# Sensitive High-Pressure Liquid Chromatographic Determination of Propranolol in Plasma

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**Abstract** □ A high-pressure liquid chromatographic method is presented for the determination of propranolol in human plasma. A reversed-phase cyanopropylsilane column was utilized with a liquid phase consisting of 70% acetonitrile and 30% 0.02 *M* acetate buffer, pH 7.0. A spectrofluorometric detector with an excitation wavelength of 276 nm and an emission filter with a 340-nm cutoff provided a detectable peak for 0.8 ng of propranolol/injection with this system. The reproducibility and precision of the method are shown from the analyses of samples containing 10–150 ng/ml of plasma.

**Keyphrases** □ Propranolol—high-pressure liquid chromatographic analysis in plasma □ High-pressure liquid chromatography—analysis, propranolol in plasma □ Cardiac depressants—propranolol, high-pressure liquid chromatographic analysis in plasma

Propranolol is used in the treatment of hypertension, angina pectoris, and cardiac arrhythmias and is used experimentally for several other conditions. Because of differences in both the pharmacokinetics and the response to the drug, the dosage regimen must be individualized. Clinical determination of plasma levels provides the most efficient and precise aid in adjusting dosage.

Plasma levels encountered during therapy with propranolol may range from a low of 20 to >100 ng/ml (1–3), a concentration range that prohibits the use of many common analytical techniques. Several methods for the quantitative determination of propranolol in plasma use electron-capture GLC, which involves derivatization of the compound (4–6). Radioimmunology (7), in spite of high sensitivity and specificity, is seldom used because of limitations in availability of antiserums. Direct fluorometric determination (8–10) is popular but lacks specificity.

This paper describes a rapid method for the determination of propranolol involving high-pressure liquid chromatographic (HPLC) separation and fluorometric detection. The combination of HPLC and fluorometry provides the needed specificity and sensitivity for the drug. Conditions are discussed for the extraction, separation, and detection of propranolol. The applicability of this procedure was demonstrated by the analysis of plasma from patients taking propranolol.

## EXPERIMENTAL

**Instrumentation**—A high-pressure liquid chromatograph[1] was equipped with a fluorometric detector[2] and a cyanopropylsilane[3] column, 300 mm long × 4 mm i.d. The degassed mobile phase was pumped through the column at 2.0 ml/min (1100–1200 psi) at ambient temperature until a stable baseline was obtained. The fluorometer was set at an excitation wavelength of 276 nm, and an emission cutoff filter (type 340) was used.

**Chemicals and Reagents**—Sodium acetate, sodium phosphate, acetic acid, phosphoric acid, dichloromethane, benzene, chloroform, and ethyl acetate were reagent grade. HPLC grade methanol and acetonitrile were used. Propranolol hydrochloride[4], 4-hydroxypropranolol[4], and cyclomethycaine sulfate[5] were obtained commercially.

**Mobile Phase**—Sodium acetate solutions (0.01, 0.02, and 0.04 *M*) were prepared in distilled water. The pH was adjusted to 4, 5, 6, or 7 with acetic acid. The mobile phase was prepared by adding an appropriate amount of acetonitrile to the aqueous buffer and degassing under vacuum. The effect on the retention time of various buffer concentrations and buffer pH was studied.

**Extraction Conditions**—The extraction of propranolol and the internal standard (cyclomethycaine) from plasma at pH 7.4 and 12 was studied using dichloromethane. The relative extractability of solutes from spiked plasma samples at pH 7.4 was compared using four solvents: benzene, hexane, ethyl acetate, and dichloromethane.

**Analytical Procedure**—To 0.5 ml of heparinized plasma (in a 15-ml screw-capped centrifuge tube) were added 0.5 ml of phosphate buffer (pH 7.4 and 0.1 *M*), 5 ml of solvent containing 0.16 μg of cyclomethycaine

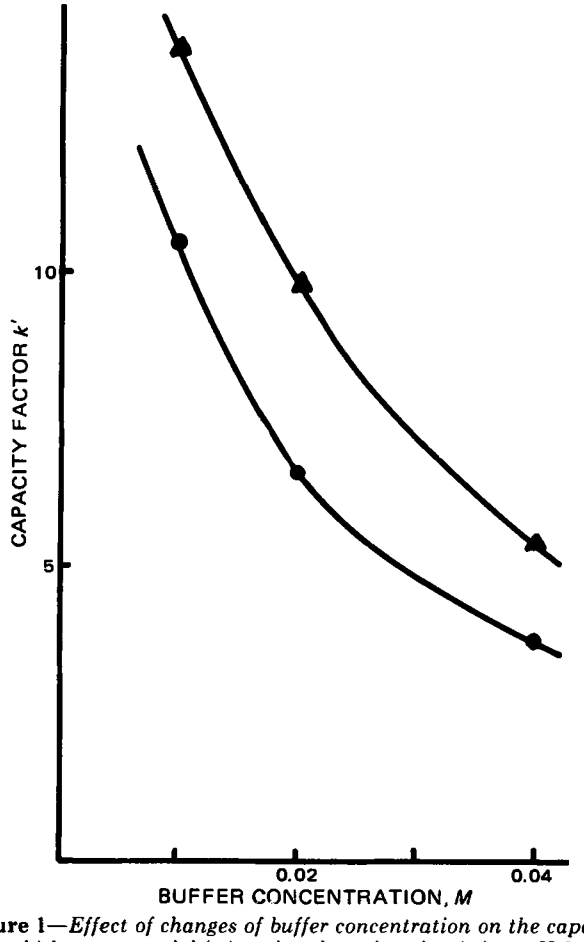


**Figure 1**—*Effect of changes of buffer concentration on the capacity factor k' for propranolol (●) and cyclomethycaine (▲) at pH 6 using 75% acetonitrile.*

[1] Model 202 chromatograph, M 600 pump, and U6K Universal injector, Waters Associates, Milford, Mass.
[2] FS-970, Schoeffel, Westwood, N.J.
[3] μBondapak CN, Waters Associates, Milford, Mass.
[4] Ayerst Laboratories, New York, N.Y.
[5] Eli Lilly and Co., Indianapolis, Ind.

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**Table I—Evaluation of Assay Precision and Accuracy for Propranolol**

| Propranolol Hydrochloride Added, ng/ml | n | Mean Propranolol Hydrochloride Measured, ng/ml | Percent Recovered | CV |
|---|---|---|---|---|
| 10 | 5 | 10.56 | 105.6 | 13.7 |
| 25 | 4 | 24.19 | 96.8 | 5.6 |
| 50 | 4 | 54.51 | 109.0 | 5.7 |
| 75 | 5 | 73.38 | 97.8 | 1.3 |
| 100 | 3 | 100.69 | 100.7 | 4.5 |
| 150 | 3 | 149.20 | 99.5 | 2.6 |
| Average | | | 101.6 | |

sulfate/ml as the internal standard, and an aliquot of the propranolol hydrochloride solution containing 5.0–75.0 ng (10–150 μl of a methanolic solution). The tubes were then vortexed for 10 sec and centrifuged for 5 min at 900×*g*. A 4.0-ml volume of the organic phase was transferred to special concentration tubes[6] and evaporated to dryness at ambient temperature under a gentle stream of nitrogen.

The residue was dissolved in 100 μl of methanol, and replicate 50-μl injections were made using a 50-μl syringe[7]. A standard curve was constructed by injecting plasma extracts simulating concentrations of propranolol hydrochloride from 10 to 150 ng/ml. The chromatograms were recorded[8] at a chart speed of 5 mm/min. The peak heights were measured, and the ratios (propranolol to cyclomethycaine) were calculated and plotted *versus* the propranolol concentrations expressed as nanograms per milliliter.

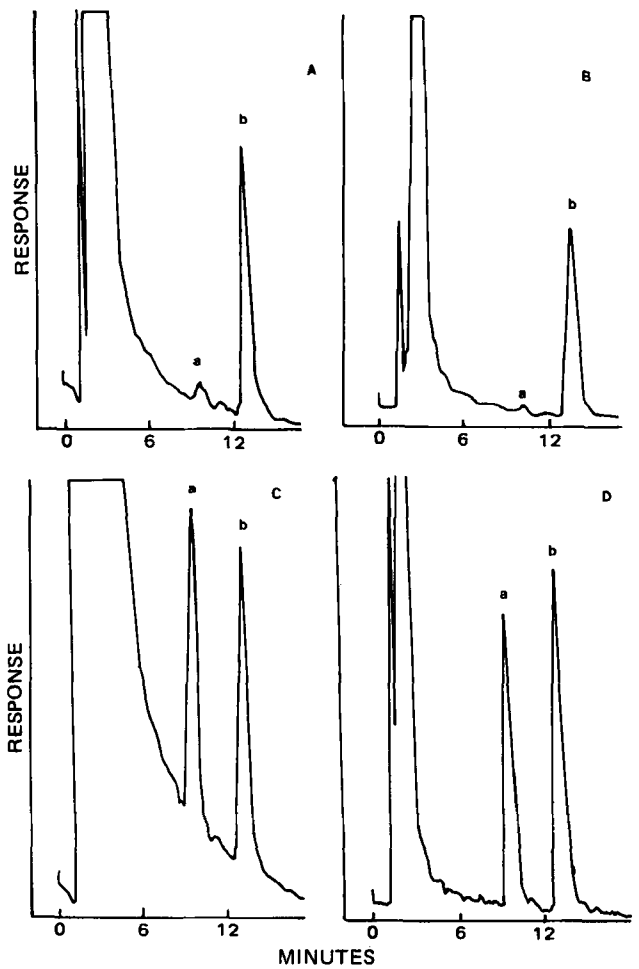


**Figure 2**—*Chromatograms of plasma extracts containing propranolol hydrochloride (a) and cyclomethycaine sulfate (b). Key: A, hexane; B, benzene; C, ethyl acetate; and D, dichloromethane.*

**Interferences**—The possible interferences from normal plasma constituents and the major metabolite (4-hydroxypropranolol) (11), as well as other drugs, were studied by adding therapeutic concentrations of these drugs to plasma containing propranol and the internal standard and then performing the analysis.

**Sample Preparation and Assay**—Heparinized plasma samples from patients receiving oral propranolol therapy were processed in duplicate as described under *Analytical Procedure*. The amount of propranolol was calculated by comparison with standards prepared daily.

## RESULTS AND DISCUSSION

Increasing the buffer molar concentration shortened the capacity factor in a nonlinear fashion (Fig. 1). The capacity factor of the compounds also was affected by pH change. A mobile phase of 70% acetonitrile and 30% acetate buffer (pH 7.0 and 0.02 *M*) gave well-resolved sharp peaks for propranolol, 4-hydroxypropranolol, and cyclomethycaine with retention times of 10.0, 7.7, and 13.5 min, respectively. Quinidine, lidocaine, procainamide, and disopyramide, commonly utilized cardiac drugs, were eluted at different retention times and thus did not interfere with the analysis under the experimental conditions. The choice of fluorometer parameters allowed a concentration of propranolol as low as 0.8 ng on-column to give a peak clearly distinguishable from baseline noise. After the study was completed, a cyanopropylsilane column from a different manufacturer[9] was utilized with only minor changes in solvent conditions.

Dichloromethane was selected as the solvent for extraction of propranolol from plasma at pH 7.4. This selection was based on the highest amount of propranolol and/or the lowest amount of interfering fluorescent material being extracted. Hexane and benzene were inefficient in the extraction of propranolol, while ethyl acetate extracted excessive fluorescent substances from plasma (Fig. 2). Dichloromethane produced excellent recovery of propanolol and minimum extraction of interfering constituents from plasma and was evaporated easily.

A high recovery of propranolol (90%) from spiked plasma samples was obtained with dichloromethane at pH 7.4. When 0.1 *N* NaOH was used as an alkalinizing agent, the ratio of the propranolol peak height to the internal standard peak height dropped appreciably. Extraction at pH 7.4 with dichloromethane using a ratio of the organic phase to the aqueous phase of 5 gave satisfactory recoveries of propanolol and the internal standard; a second extraction yielded only trace quantities of the two compounds.

The ratio of the peak height of propranolol to the peak height of the internal standard was calculated. Statistical analyses (Table I) by linear regression indicated excellent linearity and reproducibility with a correlation coefficient of 0.994, a slope of 0.00297, and an intercept of 0.000302 in the range of 1.8–26.4 ng of propranolol on-column. This range corresponds to 10–150 ng of propranolol hydrochloride/1.0 ml of plasma and includes the therapeutic range of the drug.

The method was applied to the determination of free propranolol in patient plasma before and after administration of a dose. No interference was noted, and the method proved to be sensitive even to the trace amount of propranolol present before dosing. The method can be recommended for the pharmacokinetic studies of propranolol in the presence or absence of other simultaneously prescribed cardiovascular drugs.

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(6) T. Walle, J. Morrison, K. Walle, and E. Conradi, *J. Chromatogr.*, **114**, 351 (1975).

[6] Concentratubes, Laboratory Research Co., Los Angeles, Calif.
[7] Hamilton Co., Reno, Nev.
[8] Model 56, Perkin-Elmer, Norwalk, Conn.
[9] DuPont, Wilmington, Del.

(7) K. Kawashima, A. Levy, and S. Spector, *J. Pharmacol. Exp. Ther.*, **196**, 517 (1976).
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# Electron-Capture GLC Assay of Dichlorphenamide

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**Abstract** □ GLC with electron-capture detection was applied to the assay of the carbonic anhydrase inhibitor dichlorphenamide and demonstrated a sensitivity of 10 ng in 0.5 ml of rabbit serum or whole aqueous humor (≃0.25 ml) from one rabbit eye. After extraction of the drug and internal standard (monochlorphenamide) from the biological fluid, these compounds were converted to their tetramethyl derivatives by a nucleophilic alkylation method. Dichlorphenamide contents of aqueous humor and serum of rabbits treated with this drug are reported.

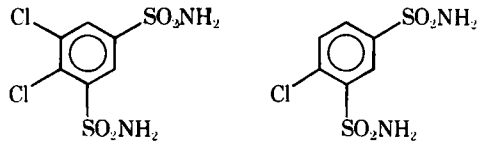
**Keyphrases** □ Dichlorphenamide—GLC analysis in biological fluids □ GLC—analysis, dichlorphenamide in biological fluids □ Carbonic anhydrase inhibitors—dichlorphenamide, GLC analysis in biological fluids

Dichlorphenamide (I), 4,5-dichloro-1,3-disulfamoylbenzene, is an orally effective carbonic anhydrase [carbonate dehydratase EC 4.2.1.1 (1)] inhibitor[1] (2). This drug possesses diuretic activity (3, 4) and decreases intraocular pressure in rabbits (5) and in normal (2) and glaucomatous (2, 6, 7) humans. Assay of dichlorphenamide in biological fluids, especially aqueous humor, requires a very sensitive method. GLC with electron-capture detection is useful for the determination of submicrogram quantities of compounds in small volumes of body fluids. However, direct GLC of polar compounds is usually not possible; derivatization techniques must be used to convert these compounds to less polar derivatives.

This paper describes a GLC technique combined with electron-capture detection for the assay of dichlorphenamide and its application to the determination of aqueous humor and serum drug levels following dichlorphenamide administration to rabbits by different routes.

## EXPERIMENTAL

**Reagents and Chemicals**—The following were used: dichlorphenamide[2] (I), 4-chloro-1,3-disulfamoylbenzene[2] (II) as the internal standard, toluene[3] (pesticide quality), ethyl acetate[3] (pesticide quality), *N,N*-dimethylacetamide[4] for synthesis, acetic acid[4] for analysis, sodium bicarbonate[4], anhydrous sodium sulfate[4], 10% aqueous tetramethylammonium hydroxide[4], methanol[4], and methyl iodide[5] (purum). A half-saturated solution of sodium bicarbonate and 0.1 *N* $NH_4OH$ were prepared with deionized water. The tetramethylammonium hydroxide was diluted fourfold with methanol.




I II

**Instrumentation**—The gas chromatograph[6] was equipped with a $^{63}Ni$ (10-mCi) electron-capture detector. A 1.5-m × 3-mm i.d. glass column containing 3% OV-17 on 100–120-mesh Gas Chrom Q[7] was used. The carrier gas was argon–methane (90:10) with a flow rate of 60 ml/min and <5 ml/min detector scavenge. Temperatures were 265° for the column and 290° for the injector and detector.

GLC–mass spectrometry of the derivative was carried out with a mass spectrometer[8] operated in the electron-impact (70 ev) mode and equipped with a 1.5-m × 3-mm column packed with 2% SE-30 on 80–100-mesh Gas Chrom Q[7] (250°).

**Drug Extraction**—The extraction method was based on that described by VandenHeuvel *et al.* (8) for hydrochlorothiazide in human plasma. An appropriate, known volume of aqueous humor or serum (containing less than 100 ng of dichlorphenamide) was diluted with water to 0.5 ml in a 7-ml disposable, silanized, glass-stoppered tube to which 75 ng of II in 0.1 ml of ethyl acetate (taken to dryness with a nitrogen

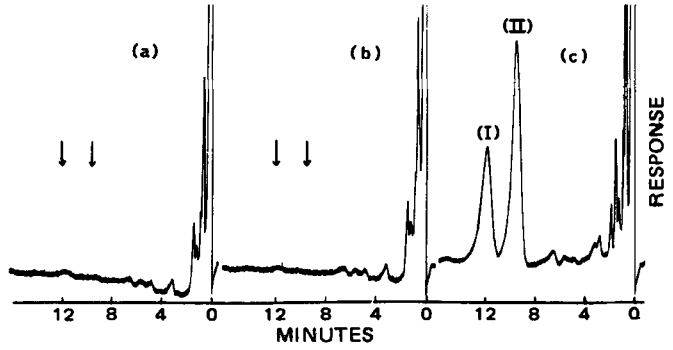



**Figure 1**—*Chromatograms (1 × $10^{-9}$ afs at 1 mv) from control aqueous humor (a), control serum (b), and control aqueous humor spiked with 50 ng of dichlorphenamide (I) and 75 ng of the internal standard (II) (c).*

[1] Daranide, Merck Sharp & Dohme.
[2] Merck Sharp & Dohme.
[3] Carlo Erba.
[4] E. Merck.
[5] Fluka.
[6] Girdel 75.
[7] Altech Associates.
[8] Finnigan 3200.

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