High-Performance Liquid Chromatographic Determination of Propranolol and 4-Hydroxypropranolol in Plasma

M. T. ROSSEEL ˣ **and M. G. BOGAERT**

Received July 24, 1980, from the *J. F. & C. Heymans Institute of Pharmacology, University of Ghent, De Pintelaan, 135, B-9000 Gent, Belgium.* Accepted for publication November 7, 1980.

Abstract □ A high-performance liquid chromatographic method for the simultaneous determination of propranolol and 4-hydroxypropranolol in plasma is presented. The method involves plasma extraction at basic pH with ethyl acetate, two brief back-extractions, chromatography on a reversed-phase column, and fluorescence detection. The within-run coefficients of variation were 3.0–7.1% for propranolol and 5.8–8.3% for 4-hydroxypropranolol. The day-to-day variations were 4.6 and 8.2% for propranolol and 4-hydroxypropranolol, respectively. The method can detect 1 ng of each compound/ml of plasma.

Keyphrases □ High-performance liquid chromatography—propranolol and 4-hydroxypropranolol, human plasma □ Propranolol—analysis, high-performance liquid chromatography, human plasma □ 4-Hydroxypropranolol—analysis, high-performance liquid chromatography, human plasma □ β-Adrenergic antagonists—propranolol and 4-hydroxypropranolol, high-performance liquid chromatography, human plasma

Propranolol, a β-adrenergic antagonist, is used to treat angina pectoris, cardiac arrhythmias, and hypertension. 4-Hydroxypropranolol, a metabolite of propranolol in humans and animals (1), is equipotent with propranolol as a β-adrenergic antagonist (2) and is present in human plasma after acute and chronic treatment with propranolol (3–7).

Several methods for the simultaneous determination of both substances have been proposed, including mass fragmentography (3), direct fluorometry (4), and reversed-phase high-pressure liquid chromatography (HPLC) with fluorescence detection (5), but none of these methods is entirely satisfactory. In other methods, the standard curve for 4-hydroxypropranolol was not linear (8) and no internal standard was used (9).

This paper describes a specific and sensitive HPLC method for the simultaneous determination of propranolol and 4-hydroxypropranolol in plasma. The procedure has been used for the analysis of plasma samples of patients taking propranolol.

EXPERIMENTAL

Chemicals and Reagents—1-Heptanesulfonic acid[1] in acetic acid was used. Propranolol, 4-hydroxypropranolol, and 4-methylpropranolol were supplied as the hydrochloride salts[2]. Methanol was Lichrosolv quality[3], and all other reagents were analytical grade.

Instrumental Conditions—The microprocessor-controlled high-performance liquid chromatograph[4] was equipped with a fluorescence detector[5]. The fluorometer was set at an excitation wavelength of 290 nm, with an emission cutoff filter allowing 90% transmission at 340 nm.

Chromatography was done on a reversed-phase column 250 mm long × 4.6 mm i.d.[6]. The helium degassed mobile phase was pumped through the column at a flow rate of 1.5 ml/min, which required pressures of 600–1000 psi, depending on column age. The column temperature was 40°. Samples were injected using an automatic injector[7] equipped with a 10-μl loop. Quantitation was done by peak area measurement[8].

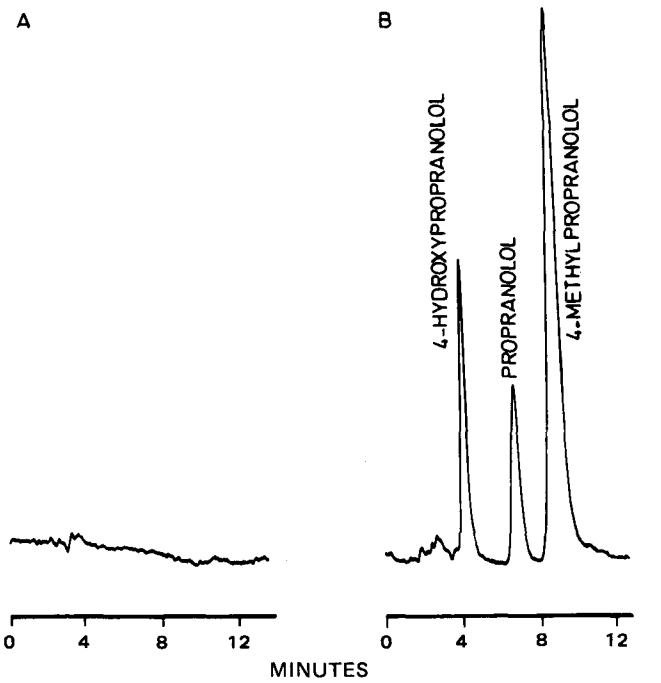

Figure 1—*(A) High-performance liquid chromatogram of the extract of 1 ml of blank plasma. (B) Chromatogram of an extract of 1 ml of blank plasma spiked with 20 ng of 4-hydroxypropranolol, 50 ng of propranolol, and 60 ng of the internal standard 4-methylpropranolol.*

Mobile Phase—1-Heptanesulfonic acid in acetic acid (25.0 ml) was mixed with 1000 ml of distilled water (Solvent A), and 25.0 ml of the same acid solution was mixed with 1000 ml of methanol (Solvent B). The mobile phase was Solvent A–Solvent B (34:66, v/v).

Assay Standards—Standard solutions of propranolol and of 4-hydroxypropranolol (10 mg/100 ml each, calculated as the base) were prepared in methanol and stored at −20°. For each assay, 100-fold aqueous dilutions of the standard solutions were made. The internal standard was 10 μg of 4-methylpropranolol/ml of methanol and was stored at −20°.

Extractions—To 1 ml of the spiked plasma or the unknown sample in a 10-ml glass-stoppered centrifuge tube, 20 mg of sodium hydrogen sulfite (20% in distilled water), 6 μl of the internal standard solution, 50 μl of 0.05 *M* triethylamine, and 500 μl of 2.5 *N* NaOH were added successively. The mixture was extracted with 4 ml of ethyl acetate by shaking horizontally for 10 min.

After centrifugation for 10 min at 6000 rpm, the organic phase was transferred with a Pasteur pipet to a 6-ml glass-stoppered conical tube. To the ethyl acetate layer, 100 μl of 0.2 *N* H_2SO_4 was added and the solution was mixed with a vortex mixer for 30 sec. After centrifugation the ethyl acetate layer was discarded and 100 μl of 2.5 *N* NaOH was added.

The basic solution was extracted immediately with 1 ml of ethyl acetate by mixing with a vortex mixer for 30 sec and centrifuging. The supernate was transferred with a Pasteur pipet to another 6-ml glass-stoppered conical tube, and the organic phase was removed under a gentle nitrogen stream at room temperature to near dryness. The residue was dissolved in 15 μl of the mobile phase and stored at −20° until analysis, *i.e.*, within a few hours.

[1] Reagent B-7, Waters Associates, Milford, Mass.
[2] Imperial Chemical Industries, Macclesfield, England.
[3] Merck, Darmstadt, West Germany.
[4] Spectra-Physics SP-8000, Eindhoven, The Netherlands.
[5] F.S. 970, Schoeffel Instruments Corp., West Germany.
[6] Lichrosorb 10RP8, Chrompack, Merksem, Belgium.
[7] Spectra-Physics *SP-8000*, Eindhoven, The Netherlands.
[8] Spectra-Physics SP-8000 data system, Eindhoven, The Netherlands.

0022-3549/81/0600-0688$01.00/0

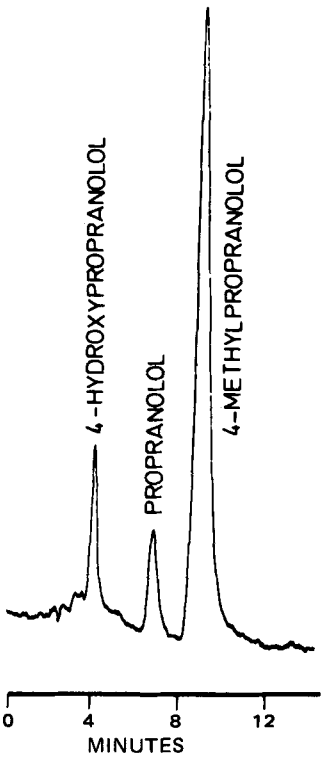

Figure 2—*High-performance liquid chromatogram of an extract of 1 ml of plasma of a patient receiving propranolol. To 1 ml of plasma, 60 ng of methylpropranolol was added as the internal standard.*

Quantitation—Human plasma samples spiked with increasing concentrations of propranolol (5–300 ng/ml) and 4-hydroxypropranolol (1–100 ng/ml) were carried through the whole procedure. Peak area ratios of propranolol or 4-hydroxypropranolol to the internal standard were plotted *versus* their concentrations, and an unweighted least-squares linear regression analysis was performed. Unknown drug concentrations were estimated from the calibration curve.

RESULTS AND DISCUSSION

Blank plasma extracts yielded no interfering peaks from endogenous plasma components (Fig. 1A). Propranolol, 4-hydroxypropranolol, and 4-methylpropranolol were separated and eluted in ~12 min (Fig. 1B). Sensitivity limits were 1 ng/ml for each compound when the entire 1-ml plasma extract was injected. The detector response was linear for plasma propranolol concentrations of at least 5–300 ng/ml and plasma 4-hydroxypropranolol concentrations of at least 1–100 ng/ml. Typical calibration curve data are listed in Table I. Over 3 weeks, the slopes of the calibration curves demonstrated little change (CV = 4.8 and 5.6% for propranolol and 4-hydroxypropranolol, respectively, n = 13). The results of accuracy and precision, both within run and day to day, are shown in Table II. 4-Hydroxypropranolol is stable in plasma, if frozen, for at least 3 weeks (Table II). This stability also was reported by others (6).

Uncorrected recovery, as assessed by comparing peak areas of extracted standards with those of unextracted compounds, was 75 ± 6% (n = 5) for 4-hydroxypropranolol and 80 ± 5% (n = 5) for propranolol.

Figure 2 illustrates a chromatogram of the plasma extract from a patient receiving 40 mg of propranolol four times a day. The blood sample was taken 10 hr after the last dose.

There was no interference of the commonly used cardiac drugs quinidine, lidocaine, procainamide, and disopyramide added *in vitro*. *N*-Desisopropylpropranolol, another reported metabolite of propranolol (10), eluted just before propranolol, but no measurable amounts were found in the plasma of patients treated with propranolol.

Table I—Calibration Curve Data ($y = c + mx$)

Compound	Range, ng/ml	Slope (m)	Intercept (c)	r
Propranolol	5–300	0.0098	0.0031	0.9990
4-Hydroxypropranolol	1–100	0.0127	0.0006	0.9992

Table II—Accuracy and Within-Run and Day-to-Day Precision for Propranolol and 4-Hydroxypropranolol

Compound	Level, ng/ml	Mean Assay, %	CV, %
	Within Run (n = 5)		
Propranolol	10	106.3	7.1
	50	99.6	3.0
	100	100.6	5.1
4-Hydroxypropranolol	5	98.2	8.3
	20	101.9	5.8
	40	97.4	6.4
	Day to Day (n = 10)[a]		
Propranolol	50	103.7	4.6
4-Hydroxypropranolol	15	98.5	8.2

[a] Pooled plasma standards, analyzed over 3 weeks.

The brief cleanup steps before chromatography, involving successive back-extractions into acid and ethyl acetate at basic pH, resulted in cleaner chromatograms than those previously reported (4, 5, 8). These cleanup steps were necessary particularly to obtain the assay sensitivity of 1 ng/ml for 4-hydroxypropranolol. In comparison to other HPLC methods for propranolol and 4-hydroxypropranolol (5, 8, 9), the described HPLC method is more sensitive, specific, and precise, particularly for the 4-hydroxypropranolol determination.

REFERENCES

(1) P. A. Bond, *Nature*, **213**, 721 (1967).

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

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

(4) P. S. Rao, L. C. Quesada, and H. S. Mueller, *Clin. Chim. Acta*, **88**, 355 (1978).

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

(6) T. W. Walle, E. C. Conradi, U. K. Walle, T. C. Fagan, and T. E. Gaffney, *Clin. Pharmacol. Ther.*, **27**, 22 (1980).

(7) L. Wong, R. L. Nation, W. L. Chiou, and P. K. Mehta, *Br. J. Clin. Pharmacol.*, **8**, 163 (1979).

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

(9) W. D. Mason, E. N. Amick, and O. H. Weddle, *Anal. Lett.*, **10**, 515 (1977).

(10) D. A. Saelens, T. Walle, and P. J. Privitera, *J. Chromatogr.*, **123**, 185 (1976).

ACKNOWLEDGMENTS

Supported by Grant 3.9001.79 from the National Fund for Medical Research, Belgium.