drug was extracted from the tablet matrix using isopropyl alcohol–0.1 *M* hydrochloric acid (9:1) and diluted to a final bromperidol concentration of 15 μg/ml with isopropyl alcohol–0.1 *M* hydrochloric acid. The bromperidol present in the sample was calculated by comparing the absorbance of the sample at 242 nm to a similarly prepared solution of standard at a known concentration. Table III shows the agreement between results obtained by both procedures using actual 3- and 6-mg bromperidol tablets. Equivalent results were obtained independent of whether ground composites or whole tablets were assayed. Table III also shows data collected on representative 10-mg tablet lots.

The data in Table IV demonstrate that peak heights can be used interchangeably with peak areas for quantitation. These data were determined using individual determinations of 3-, 6-, and 10-mg tablets calculated by both techniques. Overall results as well as differences between individual results were within experimental error.

To demonstrate the stability-indicating properties of the method, the chromatographic response of several compounds structurally related to bromperidol were compared to bromperidol and octacosane (the internal standard) to establish resolution properties. Each compound was dissolved in ethyl acetate–cyclohexane (9:1), and the resulting solutions were chromatographed. Besides bromperidol, only seven compounds produced discernible peaks. All compounds were chromatographed individually; Table V presents the relative retention times and weight responses relative to bromperidol (the first compound). Compounds not observed were concluded to have either eluted with the solvent front or been strongly retained by the packing. However, none of the compounds interfered with the determination of bromperidol.

To defend further the resolution properties of the method and, therefore, the stability-indicating properties, a weighed amount of bromperidol drug substance was heated in a capillary tube at 260° (melting point of bromperidol is 158°). After ~30 min at this extreme temperature, the sample was partially discolored near the top of the tube; analysis for bromperidol indicated a drug loss of ~25%. However, no extra peaks were observed in the resulting chromatogram. Examination of the analytical regions of the resultant chromatogram using GLC–mass spectrometry indicated that thermal stress reaction products of bromperidol did not elute with the drug or internal standard.

In addition to the thermal stress experiment, an accurately weighed 25-mg sample of bromperidol was mixed with 2 ml of 30% hydrogen peroxide and allowed to stand for 1 hr. The sample was mixed with 10 ml of methanol to consume excess peroxide and evaporated to dryness under nitrogen. GLC analysis of the residue indicated as little as 10% intact drug remaining. Examination of the oxidized sample of bromperidol by GLC–mass spectrometry in the analytical regions indicated that reaction products of bromperidol did not elute with the internal standard or bromperidol.

This GLC method is specific for bromperidol in the presence of structurally related compounds and stress reaction products of bromperidol. In addition, the procedure is accurate, reproducible, and suitable for stability or release analyses of bromperidol tablets.

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High-Performance Liquid Chromatograhic Determination of Metoprolol in Plasma

M. T. ROSSEEL *x, F. M. BELPAIRE *, I. BEKAERT ‡, and M. G. BOGAERT *

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Abstract □ A high-performance liquid chromatographic method is presented for the determination of metoprolol in human plasma. Metoprolol was extracted from plasma by a single extraction procedure with 4-methylpropranolol as the internal standard. Chromatography was done on a reversed-phase column with fluorescence detection. The minimum detectable concentration was 5.0 ng/ml of plasma. The standard curve was linear in the range evaluated, 10–300 ng/ml. The within-run coefficient of variation was 2.3–6.0%, and the day-to-day variation was 6.8%. The method is free from interference by major metoprolol metabolites.

Keyphrases □ Metoprolol—high-performance liquid chromatographic analysis in plasma □ β-Adrenergic blocking agents—metoprolol, high-performance liquid chromatographic analysis in plasma □ High-performance liquid chromatography—analysis of metoprolol in plasma

Metoprolol is a cardioselective β-adrenergic blocking agent used in the treatment of hypertension and angina. Analysis of metoprolol in plasma has been performed with electron-capture GLC after derivatization (1, 2), and a GC–mass spectroscopy method was recently developed (3).

This report describes a high-performance liquid chromatographic (HPLC) method for the determination of metoprolol in plasma using reversed-phase ion-pair chromatography with fluorescence detection. The same approach has been used for other β-adrenergic blocking drugs such as propranolol (4, 5), atenolol (6, 7), and sotalol (8).

EXPERIMENTAL

Reagents—The following were used: metoprolol tartrate[1], α-hydroxymetoprolol[1], and *O*-desmethylmetoprolol[1] as the *p*-hydroxybenzoic acid salts; and 4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetic acid[1], 2-hydroxy-3-(4-methyoxyethylphenoxy)propanoic acid[1], and 4-methylpropranolol[2] as the hydrochloride salts. Methanol[3] was HPLC grade. 1-Heptanesulfonic acid[4] in acetic acid was used. Glass double-distilled water was passed through a 0.45-μm filter[5]. Methylene chloride was reagent grade and distilled just before use.

Instrument Conditions—The microprocessor-controlled high-per-

[1] Hässle, Mölndal, Sweden
[2] Imperial Chemical Industries, Macclesfield, England.
[3] Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Reagent B-7, Waters Associates, Milford, Mass.
[5] Type HA Millipore Corp., Bedford, Mass.

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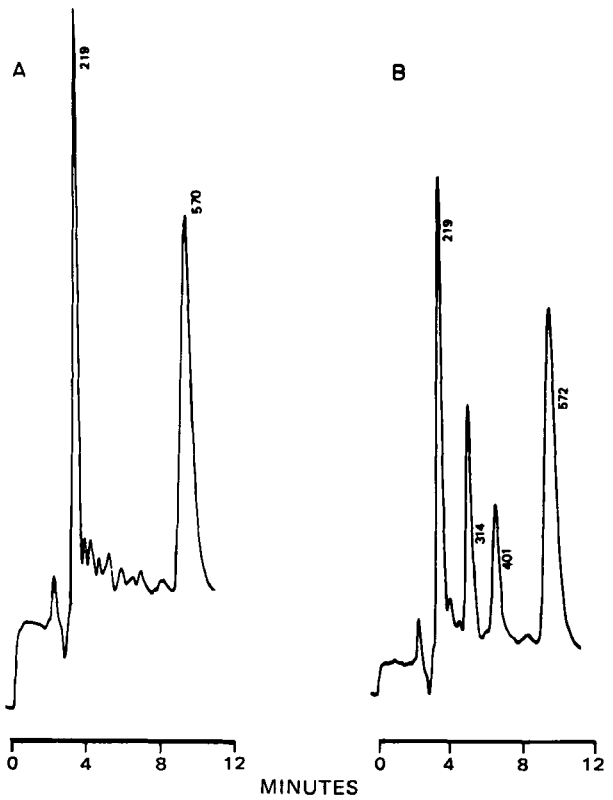

Figure 1—*Liquid chromatograms of 1-ml plasma extracts. Key: A, blank plasma sample of a cardiac patient spiked with 30 ng/ml of the internal standard (570 sec); and B, plasma of the same patient 4 hr after oral intake of 100 mg of metoprolol and spiked with 30 ng/ml of the internal standard. Retention times were 401 and 572 sec for metoprolol and the internal standard, respectively. The plasma metoprolol concentration in this patient was calculated to be 46 ng/ml. The peak at 314 sec is probably α-hydroxymetoprolol.*

formance liquid chromatograph[6] was equipped with a fluorescence detector[7]. The effluent was monitored at an excitation wavelength of 220 nm with a 295-nm UV interference filter to set the emission wavelength.

A reversed-phase column[8] (25-cm long × 4-mm i.d.) was maintained at 40°. The flow rate of the helium-degassed mobile phase was 1.0 ml/min. Samples were injected into the chromatograph *via* an injector[9] equipped with a 10-μl loop.

Mobile Phase—1-Heptanesulfonic acid (25 ml) in acetic acid was mixed with 1 liter of distilled water (Solvent A), and 25 ml of the same acid solution was mixed with 1 liter of methanol (Solvent B). The mobile phase was a mixture of Solvents A and B (15:85 v/v).

Procedure—One milliliter of plasma, 30 μl of the internal standard solution (1 μg/ml in methanol), 0.5 ml of 1.0 *N* NaOH, and 4 ml of methylene chloride were added to a 10-ml glass-stoppered centrifuge tube. The mixture was vortexed for 30 sec and then centrifuged for 5 min at 5000 rpm. The organic phase was transferred to a 6-ml glass-stoppered conical tube, evaporated to dryness under nitrogen at room temperature, and reconstituted in 15 μl of the mobile phase. An aliquot of this solution was injected.

RESULTS AND DISCUSSION

The efficiency of the extraction recovery for metoprolol (100 ng/ml) was 76.9% with a coefficient of variation of 3.1%. Over the 10–300-ng/ml plasma range studied, the standard curve was linear. The standard curves analyzed over 1 month (n=11) gave a mean regression (±*SD*) of y = 0.0104 (±0.0008)x + 0.0054 (±0.0050) with r = 0.9987 (±0.0011), where y is the metoprolol peak height divided by the 4-methylpropranolol peak height and x is the concentration of metoprolol (nanograms of free base per milliliter).

Table I—Within-Run Accuracy and Precision for Metoprolol [a]

Concentration, ng/ml	Mean Result, %	*CV*,%
30	103.1	6.0
50	105.6	5.3
100	100.9	2.3

[a] n = 5.

The reproducibility of the method was demonstrated by a day-to-day coefficient of variation of 6.8% (n=11) using pooled plasma spiked with metoprolol (50 ng/ml) and analyzed over 1 month. The within-run coefficients of variation and accuracy are given in Table I.

The detection limit of this assay with the described instrumentation and procedure was ~5 ng/ml of plasma.

Assay of samples spiked with a number of commonly used cardiovascular drugs showed that procainamide interferes with the internal standard. Plasma samples obtained from 40 cardiac patients taking drugs such as nitrates and other antianginal agents, antiarrhythmic agents, diuretics, antibiotics, theophylline, aspirin, dipyridamole, and benzodiazepines were analyzed. Only in quinidine-treated patients was there a problem since quinidine metabolites interfered with metoprolol on the chromatogram.

An example of the chromatogram obtained from the plasma of a cardiac patient is shown in Fig. 1 along with the chromatogram of plasma of the same patient sampled 4 hr after receiving 100 mg of metoprolol orally.

The metabolites of metoprolol, α-hydroxymetoprolol, *O*-desmethylmetoprolol, 4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetic acid, and 2-hydroxy-3-(4-methoxyethylphenoxy)propanoic acid, eluted at 322, 342, 312, and 215 sec, respectively, which separated them from metoprolol (411 sec) and the internal standard (587 sec). Only the two basic metabolites, α-hydroxymetoprolol and *O*-desmethylmetoprolol, were recovered from extracted spiked plasma. In humans, *O*-desmethylmetoprolol concentrations are low, but α-hydroxymetoprolol concentrations can be detected (9–11). In some patient samples, a peak was seen that could be attributed to α-hydroxymetoprolol (Fig. 1).

The described HPLC assay provides a specific, sensitive, and quantitative method for the determination of metoprolol in plasma.

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