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High-Performance Liquid Chromatographic Determination of Metoprolol and α-Hydroxymetoprolol Concentrations in Human Serum, Urine, and Cerebrospinal Fluid

F. M. GENGO ˣ, M. A. ZIEMNIAK, W. R. KINKEL, and W. B. McHUGH

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Abstract □ A sensitive and simplified high-performance liquid chromatographic procedure was developed for the simultaneous quantification of metoprolol and α-hydroxymetoprolol in human serum, as well as cerebrospinal fluid and urine. Following protein precipitation with trichloroacetic acid, the sample was alkalinized with 1 M NaOH and extracted with dichloromethane. The mobile phase consisted of acetonitrile–water (50:50) containing 0.005 M 1-heptanesulfonic acid in 0.001% acetic acid. Using pronetalol as an internal standard, compounds were quantitated using fluorescence detection at 230 nm with a 300-nm emission filter and 0.02 AUFS. Extraction recovery is ~80% for both compounds. The lower limits of detection are 5 ng/mL and 4 ng/mL for metoprolol and α-hydroxymetoprolol, respectively.

Keyphrases □ Metoprolol—HPLC, α-hydroxymetoprolol, human serum, urine, and cerebrospinal fluid □ α-Hydroxymetoprolol—HPLC, metoprolol, human serum, urine, and cerebrospinal fluid

Metoprolol, a selective β-1-receptor blocker with an active hydroxy metabolite, is widely used in the treatment of several cardiovascular and neurological disorders (1–3). Many patients treated with standard oral doses of β-blockers experience either adverse toxic effects or receive no therapeutic benefit (4). Evaluation of the serum concentration profile of metoprolol may enable optimization of therapy for some patients. Several recent reports suggest that many of the pharmacological actions of metoprolol are paralleled by serum concentrations of metoprolol and/or α-hydroxymetoprolol (5, 6). Although α-hydroxymetoprolol may have relatively weak β-adrenergic blocking potency, its contribution to the other pharmacological actions of metoprolol remain to be determined (1, 2, 7). It is known that α-hydroxymetoprolol accumulates in the serum of patients with certain disease states, such as renal failure (7).

The current assay methods for the analysis of metoprolol include GC–MS (8), GC–EC detection (9), as well as high-performance liquid chromatography (HPLC) (10, 11). Most were designed primarily for quantification of only the parent drug. The assay procedure reported here is sensitive and specific for both metoprolol and the active hydroxy metabolite. Additionally, this procedure is useful for quantification of both compounds from serum and cerebrospinal fluid (CSF).

EXPERIMENTAL SECTION

Reagents—All chemicals and reagents were analytical grade unless otherwise indicated. Metoprolol tartrate[1], α-hydroxymetoprolol[2], and pronetalol[3] were all received as powders; stock solutions were prepared in methanol. Dichloromethane, acetonitrile[4], 30% trichloroacetic acid solution, sodium hydroxide[5], and sodium-1-heptanesulfonate[6] were used as received.

The HPLC system consisted of a delivery system[7], universal injector data module[7], and a variable-wavelength fluorescence detector[8]. Separation was conducted on a reverse-phase C_{18} column, 3.9 mm × 30 cm[9]. The mobile phase consisted of 25 mL of 0.005 M sodium-1-heptanesulfonate in 1.0% acetic acid diluted to 1 L with distilled deionized water (solvent A). The same amount of heptanesulfonic acid solution (25 mL) was diluted to 1 L with acetonitrile (solvent B). The mobile phase was a mixture of solvents A and B (50:50). A flow rate of 2 mL/min was maintained. The effluent was monitored at an excitation wavelength of 230 nm with a 300-nm UV interference filter, to set the emission wavelength, with an attenuation of 0.02.

Extraction Procedure—To 1 mL of serum (in a glass tube) was added 10 μL of a 3.0-μg/mL solution of the internal standard (pronetalol) and 200 μl of 30% trichloroacetic acid solution. This mixture was vortexed and then centrifuged[10] at 5000 rpm for 10 min. The clear supernatant (750 μL) was transferred and 375 μL of 1 M NaOH and 4.0 mL of dichloromethane were added. The mixture was vortexed for 30 s, then centrifuged for 10 min at 5000 rpm. The upper aqueous phase was aspirated and discarded; the lower organic phase was transferred, evaporated to dryness under a stream of nitrogen at 30°C, and reconstituted in 100 μL of the mobile phase. The extraction procedure for urine and (CSF) is exactly the same as described for serum.

Recovery Studies—Human serum and methanol samples were each spiked with two different concentrations of metoprolol and α-hydroxymetoprolol. The methanol samples were evaporated under a stream of nitrogen at 30°C, with internal standard added just prior to injection. The serum samples were extracted as described, with the exception that internal standard was again added prior to injection. Percent recovery was determined by comparison of peak height ratios between extracted samples and methanol samples. Five replicate determinations were made at each concentration. Similar studies were conducted using blank urine and CSF. To determine the within-day

[1] Ciba Pharmaceutical Co., Summit, N.J.
[2] Hassle Subsidiary, Molndal, Sweden.
[3] Imperial Chemical Industries, Hurdsfield Indust. Estate, Macclesfield, Cheshire, U.K.
[4] Burdick and Jackson Laboratories, Muskegon, Mich.
[5] Fisher Scientific Co., Fair Lawn, N.J.
[6] Eastman Kodak Co., Rochester, N.Y.
[7] Waters Associates, Milford, Mass.
[8] Model FS970; Schoeffel Instruments, Westwood, N.J.
[9] μBondapak C_{18}; Waters Associates, Milford, Mass.
[10] GLC-1 Centrifuge; Sorvall, Newton, Conn.

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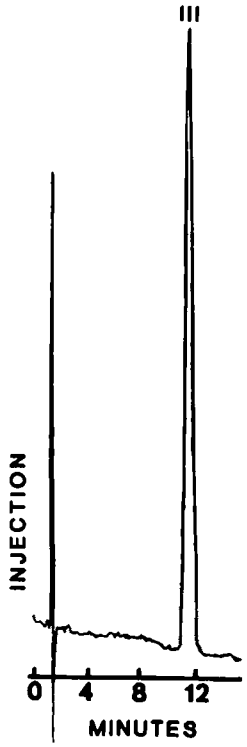

Figure 1—*Chromatogram obtained after extracting 1.0 mL of blank serum. Key: (III) internal standard.*

variation, a serum sample with known amounts of metoprolol and α-hydroxymetoprolol was assayed five times. To assess the between-day variation, another serum sample with known amounts was assayed six times over a period of 10 d.

Human Study—A patient diagnosed as having essential tremor was treated for 1 week with a regimen of 75 mg of metoprolol b.i.d. On the day 7, blood samples were collected at various times after the morning dose. The serum was stored at −30°C prior to analysis.

Stability Study—To determine the effects of temperature on the stability of metoprolol and α-hydroxymetoprolol, 1.0-mL aliquots of spiked serum were incubated at −30°C, 5°C, 25°C, and 37°C. Samples were assayed periodically over 30 d.

RESULTS

Chromatography—Figure 1 illustrates the chromatogram obtained after extracting 1.0 mL of blank serum as described. There were no additional peaks eluting that interfered with the determination of metoprolol or α-hydroxymetoprolol. A serum sample spiked with 44.4 ng/mL of metoprolol and 35.2 ng/mL of α-hydroxymetoprolol was extracted, and the resulting chromatograph is shown in Fig. 2. The retention times of metoprolol, α-hydroxymetoprolol, and the internal standard were 9.3, 5.9, and 12.0 min, respectively. A typical chromatogram of serum obtained from a patient receiving metoprolol is shown in Fig. 3.

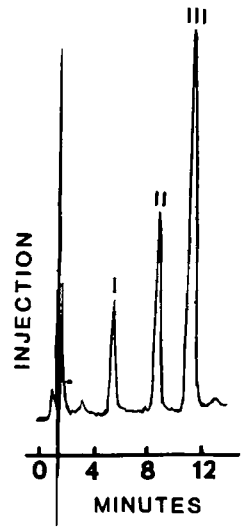

Figure 2—*Chromatogram obtained after extracting 1.0 mL of spiked serum. Key: (I) α-hydroxymetoprolol; (II) metoprolol; (III) internal standard.*

Table I—Slope of Intercept Values [a] Derived from Concentration and Peak-Height Ratio Relationship for Metoprolol and α-Hydroxymetoprolol in Various Biological Fluids

Biological Fluid	Metropolol		α-Hydroxymetoprolol	
	Slope	Intercept	Slope	Intercept
Serum	0.0025 + 0.0006	0.073 + 0.054	0.0024 + 0.0002	0.161 + 0.013
Urine	0.004 + 0.0002	0.0146 + 0.0006	0.003 + 0.0004	0.004 + 0.0004
CSF	0.005 + 0.0003	0.005 + 0.0004	0.005 + 0.0005	0.005 + 0.0002

[a] All correlation coefficients >0.99.

Table II—Recovery of Metoprolol and α-Hydroxymetoprolol from Human Serum [a]

	Drug conc., ng/mL	Mean Recovery, %	*CV*, %
Metoprolol	22.2	79.0	9.7
	266.4	78.2	7.9
α-Hydroxymetoprolol	17.6	80.0	9.1
	211.1	80.0	7.9

[a] $n = 5$.

Calibration curves, were prepared daily by plotting peak height ratios (drug or metabolite/internal standard) *versus* concentrations of spiked serum samples. These plots were linear over a 5–600-ng/mL concentration range. The mean ±*SD* slope value and intercept values are given in Table I. The lower limits of detection for metoprolol and the α-hydroxy metabolite were 4.9 and 3.8 ng/mL, respectively. Linearity was also demonstrated for metoprolol and α-hydroxymetoprolol when extracted from CSF and urine in the 15–150-ng/mL concentration range (Table I). Chromatograms of samples extracted from urine and CSF showed no additional peaks that interfere with quantification of the drug, metabolite, or internal standard (Fig. 4).

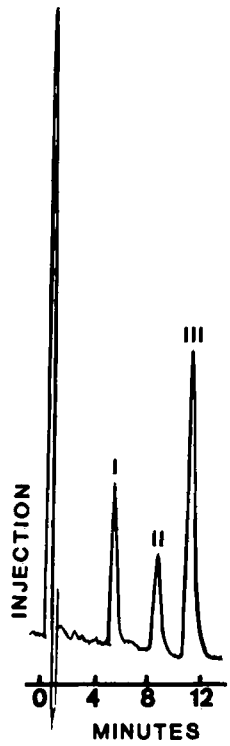

Figure 3—*Chromatogram of extracted serum from a patient receiving metoprolol. Key: (I) α-hydroxymetoprolol; (II) metoprolol; (III) internal standard.*

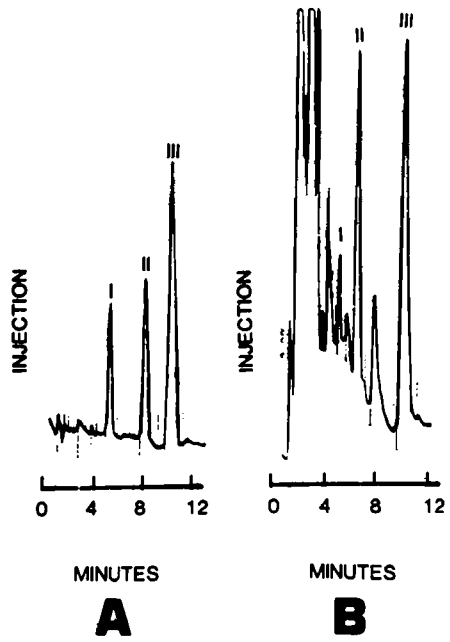

Figure 4—*Chromatograms of extracted CSF (A) and urine (B) containing α-hydroxymetoprolol (I), metoprolol (II), and internal standard (III).*

Recovery Experiments—The results of the recovery experiments are illustrated in Table II for both high and low concentrations. The overall recovery of metoprolol and α-hydroxymetoprolol ranged from 78.2 to 80.0% in serum, urine, and CSF over a 10-fold concentration range. Both metoprolol and α-hydroxymetoprolol also demonstrated similar within-day and between-day variations, as illustrated in Table III. The coefficient of variation ranged from 5.4 to 6.0% for within-day variation experiments, while the between-day studies ranged from 8.6 to 9.3%.

Human Serum Concentration Analysis—Figure 5 illustrates a serum concentration *versus* time profile for metoprolol and α-hydroxymetoprolol in the tremor patient following a 75-mg oral dose of metoprolol. After 2 h, peak concentrations were seen for the drug and its metabolite, 53.8 ng/mL and 88.1 ng/mL, respectively. After 7 h, both metoprolol and α-hydroxymetabolite decreased monoexponentially to 13.1 and 50.5 ng/mL, respectively.

Stability Study—To assess proper handling and storage conditions for

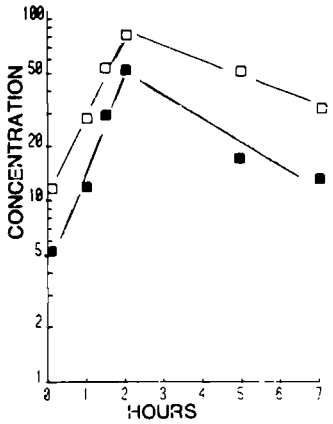

Figure 5—*Serum concentration* versus *time profiles for metoprolol (■) and α-hydroxymetoprolol (□) after a 75-mg oral dose of metoprolol.*

Table III—Within-Day and Between-Day Variation in the Determination of Metoprolol and α-Hydroxymetoprolol in Human Serum

	Mean, ng/mL	*CV*, %
Within-day,[a]		
Metoprolol	191.8	5.9
α-Hydroxymetoprolol	148.0	5.4
Between-day[b]		
Metoprolol	181.9	9.2
α-Hydroxymetoprolol	147.1	8.6

[a] $n = 5$. [b] $n = 6$.

metoprolol and α-hydroxymetoprolol, the effect of temperature on the degradation rate was monitored. Over the 30-d test period, the samples frozen at −30°C remained stable. At 37°C, 25°C, and 5°C there was degradation within 14 d.

DISCUSSION

The metoprolol assay described in this report is specific, selective, and reproducible. Both drug and active metabolite can be specifically and reproducibly quantitated in urine, serum, and CSF in concentrations that are achieved by standard doses of metoprolol.

The procedure reported here is a modification of other previously reported metoprolol assays (10, 11). Modifications in the extraction process circumvents the need to distill reagents daily. These modifications also reduce background interference peaks in blood samples and prevent emulsification of reagents during the extraction. Modification in the mobile phase enables excellent separation of the drug, metabolite, and internal standard within a 15-min time period.

This procedure quantitates drug and metabolite concentrations from urine and CSF using the same experimental procedure used for serum. This procedure has been tested in patients concomitantly taking several medications. Drugs such as thiazide, benzodiazepines, digoxin, and thyroid hormone produced no interference; however, other β-blockers such as alprenolol, atenolol, naldolol, propranolol, sotalol, and timolol did interfere. In summary, this method provides a simplified and clinically comprehensive procedure for the analysis of metoprolol and α-hydroxymetoprolol.

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