Journal of Chromatography, 228 (1982) 215—222
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1159

DETERMINATION OF METOPROLOL AND α-HYDROXYMETOPROLOL IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

DAVID B. PAUTLER and WILLIAM J. JUSKO*

Departments of Chemistry and Pharmaceutics, State University of New York at Buffalo, Cooke Hall 319, School of Pharmacy, Amherst, NY 14260 (U.S.A.)

(First received August 18th, 1981; revised manuscript received October 28th, 1981)

SUMMARY

A sensitive, selective and reproducible high-performance liquid chromatographic assay for the simultaneous determination of metoprolol and α-hydroxymetoprolol in plasma was developed with (±)-ethyl-2-(4-(3-isopropyl-amino-2-hydroxy-propoxy)phenyl)-ethyl carbamate as the internal standard. Samples were acidified and subjected to an organic wash to remove interfering neutral and acidic components. Final extraction is made from the alkalinized aqueous phase with methylene chloride. The samples were chromatographed on a microparticulate silica gel column with fluorescent detection. Sensitivity was 3 ng/ml for metoprolol and 12.5 ng/ml for α-hydroxymetoprolol. Specificity was established by comparison of retention times of samples with standard materials. A typical absorption/disposition profile for metoprolol and α-hydroxymetoprolol is presented for one subject who received 50 mg of metoprolol tartrate (Lopressor®).

INTRODUCTION

Metoprolol, a selective β_1-receptor blocker, is eliminated from the body primarily by hepatic metabolism. The major urinary metabolites of metoprolol, identified by Borg et al. [1], are shown in Fig. 1. Borg also found that two metabolites, *o*-demethylmetoprolol (H 105/22) and α-hydroxymetoprolol, possessed β_1-receptor blocking activity in the cat when given in doses 5—10 times that of metoprolol [1]. In studies with healthy subjects undergoing chronic oral administration it was found that plasma concentrations of α-hydroxymetoprolol reached approximately 50% of the concentration of metoprolol [2, 3]. The other metabolite *o*-demethylmetoprolol was found to be rapidly oxidized to an inactive amino acid (H 117/04) and was recovered in only minor amounts in human body fluids [3].

The existence of significant plasma levels of α-hydroxymetoprolol in normal subjects and the accumulation of substantial concentrations during long-term

0378-4347/82/0000—0000/$02.75

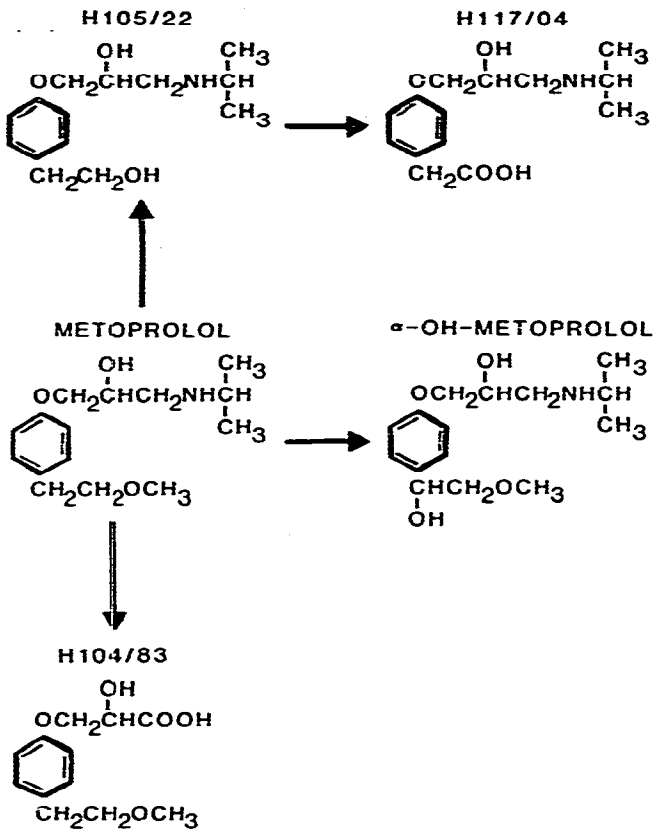

Fig. 1. Major routes of metabolism of metoprolol in man. From Borg et al. [1].

treatment of patients with severely impaired renal function [3] indicate a need for adequate monitoring of both compounds.

This paper describes a high-performance liquid chromatographic (HPLC) method with fluorescence detection for the simultaneous determination of metoprolol and α-hydroxymetoprolol in plasma. It offers advantages over previous methods for these compounds in functioning on readily available equipment [4], permitting drug and metabolite assay in one procedure [5], obviating derivatization steps [5,6], and providing greater sensitivity than another HPLC method [7].

EXPERIMENTAL

Materials

The HPLC system consisted of a Model 870 pump module (Dupont Instruments, Wilmington, DE, U.S.A.), a Model 7125 injection valve (Rheodyne, Berkeley, CA, U.S.A.) a Model FS970 fluorescence detector (Schoeffel Instrument, Westwood, NJ, U.S.A.) and an Omniscribe recorder (Houston Instruments, Austin, TX, U.S.A.). A 25 cm × 0.46 I.D. stainless-steel column packed with 5-μm silica B/5 particles (Perkin-Elmer, Norwalk, CT, U.S.A.) was used.

Mobile phase and extraction solvents were purchased from either Burdick and Jackson Labs. (Muskegon, MI, U.S.A.) or Fisher Scientific (Fairlawn, NJ, U.S.A.). Analytical standards were obtained from the following sources: metoprolol tartrate (Lopressor®), gift of Ciba Pharmaceutical (Division Ciba-Geigy, Summit, NJ, U.S.A.); α-hydroxymetoprolol *p*-hydroxybenzoate, gift of Hassle (Mölndal, Sweden); and H 93/93 [(±)-ethyl-2-(4-(3-isopropyl-amino-2-hydroxypropoxy)phenyl)-ethyl carbamate], gift of Astra Pharmaceutical Products (Worcester, MA, U.S.A.). Pharmaceutical grade decolorizing carbon was obtained from Amend Drug and Chemical Co. (Irvington, NJ, U.S.A.).

Preparation of standards

Decolorizing carbon (5 g) was added to 100 ml of human plasma and stirred for 2 h at room temperature. Charcoal was then removed from the plasma by centrifugation at 27,500 *g* for 1 h followed by filtering through 5-μm and 0.45-μm membrane filters (Millipore, Bedford, MA, U.S.A.). To this stripped plasma were added metoprolol and α-hydroxymetoprolol in methanol to give concentrations of 25—200 ng/ml of the free base form of each drug.

Extraction procedure

Samples of plasma (1 ml) were placed in 8-ml glass centrifuge tubes and 50 μl of a 2 μg/ml solution of H 93/93 added as internal standard. Then 0.6 ml of 0.05 *M* hydrochloric acid, 1 ml of hexane—butanol (4:1) and 1 ml of ethyl acetate were added. The tubes were capped and vortexed for 30 sec. After centrifugation at 1300 *g* for 3 min, the upper organic layer and yellow interface were aspirated. The remaining aqueous phase was made alkaline with 0.3 ml of concentrated ammonium hydroxide and 5 ml of methylene chloride added. The tubes were rotated for 1 h at the zero speed position on a Fisher Roto Rack Model 343 (Fisher Scientific, Rochester, NY, U.S.A.) and then centrifuged for 10 min at 1300 *g*. The aqueous layer and creamy interface were aspirated and the remaining organic phase evaporated to dryness at 30°C under a stream of nitrogen gas.

Chromatography

The extraction residue was reconstituted with approximately 100 μl of mobile phase for injection. The mobile phase was composed of hexane—isopropanol—methanol—concentrated ammonium hydroxide (850:100:50:1) at a column flow-rate of 3 ml/min. The detector excitation wavelength was set at 224 nm and no emission filter was employed. Concentrations of metoprolol and α-hydroxymetoprolol were determined by comparison of the peak height ratio of drug to H 93/93 and to the peak height ratio of known standard concentrations of the drugs.

Extraction recoveries

To 1 ml of plasma containing either 25 ng/ml or 200 ng/ml of drug were added 0.6 ml of 0.05 *M* hydrochloric acid, 1 ml of hexane—butanol (4:1) and 1 ml of ethyl acetate. The samples were then vortexed, centrifuged and aspirated as in the extraction procedure. A 1-ml aliquot of the aqueous phase was obtained and alkalinized with 0.3 ml of concentrated ammonium hydroxide

and 5 ml of methylene chloride added. After rotation, centrifugation and aspiration, a 4-ml aliquot of the remaining methylene chloride phase was obtained. External standard (100 μl of 2 μg/ml solution of H 93/93 for the 200 ng/ml sample or 50 μl for the 25 ng/ml sample) was added and the solution evaporated to dryness. The residue was reconstituted in mobile phase for injection on the system described above. A non-extraction standard was made by taking a 1-ml aliquot of a methanol solution of metoprolol and α-hydroxymetoprolol of the same concentration as the extraction standard, adding external standard, evaporating and injecting. Calculation of percent recovery was made from the following equation:

$$\text{Percent recovery} = \frac{\text{peak height ratio extracted sample}}{\text{peak height ratio non-extracted sample} \times 0.5} \cdot 100\%$$

The number 0.5 is a result of the aliquots taken: $\frac{1\text{ ml}}{1.0\text{ ml}} \cdot \frac{4\text{ ml}}{5\text{ ml}}$

Five replicates of both the extracted and non-extracted samples were made.

RESULTS

A chromatogram of an extract of charcoal-stripped plasma spiked with metoprolol, H 93/93 and α-hydroxymetoprolol is shown in Fig. 2. This chromatogram illustrates the response to approximately 50 ng/ml plasma of metoprolol and α-hydroxymetoprolol, and to 100 ng/ml H 93/93 which was used as the internal standard. In Fig. 3a, the chromatogram of a plasma sample taken before administration of a 50-mg dose of metoprolol tartrate (Lopressor®) is shown without internal standard. The recovery of metoprolol and α-hydroxy-

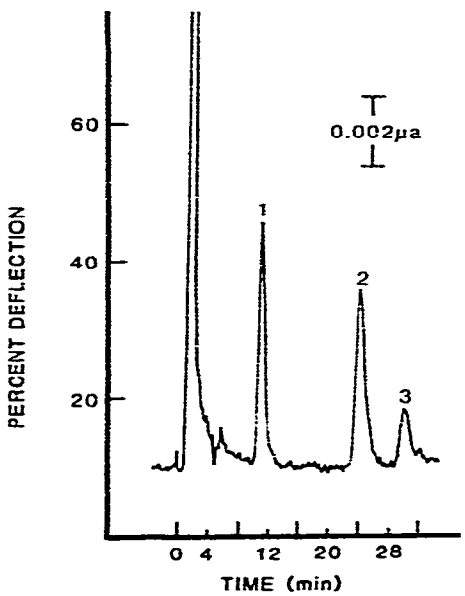

Fig. 2. Chromatogram of an extract of 1.0 ml of human plasma spiked with 50 ng of (1) metoprolol, (3) α-hydroxymetoprolol, and (2) H93/93, internal standard, 100 ng.

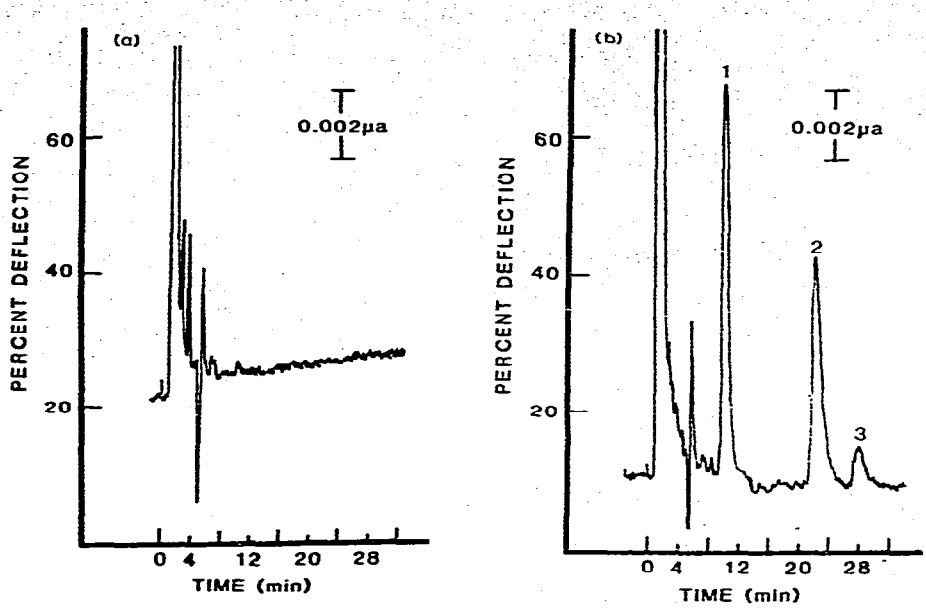

Fig. 3. Chromatograms of extracts of plasma: (a) taken prior to a dose of metoprolol, and (b) taken 1.5 h after a single oral dose of 50 mg of metoprolol tartrate. Peaks as in Fig. 1.

metoprolol from plasma (Table I) was greater than 83%. Fig. 3b illustrates the response to drug concentrations 1.5 h after the 50-mg dose of metoprolol and represents concentrations of 72 ng/ml of metoprolol and 28 ng/ml of α-hydroxymetoprolol. The sensitivity limit of the assay was 3 ng/ml for metoprolol and 12.5 ng/ml for α-hydroxymetoprolol when a signal-to-noise ratio of 2.5 or greater was used as a criterion for significant response and noise is measured as peak-to-peak baseline noise. The response of the HPLC system was linear over the 0—200 ng/ml concentration range studied. As shown in Fig. 4, a greater response was obtained for metoprolol than with α-hydroxymetoprolol.

The intra- and inter-day precision of the method was determined by assay of ten samples prepared by spiking stripped plasma with known amounts of metoprolol and α-hydroxymetoprolol. Table II presents the results of these precision studies.

Assay specificity was determined by comparing retention times of standards to those of samples. An additional assurance of specificity was provided by observing that the 227 nm to 205 nm peak height ratios of standard and patient samples were the same. Other β-blocking agents, antihypertensives and diuretics which might be co-administered with metoprolol to patients were examined.

TABLE I

EXTRACTION RECOVERY OF METOPROLOL AND α-HYDROXYMETOPROLOL FROM PLASMA

Concentration (ng/ml)	Recovery (%)	
	Metoprolol	α-Hydroxymetoprolol
25	95.32 ± 10.01	90.23 ± 8.46
200	83.06 ± 2.33	85.96 ± 2.52

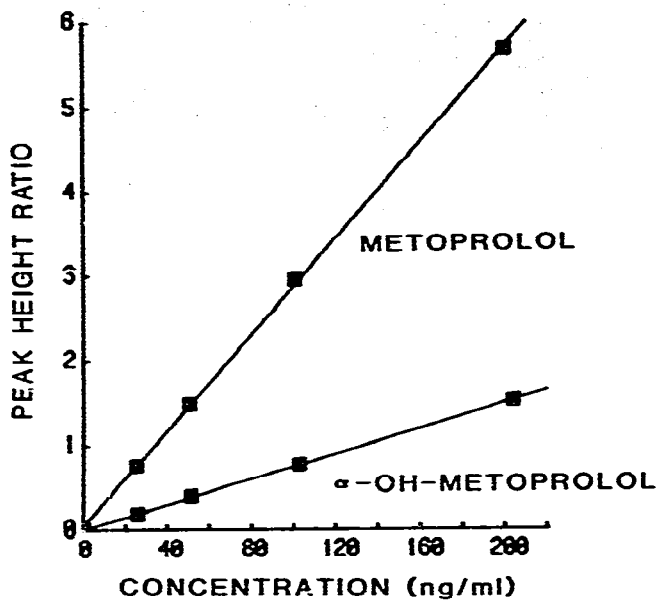

Fig. 4. Calibration curve for the determination of metoprolol and α-hydroxymetoprolol in a 1.0-ml plasma extract.

TABLE II

ASSAY PRECISION

Concentration (ng/ml)	Coefficient of variation (%)			
	Inter-day		Intra-day	
	Metoprolol	α-Hydroxymetoprolol	Metoprolol	α-Hydroxymetoprolol
25	3.12	2.61	2.34	5.49
200	2.69	3.18	4.90	1.69

TABLE III

RELATIVE RETENTION TIMES OF SELECTED DRUGS

Drug	Relative retention time
Canrenone	0.37*
Clonidine	0.40*
Spironolactone	0.43*
Reserpine	0.45
Alprenolol	0.57
Propranolol	0.69
Oxprenolol	0.89*
Metoprolol	1.00
Prazosin	1.20
4-Hydroxyalprenolol	1.56
4-Hydroxypropranolol	1.93
H 93/93	2.11
Hydrochlorothiazide	2.59*
α-Hydroxymetoprolol	2.68
Nadolol	4.01
Sotalol	4.10

*Gave negative deflection at time specified.

These materials had retention times relative to that of metoprolol as listed in Table III. Other drugs which were tested in this mobile phase system but gave no response were furosemide, hydralazine, methyldopa, methyldopa ethyl ester and guanethidine.

DISCUSSION

The determination of metoprolol and α-hydroxymetoprolol by this HPLC method has proved to be precise, sensitive and selective. The procedure allows the pharmacokinetic characterization of metoprolol and its active metabolite α-hydroxymetoprolol. The data in Fig. 5 were obtained in a male volunteer undergoing treatment for essential tremor who received a 50-mg oral dose of metoprolol tartrate (Lopressor®) as part of his normal treatment. As can be seen the data are adequate to depict a typical absorption/disposition profile [2] and observe formation of the metabolite.

The most recent pharmacokinetic studies of metoprolol and α-hydroxymetoprolol have utilized gas chromatography—mass spectrometry as an analytical tool [4]. The reported detection limit for metoprolol, α-hydroxymetoprolol, and *o*-demethylmetoprolol is 0.3 ng/ml with a relative standard deviation of less than 10%. The necessary derivatization step is rapid and reproducible. The major drawback of the method is the unavailability of the gas chromatography—mass spectrometry technology in the typical laboratory. The electron-capture—gas—liquid chromatography methods of Ervik [6] for metoprolol and of Quarterman et al. [5] for *o*-demethylmetoprolol and α-hydroxymetoprolol give sensitivities and precision comparable to ours. However, the conditions specified in these procedures preclude the simultaneous analysis of

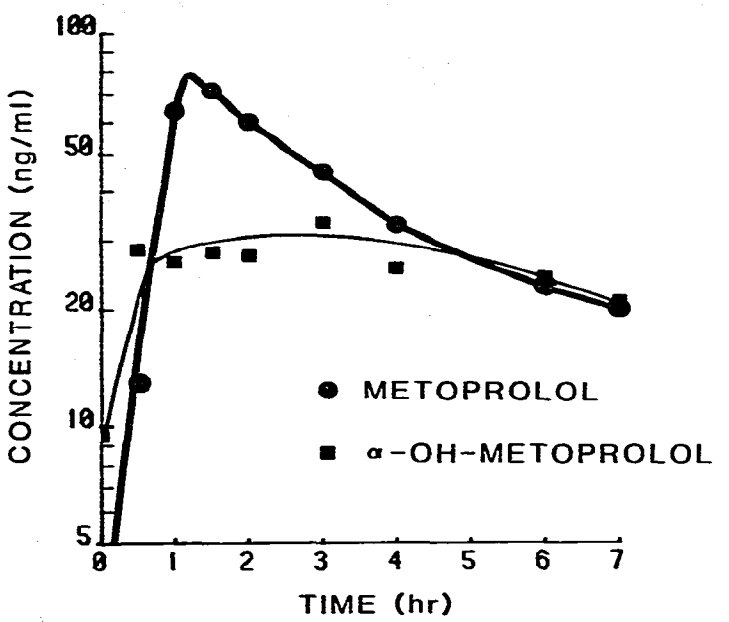

Fig. 5. Plasma concentration—time profile of a human volunteer given a 50-mg oral dose of metoprolol tartrate.

metoprolol and its metabolites and the derivatization procedures used are rather time consuming. The HPLC procedure of Lefebvre et al. [7] measures only metoprolol although extension to the measurement of the metabolites could be accomplished. However, the stated detection limit is only one-third of that reported here.

Of the above methods only Ervik [6] presents any data on possible interference from other β-blocking, antihypertensive or diuretic agents which might be co-administered with metoprolol to patients.

The HPLC procedure reported here has proven to be specific and precise in general use. It makes use of technology readily available in most laboratories. The sensitivity exhibited is sufficient to carry out both routine monitoring or pharmacokinetic studies.

ACKNOWLEDGEMENTS

We appreciate the clinical assistance of Dr. Patricia Christoff, Dr. L. Jacobs, and Dr. R. Newman. Supported in part by NIH grant No. 20852 and 24211.

REFERENCES

1 K.O. Borg, E. Carlsson, J.-J. Hoffmann, T.-E. Jönsson, H. Thorin and B. Wallin, Acta Pharmacol. Toxicol., 36 (1975) 125.
2 L. Jordö, P.O. Attman, M. Aurell, L. Johansson, G. Johnsson and C.G. Regårdh, Clin. Pharmacokin., 5 (1980) 169.
3 K.-J. Hoffmann, C.G. Regårdh, M. Aurell, M. Ervik and L. Jordö, Clin. Pharmacokin., 5 (1980) 181.
4 M. Ervik, K.-J. Hoffmann and K. Kylberg-Hanser, Biomed. Mass Spectrom., 8 (1981) 322.
5 C.P. Quarterman, M.J. Kendall, and D.B. Jack, J. Chromatogr., 183 (1980) 92.
6 M. Ervik, Acta Pharmacol. Toxicol., 36, Suppl. V (1975) 136.
7 M.A. Lefebvre, J. Girault and J.B. Fourtillan, J. Liquid Chromatogr., 4 (1981) 483.