the sensitivity of the method can no doubt be extended making it an attractive technique for studying the bioavailability and pharmacokinetics of flurbiprofen in pediatric populations where small sample volumes and lower doses are required.

## REFERENCES


(1) S. S. Adams and K. F. McCullough, 7th European Rheumatology Congress, London, England, Abstr. 32.2 (1971).

(2) I. M. Chalmers, B. J. Cathcart, E. B. Kumar, W. Carson Dick, and W. W. Buchanan, *Ann. Rheum. Dis.*, **31**, 319 (1972).

(3) I. M. Chalmers, M. A. Bell, and W. W. Buchanan, *Ann. Rheum. Dis.*, **32**, 58 (1973).

(4) B. G. Snider, L. J. Beaubein, D. J. Sears, and P. D. Rahn, *J. Pharm. Sci.*, **70**, 1347 (1981).

(5) D. G. Kaiser, S. R. Shaw, and G. J. Vangiessen, *J. Pharm. Sci.*, **63**, 567 (1974).

(6) P. C. Risdall, S. S. Adams, E. L. Crampton, and B. Marchant, *Xenabiotica*, **8**, 691 (1978).


# Direct, Simultaneous Determination of Propranolol and Its 4-Hydroxy Metabolite by Liquid Chromatography


**G. TAMAI, I. MORITA, T. MASUJIMA, H. YOSHIDA, ˣ and H. IMAI**



Received August 3, 1983, from the *Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima 734, Japan.* Accepted for publication November 28, 1983.



**Abstract** □ Propranolol and its active 4-hydroxy metabolite are determined by direct injection of spiked plasma samples onto a protein-coated ODS liquid chromatographic column. The recovery of propranolol and the 4-hydroxy metabolite is essentially quantitative and the reproducibility is good. The reproducibility and simplicity may be superior to the conventional HPLC analyses which include pretreatment, *i.e.*, solvent extraction or deproteinization.



**Keyphrases** □ Protein-coated ODS column—reverse-phase, direct injection analysis of plasma sample □ Propranolol—analysis and its 4-hydroxy metabolite, plasma


Propranolol, a $\beta$-adrenergic blocking drug, is widely used for the treatment of cardiac arrhythmia, sinus tachycardias, hypertension (1), and other pathological states (2, 3). The therapeutic drug level of propranolol is considered to be in the range of 50–100 ng (193–386 pmol)/mL of plasma (4). The 4-hydroxy metabolite, formed by hydroxylation of propranolol (5), has been reported to be an equipotent $\beta$-adrenergic blocker (6). Thus, for drug level monitoring, both propranolol and the 4-hydroxy derivative should be determined. Determination of propranolol (7, 8) and the 4-hydroxy metabolite using a reverse-phase HPLC method including sample pretreatment, such as solvent extraction or deproteinization has been reported previously (9–11). We reported that the protein-coated ODS column had no affinity for proteins, but did have an affinity for small hydrophobic molecules (12). The simultaneous plasma determination of propranolol and the 4-hydroxy metabolite reported herein is rapid and reproducible, and deproteinization can be carried out by the direct injection of plasma samples onto a protein-coated ODS liquid chromatographic column.

## EXPERIMENTAL SECTION

**Chemicals and Reagents**—Propranolol hydrochloride[1], the 4-hydroxy metabolite (as the hydrochloride)[2], and bovine serum albumin[3] were obtained commercially. All other chemicals were analytical reagent grade.

**Preparation of the Protein-Coated Octadecylsilane Column**—An ODS column (pore size, 10-nm) was packed by the balanced-slurry method (slurry solvent, chloroform; purge solvent, 50% methanol). After the ODS column was equilibrated with phosphate-buffered saline (pH 7.4), 6% bovine serum albumin solution was injected to saturate the column. After the column was equilibrated with 0.1 M phosphate solution (pH 3.0), bovine serum albumin solution (6%) was again injected, and the column was then washed with absolute methanol. This procedure was repeated several times. The same procedure was repeated, using dialyzed rabbit plasma instead of the bovine serum albumin solution. The column treated by the aforementioned procedure is referred to as the protein-coated ODS column below.

**Liquid Chromatographic Apparatus**—An HPLC apparatus with a single plunger pump[4] and a sample injector[5] was assembled in our laboratory. For stepwise elution, solvent switching was performed by a solvent selector[6] and controlled by a sequence programmer[7]. For double-column analysis, a flow-direction switch valve[8] was placed between the precolumn and the analytical column. The valve was controlled by the sequence programmer[7]. Two fluorometers[9] were used in series: one for detection of propranolol (Ex, 297 nm; Em, 347 nm); the other for the 4-hydroxy metabolite (Ex, 327 nm; Em, 427 nm).

**Preparation of Standard and Sample Solutions**—A stock solution of propranolol and the 4-hydroxy metabolite containing 2% sodium metabisulfite (13) was prepared in distilled water at $10^{-5}$ M. Heparinized human and rabbit plasma samples were obtained by centrifuging whole blood (1000×$g$, at 25°C, for 10 min) and filtering[10] to remove solid materials. A plasma sample (0.25 mL) with or without propranolol and the 4-hydroxy derivative at the drug monitoring level, was injected onto the column. If the pressure of the column increased during successive analyses, the inlet filter of the column was washed with 1 M NaOH.

**Single-Column Analysis**—An ODS column of 100 mm × 6 mm i.d.[11] (particle size 5 μm, pore size 10 nm) was protein-coated and then used at room temperature. The protein-coated ODS column was equilibrated with phosphate-buffered saline (flow rate, 2 mL/min). The plasma sample spiked with propranolol and its 4-hydroxy derivative (0.25 mL) was injected onto the column. After eluting plasma proteins and hydrophilic components with phosphate-buffered saline for 5 min, the column was washed with phosphate-buffered saline containing 22% acetonitrile for 4 min. Propranolol and the 4-hydroxy derivative were then eluted with 0.1 M citrate buffer (pH 4.0) containing 22% acetonitrile.

[1] Supplied by Sumitomo Chemicals, Osaka, Japan.
[2] Supplied by I.C.I. Pharmaceutical Ltd., Osaka, Japan.
[3] Fraction V Powder; Sigma, St. Louis, Mo.

[4] Model INTD18-40-3S2K; Toyo Soda, Tokyo, Japan.
[5] Model 7125; Rheodyne, Calif.
[6] Model 8V; Kyowa Seimitsu, Tokyo, Japan.
[7] Model SCY-PO; Omron, Tokyo, Japan.
[8] Model 7010; Rheodyne.
[9] Model RF-530; Shimadzu, Kyoto, Japan.
[10] Millex-HA; Millipore Corp., Bedford, Mass.
[11] Shodex ODSpak; Showa Denko, Tokyo, Japan.


0022-3549/84/1200-1825$01.00/0


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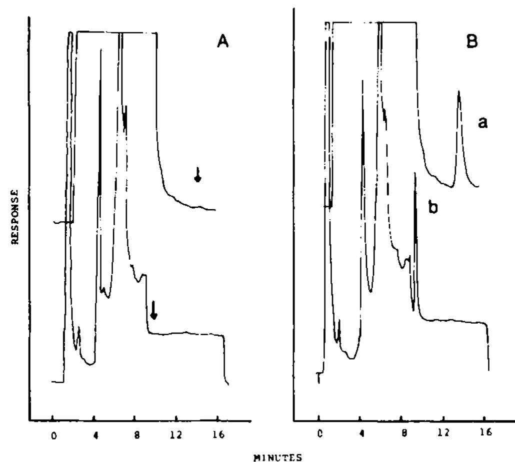


**Figure 1**—*Chromatograms obtained by a single protein-coated ODS column. Key: (A) human plasma blank; (B) human plasma sample spiked with propranolol (a) (Ex 297 nm, Em 347 nm) and the 4-hydroxy metabolite (b) (Ex 327 nm, Em 427 nm). Samples were 100 pmol of drug/0.25 mL of plasma.*

**Double-Column Analysis**—A protein-coated ODS column, 60 mm × 4 mm i.d.[12] (particle size 20 μm, pore size 10 nm), was used as the precolumn at room temperature. A 60 mm × 4 mm i.d. column[13] (particle size 5 μm, pore size 10 nm) was used as the analytical column at 35°C. The precolumn was equilibrated with phosphate-buffered saline (flow rate, 1.2 mL/min). A 0.25-mL plasma sample spiked with propranolol and the 4-hydroxy metabolite was injected onto the precolumn. Plasma proteins and hydrophilic compounds were eluted with phosphate-buffered saline for 5 min, and then the column was washed with phosphate-buffered saline containing 15% acetonitrile for 2 min. After being washed with 0.1 M citrate buffer (pH 4.0) containing 5% acetonitrile for 4 min, the precolumn was connected to the analytical column which was equilibrated with 0.1 M citrate buffer (pH 4.0) containing 15% acetonitrile. The propranolol and 4-hydroxy derivative retained on the precolumn were eluted with 0.1 M citrate buffer (pH 4.0, containing 15% acetonitrile) through the analytical column (flow rate, 1.7 mL/min). For elution from the analytical column, the 15% acetonitrile concentration was suitable for separatory elution. Chromatograms obtained by the flow-through and back-flush methods were compared.

## RESULTS AND DISCUSSION

**Determination By the Single-Column Technique**—Propranolol, a basic drug [p$K_a$ 9.45 (14)], has an affinity for ODS resin at pH 7.4. After the protein-coated ODS column was equilibrated with phosphate-buffered saline, a plasma sample (0.25 mL) spiked with propranolol and the 4-hydroxy metabolite (100–400 pmol/mL of plasma) was injected onto the column. Plasma proteins and hydrophilic compounds were eluted by phosphate-buffered saline, but propranolol and the 4-hydroxy metabolite were retained on the column. It was confirmed that the 4-hydroxy metabolite was eluted by phosphate-buffered saline containing 22% acetonitrile within 7 min, and propranolol was eluted after 30 min. The column was then washed with phosphate-buffered saline containing 22% acetonitrile for 4 min. Propranolol and the 4-hydroxy metabolite were eluted with 0.1 M citrate buffer (pH 4.0) containing 22% acetonitrile. In Fig. 1 are shown chromatograms of a human plasma blank and a plasma sample spiked with propranolol and the 4-hydroxy derivative. A calibration curve is shown in Fig. 2. The recoveries were 101.8 and 101.4%, respectively, with good reproducibilities (*CV*, 1.6 and 1.2%, respectively; $n$ = 5). The practical drug monitoring level of propranolol and the 4-hydroxy metabolite was determined with good reproducibility and recovery. The levels of propranolol and the 4-hydroxy metabolite, analyzed by the present method, were totals in plasma, regardless if free or bound to proteins [binding of propranolol to plasma proteins has been reported to be as high as 90–96% (15)]. In the determination of propranolol with a single protein-coated ODS column, at least 50 samples were analyzed without deterioration of the column.

**Determination By the Double-Column Technique**—Representative chromatograms showing the separation of propranolol and the 4-hydroxy metabolite by the flow-through and back-flush methods are shown in Figs. 3 and 4. With the flow-through method, a slight peak broadening was observed after

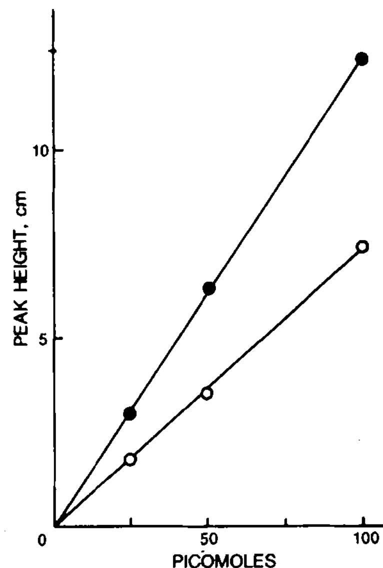


**Figure 2**—*Calibration curve obtained by a single protein-coated ODS column. Key: (○) propranolol; (●) 4-hydroxy metabolite.*

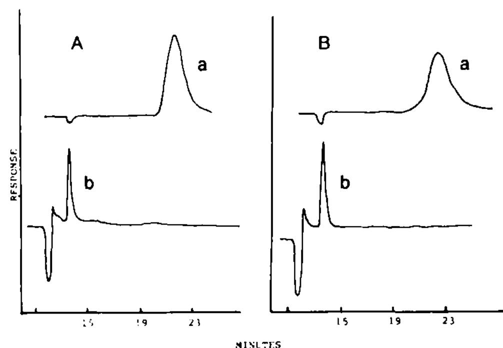


**Figure 3**—*Chromatograms obtained by the flow-through method. Key: (A) chromatogram of first analysis; (B) chromatogram after 150 successive analyses. The eluate from the analytical column was monitored for propranolol (a) and the 4-hydroxy metabolite (b).*

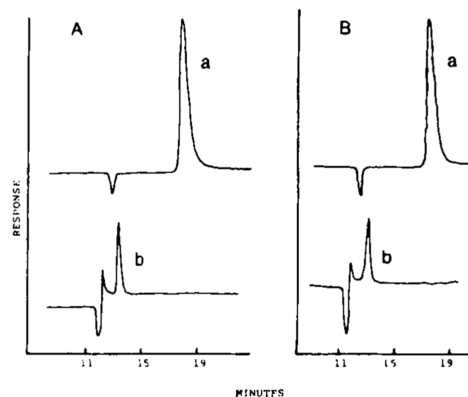


**Figure 4**—*Chromatograms obtained by the back-flush method. Key: (A) chromatogram of first analysis; (B) chromatogram after 150 successive analyses. The eluate from the analytical column was monitored for propranolol (a) and the 4-hydroxy metabolite (b).*

150 successive analyses (Fig. 3). The reason might be due to reduced efficiency of the precolumn. With the back-flush method, sharp chromatograms were obtained and 150 successive analyses could be performed without deterioration of the column (Fig. 4). Propranolol was concentrated at the top of the precolumn by eluting with phosphate-buffered saline containing 15% acetonitrile. Both methods gave quantitative recoveries of propranolol and 4-hydroxy

[12] Gel 3050; Hitachi, Tokyo, Japan.
[13] TSK gel ODS-120T; Toyo Soda, Tokyo, Japan.

Table I—Comparison of Deproteinization Methods [a]

| Deproteinization Method | Recovery, % | CV, % |
|---|---|---|
| Protein-coated ODS column | 100.1 | 0.9 |
| 5% Acetonitrile | 101.0 | 3.2 |
| 5% Trichloroacetic acid | 25.9 | 4.0 |

[a] Conditions: 300 ng of propranolol/mL of plasma; injection of 100 μL of plasma; $n = 5$.

metabolite; therefore, an internal standard was not required. By using a flow direction switching valve, the deterioration of the analytical column was minimized. At least 150 successive analyses with almost no column deterioration were carried out by the back-flush method.

Table I shows the comparison of the methods of deproteinization. Note that in the present method the deproteinization was performed at the initial stage after the injection of sample onto the column. In comparison with common HPLC analyses that include pretreatment, *i.e.*, solvent extraction or deproteinization, the proposed method proved superior in simplicity and accuracy.

## REFERENCES

(1) D. G. Shand, *N. Engl. J. Med.*, **293**, 280 (1975).

(2) A. Mitrani, M. Oettinger, E. G. Abinader, M. Sharf, and A. Klein, *Br. J. Obstet. Gynaecol.*, **82**, 651 (1975).

(3) R. B. Weber and O. M. Reinmuth, *Neurology*, **22**, 366 (1972).

(4) A. S. Nies and D. G. Shand, *Circulation*, **52**, 6 (1975).

(5) J. W. Paterson, M. E. Conolly, C. T. Dolley, A. Hayes, and R. G. Cooper, *Pharmacol. Clin.*, **2**, 127 (1970).

(6) J. D. Fitzgerald and S. R. O'Donnell, *Br. J. Pharmacol.*, **43**, 222 (1971).

(7) G. Nygard, W. H. Shelver, and S. K. W. Khalil, *J. Pharm. Sci.*, **68**, 379 (1979).

(8) M. Simon and R. Terry, *Ther. Drug Monitor.*, **1**, 265 (1979).

(9) R. L. Nation, G. W. Peng, and W. L. Chiou, *J. Chromatogr.*, **145**, 429 (1978).

(10) S. R. Parinam, C. Q. Louise, and S. M. Hiltrud, *Clin. Chim. Acta*, **88**, 355 (1978).

(11) D. W. Schneck, J. F. Pritchard, and A. H. Hayes, *Res. Commun. Chem. Pathol. Pharmacol.*, **24**, 3 (1979).

(12) H. Yoshida, I. Morita, T. Masujima, and H. Imai, *Chem. Pharm. Bull.*, **30**, 2287 (1982).

(13) J. F. Pritchard, D. W. Schneck, and A. H. Hayes, *J. Chromatogr.*, **162**, 47 (1979).

(14) W. Sadee' and G. C. Beelen, "Drug Level Monitoring," Wiley-Interscience, New York, N.Y., 1980.

(15) T. P. Moyer and S. G. Sheps, *Ther. Drug Monitor.*, **3**, 217 (1981).

# Determination of "Brompton's Cocktails" by Circular Dichroism

W. MARC ATKINSON, JOHN M. BOWEN, and NEIL PURDIE [x]

Received November 21, 1983, from the *Chemistry Department, Oklahoma State University, Stillwater, OK 74078.* Accepted for publication February 23, 1984.

**Abstract** □ Circular dichroism spectropolarimetry has been applied to the simultaneous determination of chiral compounds in binary mixtures without separation or sample preparation steps. A strategy which uses data measured at equivalent wavelength pairs simplifies the calculations. Correspondence is within ±2% of the compositions of prepared standard mixtures.

**Keyphrases** □ Circular dichroism—spectropolarimetry, UV absorption, Brompton's solutions, *R*-(−)-cocaine hydrochloride, morphine sulfate, (±)-methadone, (−)-fructose, β-cyclodextrin □ Brompton's solutions—circular dichroism, spectropolarimetry, UV absorption

A medically approved practice used to relieve the physical distress from chronic pain, such as is experienced by some terminally ill cancer patients, is to administer a mixture of controlled, addictive substances in an oral preparation commonly referred to as "Brompton's cocktails." Cocaine is common to all preparations. Other components differ, but are most often either morphine or (±)-methadone. The mixture is served in a fruit-flavored alcohol base.

Although documented evidence for the abuse of this prescription is limited at this time, the potential exists. We have developed a simple protocol for a rapid direct determination of the drugs by circular dichroism (CD) spectropolarimetry which requires no separation or sample prepreparation. We have previously reported data for opium alkaloids (1, 2), *R*-(−)-cocaine (3, 4), (+)-lysergide (D-LSD) (5), and tetracycline (6). The CD technique focuses only on those components which absorb light and which are simultaneously chiral. Chirality may be either intrinsic to the molecule (1-6) or induced by its complexation with a chiral substrate molecule (4).

By being able to focus on only the CD-active components, the problem of deconvoluting the cumulative UV spectra of the mixtures is considerably simplified. Absorption by the other CD-inactive components only affects the signal-to-noise ratio of the detector.

## EXPERIMENTAL SECTION

Standard samples of *R*-(−)-cocaine hydrochloride[1], morphine sulfate[1], and (±)-methadone[2] were used without further purification. The fruit-flavored syrups commonly used contain (−)-fructose. Standard (−)-fructose[3] was obtained commercially. In-house standard mixtures were prepared by weight. Samples of unknown composition were either blind in-house preparations, or were provided[4]. Chirality is induced into (±)-methadone by complexation with the chiral cyclic oligosaccharide β-cyclodextrin[5] present in large excess in aqueous solution, $\sim 10^{-2}$ M.

CD measurements were made on an automatic recording spectropolarimeter[6], and data analyses were made on the ancillary data processor[7]. Daily calibration of the ellipticity scale was made against a standard solution of androsterone in dioxane, as recommended. Samples were diluted with the appropriate volume of dilute hydrochloric acid or water, usually 1:10.

[1] Mallinckrodt Inc.
[2] Drug Enforcement Administration.
[3] Fisher Scientific.
[4] Arizona Department of Public Safety and the local hospital pharmacy.
[5] Kodak.
[6] Model 500A; JASCO.
[7] Model DP-500N.

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