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DETERMINATION OF METOPROLOL AND ITS α-HYDROXYLATED METABOLITE IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic method has been developed for the simultaneous determination of metoprolol and its α-hydroxylated metabolite in plasma. Metoprolol, α-hydroxymetoprolol and alprenolol (internal standard) are extracted from plasma at alkaline pH with diethyl ether—dichloromethane (4:1, v/v) and back-extracted with 0.01 *N* sulfuric acid. A 100-μl volume of the acidic extract is injected into the chromatographic system. The compounds are eluted in about 12 min with acetonitrile—acetate buffer (75:25, v/v) on a LiChrosorb RP-8 (5 μm) column. The quantitative determinations are made fluorometrically. Concentrations down to 35 nmol/l (10 ng/ml) of metoprolol base and 30 nmol/l (8 ng/ml) of α-hydroxymetoprolol base in plasma can be determined with good precision and accuracy.

INTRODUCTION

Metoprolol, a selective β-adrenergic receptor antagonist, is used in the treatment of hypertension and angina pectoris [1]. Its metabolism in humans is well documented [2, 3]. Two of its metabolites (Fig. 1), α-hydroxymetoprolol and O-demethylmetoprolol, display β-blocking activities, but are less potent than the parent drug [2, 4]. O-Demethylmetoprolol has been detected in plasma at very low concentrations, often below 5 nmol/l [3]. This metabolite would not normally contribute to the β-blocking effect of metoprolol [3]. The relative amount of α-hydroxymetoprolol in plasma has been reported to be 0.5—1 times that of metoprolol [1—4]. The object of the present study was to determine both metoprolol and α-hydroxymetoprolol in plasma.

Several methods of determining metoprolol by either gas chromatography

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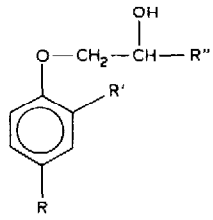
(GC) [5—12] or high-performance liquid chromatography (HPLC) [13—19] have been described. Few of them permit the simultaneous determination of metoprolol and α-hydroxymetoprolol [5, 9, 17]. GC methods [5, 9] need a derivatization step after extraction and evaporation to dryness. One of them [9] is very selective, but it uses a mass spectrometric detector. In the HPLC method [17] the sample is rotated for 1 h for extraction, before evaporating the organic phase to dryness, and the chromatography lasts about 30 min per sample. Its sensitivity is 3 ng of metoprolol and 12 ng of α-hydroxymetoprolol per ml of plasma. The method proposed below is very simple, as it requires no special washing of the glassware and only two extraction steps without evaporation to dryness before chromatography, which lasts 10 min.

EXPERIMENTAL

Chemicals and reagents

Metoprolol tartrate and the internal standard, alprenolol hydrochloride, were supplied by Ciba-Geigy (Basle, Switzerland). α-Hydroxymetoprolol in the form



	R	R'	R''	M.W.
Metoprolol	$CH_2—CH_2—OCH_3$	H	$CH_2—NH—CH(CH_3)_2$	267
α — Hydroxymetoprolol	$CH(OH)—CH_2—OCH_3$	H	$CH_2—NH—CH(CH_3)_2$	283
O-Demethylmetoprolol (H 105/22)	$CH_2—CH_2—OH$	H	$CH_2—NH—CH(CH_3)_2$	253
H 117/04	CH_2COOH	H	$CH_2—NH—CH(CH_3)_2$	267
H 104/83	$CH_2—CH_2—OCH_3$	H	COOH	240
Alprenolol	H	$CH_2—CH=CH_2$	$CH_2—NH—CH(CH_3)_2$	249

Fig. 1. Chemical structure of metoprolol, its known metabolites and alprenolol, with their molecular weights (M.W.).

of its *p*-hydroxybenzoic acid salt was supplied by Hässle (Mölndal, Sweden). The structures of these compounds are given in Fig. 1. All solvents and reagents were of analytical grade (E. Merck, Darmstadt, F.R.G.). Acetonitrile was of spectroscopy quality (Merck 16).

The alkaline solution for extraction was prepared by dissolving 30 g of $K_3PO_4 \cdot 3H_2O$ and 16.8 g of KOH in 100 ml of distilled water.

Internal standard and reference solutions

The internal standard solution was prepared in water from alprenolol hydrochloride (concentration: 17.5 μmol/l = 5 μg/ml). Reference solutions of metoprolol and α-hydroxymetoprolol for calibration were prepared from the respective salts dissolved in the aqueous solution of the internal standard. This solution was also used for preparation of validation solutions.

Sample preparation

The schematic outline of the sample preparation is given in Fig. 2. Into a 10-ml polypropylene tube are added 1 ml of plasma, 50 μl of the internal standard solution (or reference solution for calibration or validation solution), 1 ml of the alkaline solution and 4 ml of diethyl ether—dichloromethane (4:1, v/v). The tube is shaken on an Infors shaker at 350 rpm for 15 min and centrifuged at 2000 *g* for 10 min. Then 3 ml of the organic phase are transferred

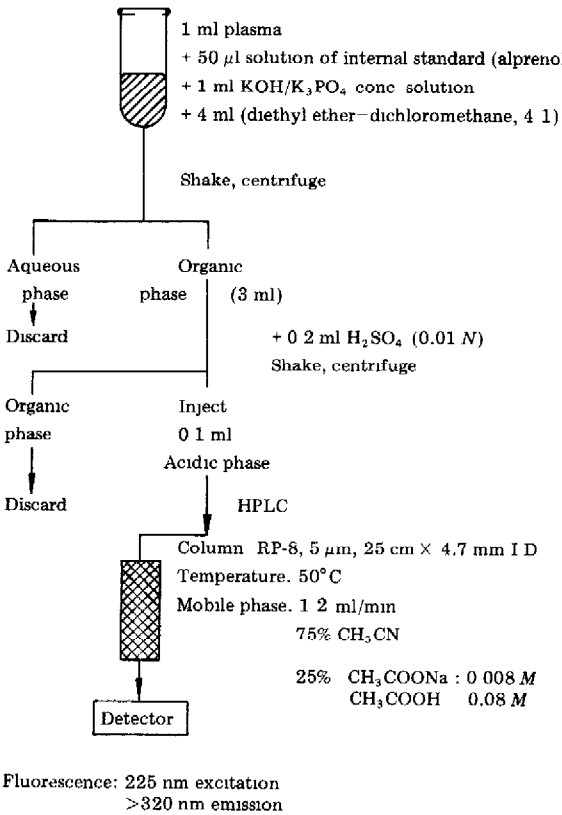


Fig. 2. Schematic outline of the sample assay.

into a 10-ml conical glass tube, and 200 μl of 0.01 N H_2SO_4 are added. The tube is shaken for 10 min and centrifuged for 2 min. About 150 μl of the acidic phase are introduced into a 0.5-ml polyethylene tube of the autosampler and 100 μl are injected onto the column.

Chromatography

The chromatography is performed on a Gilson 302 pumping system equipped with a Gilson 802 manometric system and a Wisp automatic injector from Waters. About 100 4-ml vials marketed by Waters were modified (see Fig. 3) to accommodate small 0.5-ml conical polyethylene tubes (micro-analysis tubes). These tubes are about ten times less expensive than the classical 0.3-ml glass tubes. This modification permits the injection of about 100 μl from 150 μl added in the plastic tube. The detector used is a Schoeffel fluorescence detector (Model FS 970) set at wavelengths of 225 nm for excitation and > 320 nm for emission. It is connected to a Spectra-Physics computing integrator (Model 4100).

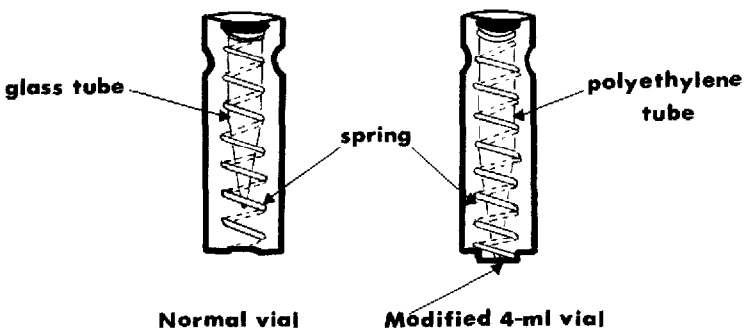


Fig. 3. Modification of a 4-ml WISP autosampler vial to accommodate 0.5-ml polyethylene micro-analysis tubes.

The analytical column (25 cm × 4.7 mm I.D.) is filled with LiChrosorb RP-8, 5 μm particle size.

The mobile phase, acetonitrile—acetate/acetic acid solution (0.008 M sodium acetate + 0.08 M acetic acid (75:25) is degassed in an ultrasonic bath before use and heated to 40—50°C during use. The column is thermostated at 50°C. The flow-rate is 1.2 ml/min. The retention times of α-hydroxymetoprolol, metoprolol and alprenolol are respectively 6.4, 8.2 and 10.1 min (Fig. 4). New LiChrosorb RP-8 batches may give longer retention times with the mobile phase composition described above. In this event, the acetate/acetic acid solution used in the mobile phase should be modified. With the aqueous solution, 0.05 M sodium acetate + 0.5 M acetic acid, the retention times and the separation of the components with new LiChrosorb batches are similar to those normally obtained.

Calibration

All metoprolol, α-hydroxymetoprolol and alprenolol amounts are expressed in pmol or in nmol of free base. They can be converted to pg or ng, respectively, by multiplying them by the molecular weights (Fig. 1).

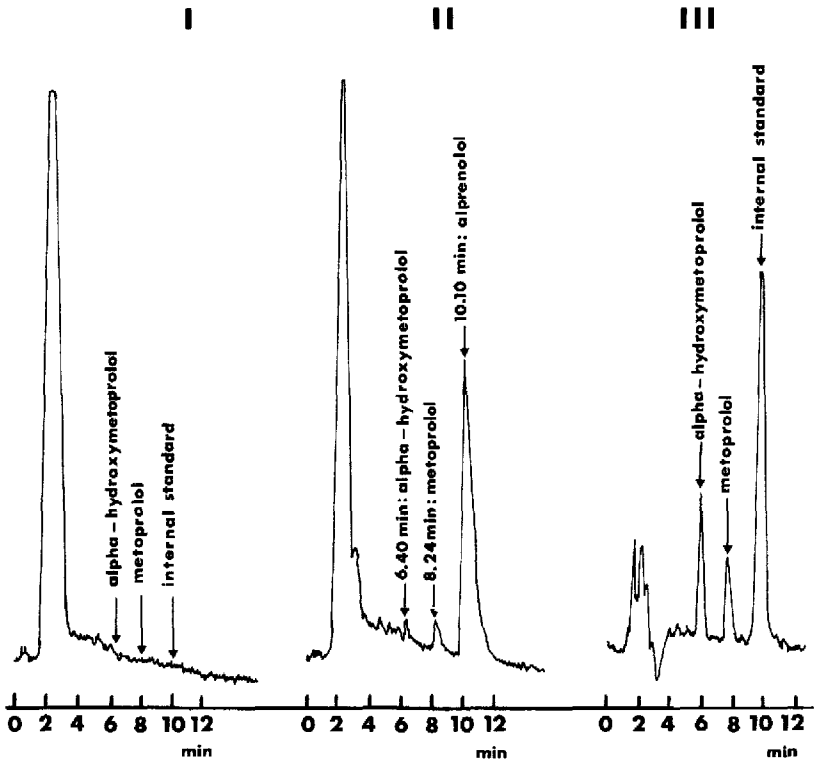


Fig. 4. Chromatograms of extracted plasma (I) human blank plasma; (II) plasma sample spiked with 40 nmol/l (11 ng/ml) metoprolol base and 28 nmol/l (8 ng/ml) α-hydroxymetoprolol; (III) actual plasma sample.

Calibration samples are prepared by adding 50 μl of calibration solutions containing known amounts of metoprolol (36.5—1462 pmol of free base), α-hydroxymetoprolol (30—1188 pmol) and alprenolol (875 pmol) to 1 ml of plasma according to the procedure described above for the sample preparation. (Calibration solutions were stable for at least one month, when kept at 5°C.)

The calibration curves are established from these calibration samples worked up in duplicate for five concentrations.

The equation of each calibration curve is that of the regression straight line of the log values of peak area ratios (metoprolol or α-hydroxymetoprolol/internal standard) versus the log value of the concentrations.

RESULTS AND DISCUSSION

Precision, accuracy and calibration

Plasma samples containing metoprolol and α-hydroxymetoprolol at different concentrations were analysed repeatedly. The results in Table I give the precision and accuracy of the method for replicates at concentration levels corresponding to those observed after administration of therapeutic doses of metoprolol. Plasma concentrations down to 35 nmol/l metoprolol (10 ng/ml) and 30 nmol/l α-hydroxymetoprolol (8 ng/ml) can be determined with a coefficient of variation lower than 10%.

TABLE I

PRECISION AND RECOVERY OF THE DETERMINATION OF METOPROLOL AND α-HYDROXYMETOPROLOL IN SPIKED PLASMA SAMPLES

	Metoprolol*				α-Hydroxymetoprolol**			
Added (nmol/l)	36.5	73	292	877	29.7	59	238	713
Found (nmol/l)	36.5	73.9	298	861	27.2	60.9	237	670
	38.8	71.7	285	861	29.7	62.0	239	689
	33.8	71.3	281	861	27.0	63.9	223	726
	32.0	70.4	285	892	30.0	62.0	231	689
	36.5	73.1	293	861		61.6	223	679
	33.3		297	843			231	689
Average	35.2	72.1	289.8	863	28.5	62.1	231	690
Coefficient of variation (%)	7.2	2	2.5	1.8	5.7	1.8	2.9	2.8
Recovery (%)	96.3	98.7	99.2	98.5	95.9	105	96.9	96.9
Overall recovery	98				99			
S.D.	4				5			

*To convert to ng/ml of metoprolol base, multiply the data by 0.267.
**To convert to ng/ml of α-hydroxymetoprolol base, multiply the data by 0.283.

The calibration curves obtained in the determination of more than 1000 clinical samples gave correlation coefficients higher than 0.9990 and slope values within the range 0.93—1.07.

Selectivity

Metoprolol and α-hydroxymetoprolol are conveniently separated from plasma components (Fig. 4). The retention times of the other metabolites (Fig. 1) were 2.3, 5.4 and 6.2 min for H 104/83, H 117/04 and H 105/22 (O-demethylmetoprolol), respectively. The last-mentioned metabolite has a retention time very close to that of α-hydroxymetoprolol. However, extremely low concentrations of this metabolite have been recorded in plasma (< 5 nmol/l) [3, 20] after intravenous or oral administration of metoprolol to healthy subjects, or to patients with renal impairment, whether undergoing dialysis or not. The highest level (12 nmol/l) was observed in a uremic patient during steady-state conditions [3]. The other two metabolites are not detected with the sample preparation described.

Adsorption on glassware and metoprolol stability in extracts

A significant concentration decrease was observed when alkaline or neutral solutions with low metoprolol concentrations were contained in glass tubes. To avoid adsorption, plastic tubes were used for the first extraction, and the decrease in concentration was no longer observed. The stability of metoprolol in the extracts was studied. No significant decrease in the concentration was found when sample extracts were kept for 15 h at room temperature. This permits the use of an automatic injector.

In conclusion, the described procedure allows the assay of metoprolol and

α-hydroxymetoprolol in plasma with suitable sensitivity and accuracy. The work-up procedure is simpler and faster than that described in the methods previously reported. It is well suited for pharmacokinetic and clinical pharmacology studies.

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