Journal of Chromatography, 272 (1983) 103—110
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1470

GAS CHROMATOGRAPHIC DETERMINATION OF METOPROLOL IN HUMAN PLASMA

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(Received July 1st, 1982)

SUMMARY

A method for the determination of metoprolol at concentrations down to 10 ng/ml in human plasma is described. After addition of oxprenolol as internal standard, both compounds are extracted into diethyl ether—dichloromethane (4 :1, v/v) at basic pH, transferred into an acidic aqueous solution and back-extracted at basic pH into diethyl ether—dichloromethane. They are then derivatized with heptafluorobutyric anhydride. The derivatives are quantitatively determined by gas chromatography using a ^{63}Ni electron-capture detector. The linearity was demonstrated, and the technique was formally validated in the concentration range 10—500 ng/ml. The technique was applied in a study of the bioavailability of metoprolol after oral administration to man; mean plasma concentrations are reported.

INTRODUCTION

Various methods for the assay of metoprolol (Fig. 1, I) have been published. A gas chromatographic—mass spectrometric determination of metoprolol and its basic metabolites in plasma has been reported [1]. Several high-performance liquid chromatographic (HPLC) methods have been described [2—5], but they are all general methods for the assay of a series of β-adrenoreceptor blocking drugs. Gas chromatographic (GC) procedures [6—8] using electron-capture detection have also been described for the determination of metoprolol in plasma, but they suffer from certain disadvantages: they require 2 ml of plasma, and the internal standard is added just before injection [6,7]. The method recently described by Kinney [8] is sensitive, but needs a very high carrier-gas flow-rate (230 ml/min), which is not attainable with the gas chromatographs generally used.

This paper describes a GC assay procedure that permits the quantitative

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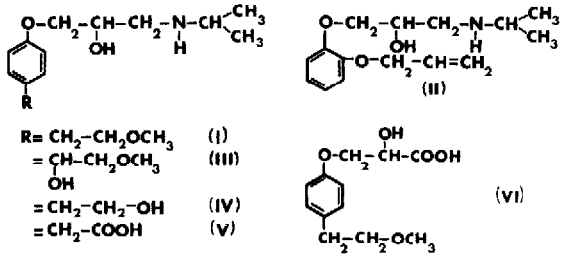

Fig. 1. Chemical structure of metoprolol (I), internal standard (oxprenolol, II), and main metoprolol metabolites (III, IV, V, VI).

determination of metoprolol in plasma down to 10 ng/ml. The technique makes use of an internal standard introduced into the plasma sample to correct for losses during extraction and derivatization. The extraction solvent used is the same as the one already described by Degen and Riess [9]. The specificity of the assay as regards the metabolites of metoprolol is demonstrated.

EXPERIMENTAL

Chemicals and reagents

Metoprolol and the internal standard oxprenolol (Fig. 1, II) were supplied by Ciba-Geigy (Basle, Switzerland).

Alkaline buffer (pH>13) was prepared by dissolving 30 g of tripotassium phosphate 3-hydrate (Merck 5102; E. Merck, Darmstadt, G.F.R.), or 38 g of tripotassium phosphate 7-hydrate (Merck 5103) and 16.8 g of potassium hydroxide (Merck 5033) in ultra-high-purity HPLC water (Alltech Assoc., Arlington Heights, IL, U.S.A.) and making the volume up to 100 ml. All reagents and solvents were of analytical grade: diethyl ether (Pestipur quality, S.D.S., Valdonne, France), hydrochloric acid (Merck 318), hexane (Pestipur quality, S.D.S.). Pyridine (Fluka 82702; Fluka, Buchs, Switzerland) was distilled at 115–116°C with potassium hydroxide pellets and stored over the same reagent. Heptafluorobutyric anhydride was purchased from Ventron (Ref. PCR, 1300-3; Ventron, Karlsruhe, G.F.R.); potassium dihydrogen phosphate (Merck 4873) was used as a saturated solution.

Calibration curves

Aliquots of 50 µl of six different aqueous solutions of metoprolol tartrate were added to 1 ml of plasma to produce reference samples in the range of concentrations 10–500 ng/ml of plasma.

The aqueous internal standard solution contained 1000 ng/ml of oxprenolol hydrochloride, and 100 µl of this solution were added to each plasma sample, resulting in a concentration of 100 ng/ml of oxprenolol in plasma.

Calibration solutions are prepared every ten days and stored at 4°C.

Equipment

All glassware was washed, dried at 100°C and immersed for 0.5 h in an ultrasonic bath, first in water and then in methanol. The dried glassware was then treated to prevent adsorption. It was immersed in a silanizing solution con-

taining hexamethyldisilazane, trimethylchlorosilane and pyridine (1% v/v each) for 15 min and rinsed with methanol. Silanization is unnecessary if quartz tubes are used instead of glass tubes.

A Hewlett-Packard (Model 5713 A) gas chromatograph equipped with a Hewlett-Packard electron-capture detector (Model 18713A) was used. The peak areas were calculated by a Hewlett-Packard computing integrator (Model 3388A) connected to the chromatograph. The column was operated at 200°C, the injector at 250°C and the detector at 300°C, with argon–methane (90:10) at a flow-rate of 60 ml/min. The glass column (2 m × 3 mm I.D.) was washed [10] and packed with 3% OV-1 on 80–100 mesh Gas-Chrom Q (Supelco 1-2096; Supelco, Bellefonte, PA, U.S.A.). The conditioning procedure has been described previously [10].

Extraction

One hundred microlitres of the internal standard solution were measured into a glass tube, to which 1 ml of plasma, 1 ml of alkaline buffer and 4 ml of diethyl ether–dichloromethane (4:1, v/v) were then added. The tube was shaken mechanically (Infors shaker) for 15 min at 300 rpm and centrifuged at 2500 *g* for 5 min. An aliquot of the organic phase was transferred to another tube, shaken with 2 ml of 0.1 *N* hydrochloric acid for 10 min at 300 rpm and briefly centrifuged. The organic phase was discarded, and 1 ml of the alkaline buffer and 4 ml of diethyl ether–dichloromethane (4:1, v/v) were added. The mixture was shaken for 10 min at 300 rpm. After centrifugation, the organic phase was removed and evaporated to dryness under a nitrogen stream at 40°C.

Derivatization and chromatography

To the dry residue were added 1 ml of 0.3% pyridine in hexane and 10 μl of heptafluorobutyric anhydride (conditioned in ampoules of 2 ml) with a glass pipette. The tube was stoppered and agitated on a Vortex mixer for 15 sec.

After 1 h at room temperature, 1 ml of a saturated aqueous solution of potassium dihydrogen phosphate was added, and the mixture was shaken for 15 min at 300 rpm, then centrifuged. The aqueous phase was frozen by immersing the tube in a methanol–dry-ice bath. An aliquot of the hexane phase was transferred to a 250-μl conical glass flask (Hewlett-Packard 5080-8779); 3 μl were injected into the gas chromatograph with a Hewlett-Packard automatic sampler (Model 7672A).

Bioavailability study in man

Twenty healthy subjects received 200 mg of metoprolol tartrate as two different formulations, given in three different dosage regimens (two 100-mg tablets every 24 h, one 100-mg tablet every 12 h, and one 200-mg sustained-release tablet every 24 h) for four days. Blood samples were collected on the first day at various times. The samples were centrifuged, and the plasma was removed and stored at −20°C until analysis.

RESULTS AND DISCUSSION

Reaction time

The duration of the derivatization reaction was varied from 15 min to 2 h. A maximum yield of derivative was reached after 1 h.

Linearity

Ln—ln straight lines were obtained for calibration curves in the concentration range of 10—500 ng/ml. At concentrations higher than 500 ng/ml, the lines began to deviate from linearity. The determinations could be extended beyond 500 ng/ml by taking less than 1 ml of plasma and diluting the plasma sample.

The method was formally validated over the concentration range 10—500 ng/ml (Table I).

The day-to-day reproducibility of the standard curves was shown in three consecutive experiments carried out on separate days. On each occasion, the

TABLE I

DAY-TO-DAY REPRODUCIBILITY OF CALIBRATION CURVES USED TO DETERMINE METOPROLOL IN PLASMA

Concentration added to plasma (ng/ml)	Peak area ratios				$100 \times \frac{E}{C}$ (%)
	Experimental (E)			Calculated from ln—ln regression line (C)	
	Day 1	Day 2	Day 3		
10	0.139			0.124	112.1
10		0.136		0.124	109.7
10			0.128	0.124	103.2
20	0.227			0.237	95.8
20		0.220		0.237	92.8
20			0.219	0.237	92.4
50	0.550			0.554	99.3
50		0.531		0.554	92.6
50			0.532	0.554	96.0
100	1.050			1.050	100.0
100		1.050		1.050	100.0
100			1.060	1.050	101.0
250	2.460			2.470	99.6
250		2.530		2.470	102.4
250			2.530	2.470	102.4
500	4.710			4.700	100.2
500		4.800		4.700	102.1
500			4.810	4.700	102.3
	Average ± C.V. (%)				100.2 ± 5.3

peak area ratio of metoprolol versus the internal standard plotted against six concentrations of metoprolol gave straight lines. A least-squares ln—ln regression line was generated from the eighteen data points of the three standard curves (Table I). It corresponds to the regression equation $\ln Y = 0.9280 \ln X$ (ng/ml) $- 4.2204$.

Reproducibility of calibration curves

A test of day-to-day reproducibility was made by expressing each data point as a percentage of the value read off the ln—ln line for the corresponding concentration (Table I). The distribution of these normalized (concentration-independent) data had an overall average (±C.V.) of 100.2 ± 5.3%, demonstrating a good reproduciblity between experiments.

The reproducibility of the calibration curves was tested over six weeks. Table II gives the slopes and intercepts on the y-axis obtained for twenty calibration curves. The results obtained show a good reproducibility; however, a variability between the curves is sometimes noted which is the reason why the calibration curve was generated daily.

TABLE II

REPRODUCIBILITY OF TWENTY CALIBRATION CURVES OVER SIX WEEKS USED TO DETERMINE METOPROLOL IN PLASMA

Determination No.	Slope (S)	Intercept on y-axis (i)	Correlation coefficient (R)
1	0.9142	−4.1114	0.9980
2	0.9353	−4.2406	0.9990
3	0.9134	−4.1725	0.9992
4	0.9103	−4.1683	0.9995
5	0.9139	−4.1515	0.9992
6	0.9285	−4.2215	0.9990
7	0.9405	−4.2805	0.9995
8	0.9465	−4.3302	0.9989
9	0.9216	−4.2418	0.9993
10	0.9154	−4.1666	0.9988
11	0.9208	−4.2112	0.9997
12	0.8949	−4.1057	0.9979
13	0.8422	−3.8882	0.9986
14	0.8885	−4.1210	0.9995
15	0.8838	−4.0393	0.9991
16	0.8750	−4.0020	0.9994
17	0.8830	−4.1282	0.9990
18	0.8844	−4.1623	0.9995
19	0.8839	−4.2037	0.9991
20	0.8488	−3.9561	0.9984
Mean	0.9022 ± 3.2%	−4.1451 ± 2.6%	

Precision and accuracy

Table III gives the results obtained when the described procedure was applied to spiked plasma samples. With a calibration curve generated on each

TABLE III

PRECISION AND RECOVERY OF THE DETERMINATION OF METOPROLOL IN SPIKED PLASMA SAMPLES

Amount added (ng/ml)	Mean amount found (ng/ml) (n=6)	Standard deviation	Recovery (mean, %)
10	10.4	±0.2	103.5
20	19.6	±0.3	97.8
50	49.6	±0.5	99.2
100	97.3	±0.8	97.3
250	256	±3	102.4
500	510	±16	101.9
			100.4 ± 2.6

day of analysis, the results demonstrate a good reproducibility of the assay down to concentrations of 10 ng/ml of plasma, which is taken as the quantitation limit of the method. Lower concentrations could still be detected.

Day-to-day validation

Over a period of six weeks, the method was validated on each working day by determining one concentration in duplicate (100 ng/ml). The assay was performed by two different analysts, and Table IV shows the day-to-day reproducibility of the method.

TABLE IV

PRECISION AND RECOVERY IN THE DAY-TO-DAY DETERMINATION OF METOPROLOL IN SPIKED PLASMA

Amount added (ng/ml)		Mean amount found (ng/ml) (n=20)	Standard deviation	Recovery (%)	C.V. (%)
100	First analyst	98.9	±19	98.9	6.5
100	Second analyst	100	±16	100	6

Plasma interference

Fig. 2 shows the chromatograms of an extract of human plasma and of the same extract spiked with 50 ng of metoprolol tartrate and 100 ng of internal standard. There is no interference from the normal components of the plasma extract.

Specificity

The four main metabolites of metoprolol, i.e. the basic metabolites

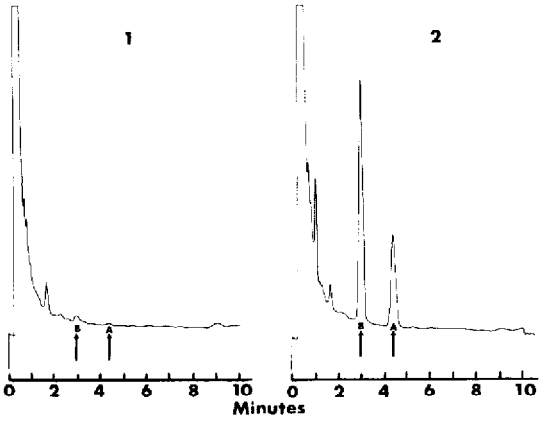

Fig. 2. Chromatograms of (1) human plasma blank (1 ml of plasma) and (2) the same plasma spiked with 50 ng/ml metoprolol tartrate (A) and 100 ng/ml internal standard (B).

α-hydroxymetoprolol and O-demethylmetoprolol (Fig. 1, III and IV), and the acidic metabolites H 117/04 and H 104/83 (Fig. 1, V and VI), were used to test the specificity. The two acidic metabolite derivatives were not detected after GC. O-Demethylmetoprolol was detected but it is reported [11] that this metabolite is only present in human urine. α-Hydroxymetoprolol was also detected; its retention time is longer than those of metoprolol and the internal standard.

Stability

Table V shows that no decrease in the metoprolol content (20 ng/ml and 250 ng/ml) occurred in plasma samples stored frozen for twelve months at −20°C.

TABLE V

STORAGE STABILITY OF METOPROLOL TARTRATE IN HUMAN PLASMA AFTER TWELVE MONTHS AT −20°C

Duration of storage at −20°C (months)	Amount of metoprolol tartrate added to plasma (ng/ml)	
	20	250
	Amount of metoprolol tartrate found (average of two assays) (ng/ml)	
0	22.3	259
1	21.5	262
2	20.3	239
3	21.5	261
6	19.6	262
9	18.6	242
12	19.9	272

Application

The technique was applied in a bioavailability study comparing commercial tablets of metoprolol with a new sustained-release formulation. Fig. 3 shows the average curves obtained from the plasma samples of twenty subjects given 200 mg of metoprolol tartrate in three dosage regimens.

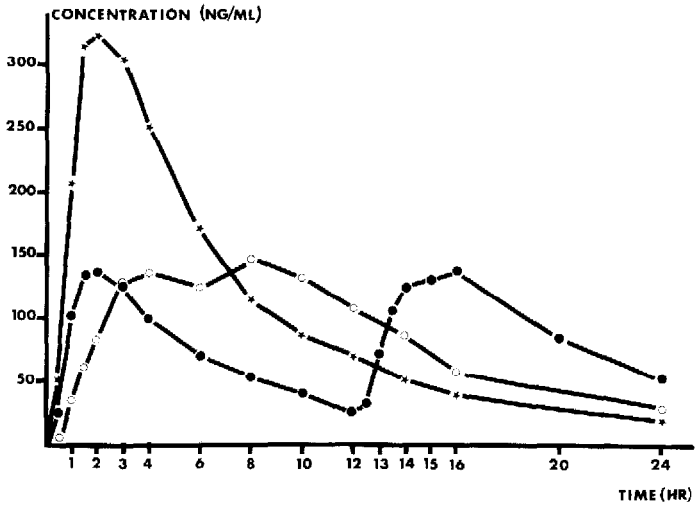

Fig. 3. Average plasma metoprolol concentrations obtained on the first day in twenty healthy subjects after administration of 200 mg of metoprolol tartrate given as: 100-mg metoprolol tablet every 12 h (●); 2 × 100-mg metoprolol tablet every 24 h (○); 200-mg sustained-release metoprolol tablet every 24 h (★).

CONCLUSION

The proposed technique permits the quantitative assay of metoprolol in human plasma at concentrations down to 10 ng/ml. It is specific, reproducible, and sufficiently sensitive for determinations of metoprolol in bioavailability studies.

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