering substances and is determined directly on the separation medium.

The method sensitivity could probably be increased by measuring the fluorescence of propranolol and keeping other parts of the procedure the same. This increase can be achieved readily with the fluorescence attachments supplied for most spectrodensitometers. At present, the UV absorbance method is as sensitive as is needed.

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