Journal of Chromatography, 622 (1993) 187–195
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 7140

Identification and determination of the enantiomers of moprolol and their metabolites in human urine by high-performance liquid chromatography and gas chromatography–mass spectrometry

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(First received July 20th, 1993; revised manuscript received October 7th, 1993)

ABSTRACT

A simple and sensitive high-performance liquid chromatographic (HPLC) method using chiral derivatization was developed to screen and determine the enantiomers of moprolol and their metabolites in human urine. The recovery of (+)- and (−)-moprolol from urine was 70.8–81.1% at different concentrations. The coefficients of variation (C.V.) were less than 3.2 and 6.5% for intra- and inter-assays, respectively. Moprolol could be detected in urine up to 24 h after oral administration of a 50-mg dose of moprolol. Unconjugated and conjugated enantiomers of moprolol and their metabolites were analyzed by gas chromatography (GC). A gas chromatographic–mass spectrometric (GC–MS) confirmatory method was established to identify the metabolites of moprolol. The double derivatization procedure for moprolol and their metabolites with S-(−)-menthyl chloroformate [(−)-MCF] and N-methyl(trimethylsilyl)trifluroacetamide (MSTFA) gave very good GC–MS properties of the derivatized compounds and provided reliable structural information for their confirmation analysis. This is the first published report on the use of a GC–MS method for the detection of the enantiomers of moprolol and their metabolites in human urine.

INTRODUCTION

β-Blocking drugs are competitive inhibitors of the effects of catecholamines at β-adreno-receptor sites. The therapeutic use of β-blocking drugs has been well established in angina pectoris, cardiac arrhythmias and hypertension. They have also been found to be efficacious in myocardial infarction, migraine and glaucoma. Patients treated with β-blocking drugs can do more exercise at lower heart rate [1]. In the last few years β-blocking drugs have been misused and abused in sports (like gunshooting, skijumping, fencing and motor racing) to reduce heart rate, muscle tremor and oxygen consumption.

Moprolol or 1-(2-methoxyphenoxy)-3-isopropylamino-2-propanol, first synthesized in 1968, is a β-blocking agent [2] which is being used in hypertension and in the topical treatment of glaucoma [3].

Although pharmacokinetic studies of moprolol have been reported in human volunteers [4,5], the stereoselective metabolism of this drug and the identification of its enantiomeric urinary metabolites in man by gas chromatography–mass spectrometry (GC–MS) has not yet been reported in the literature.

This paper reports the detection of moprolol

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0378-4347/93/$06.00

enantiomers and two enantiomeric urinary metabolites following oral administration of 50 mg of racemic moprolol to human. In this study, the emphasis was laid on the amounts of unconjugated and conjugated parent compound, the identification of enantiomers and their respective metabolites in human urine. Their identity was assessed by comparison with authentic reference compounds and on the basis of the GC–MS properties of their derivatives with the chiral reagent S-(−)-menthyl chloroformate (MCF) and N-methyl(trimethylsilyl)trifluoroacetamide (MSTFA). The gas chromatographic (GC) data also illustrate that, in human, most of the moprolol enantiomers and their metabolites are excreted in urine in the form of conjugates.

EXPERIMENTAL

Materials and reagents

Racemic (±)-moprolol and (−)-moprolol were obtained from Simes (Milan, Italy). (±)-Toliprolol or 1-[(1-methylethyl)amino]-3-(3-methylphenoxy)-2-propanol was from ICI (Cheshire, UK). S-(−)-Menthyl chloroformate [(−)-MCF] was purchased from Aldrich (Milwaukee, WI, USA). N-methyl(trimethylsilyl)trifluoroacetamide (MSTFA) was obtained from Regis (Morton Grove, IL, USA) and trimethylsilyl iodide (TMSI) from Aldrich. All the organic solvents (HPLC grade) were used as received (Caledon Labs., Georgetown, Canada). Inorganic salts were of analytical reagent grade (J.T. Baker, Phillisburg, NJ, USA, or Caledon Labs.). β-Glucuronidase type H-1 from *Helix pomatia* was purchased from Sigma (St. Louis, MO, USA). Distilled water was further treated with a four-stage Milli-Q water purification system (Continental Water System, Oakville, Canada) before used for HPLC (this is simply referred as water hereafter).

Standard solutions

Stock solutions of moprolol (0.1 and 1.0 mg/ml) and toliprolol used as internal standard (I.S.) (1.0 mg/ml) were prepared in methanol. A solution of triethylamine (0.4%) was prepared in acetonitrile–methanol (50:50, v/v). The (−)-MCF solution (1%) was prepared every week in acetonitrile which was previously dried with anhydrous sodium sulfate. All the stock solutions and reagent solutions were sealed and stored at −20°C in the dark.

Human studies

Control urine samples were collected before the administration of a single 50-mg oral dose of racemic (±)-moprolol to a healthy male volunteer (aged 33 and weighing 60 kg). Urine samples were then collected for the next 72 h and were frozen in the dark at −20°C immediately after collection. To the same volunteer, a single 25-mg oral dose of (−)-moprolol was given 14 days after the administration of (±)-moprolol, and the urine samples were collected accordingly.

Extraction and chiral derivatization for HPLC analysis

In a typical extraction procedure, aliquots of 2.0 ml of urine were basified to pH 12 with 0.9 ml of 2 *M* potassium carbonate solution. About 1.0 g of sodium chloride was then added and the resulting sample was extracted with 5 ml of ethyl acetate. The organic layer was decanted and dried with sodium sulfate. Before the evaporation of the organic solvent under a stream of nitrogen at 50°C, 15 μg of (±)-toliprolol were added as I.S. To the dried extract, 100 μl of 0.4% triethylamine solution and 100 μl 1% (−)-MCF were added. The resulting solution was then kept at room temperature for 1 h. Before HPLC analysis, the sample was diluted with 100 μl of acetonitrile.

High-performance liquid chromatography

An HP 1090 liquid chromatograph was equipped with a diode array UV detector and an HP 79994 HPLC ChemStation data system (Hewlett-Packard, Palo Alto, CA, USA). The separation of the (−)-MCF derivatives of (±)-moprolol and (±)-toliprolol was performed on an HP ODS Hypersil (C_{18}) column (200 mm × 4.6 mm I.D., 5 μm particle size) at ambient temperature. A laboratory packed HP ODS Hypersil pre-column (20 mm × 2.1 mm I.D., 30 μm particle size) was used to protect the analytical column. The mobile phase consisted of

solvent A and B. Solvent A (0.02 *M* phosphate buffer, pH 3.2) was prepared by dissolving 6.9 g of monobasic sodium phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) and 1.59 g of propylamine hydrochloride in 1 l of water and adjusting the pH to 3.2 with concentrated phosphoric acid. It was filtered over a Millipore 0.45-μm HA filter and degassed with helium for 30 min before use. Solvent B was acetonitrile. The analysis was accomplished by gradient elution starting with 15% solvent B (held for 1 min) and then linearly increasing to 80% solvent B at 10 min (held for 12 min). The flow-rate of the mobile phase was kept at 1.3 ml/min. The UV absorbance of the column effluent was monitored at 203 nm (band width of 4 nm) with a reference wavelength of 450 nm (band width of 20 nm). Spectral scanning was performed from 190 to 400 nm.

Calibration curve

Aliquots of 2 ml of blank urine samples were spiked with (±)-moprolol (concentration: 0.3 to 7.5 μg/ml for each enantiomer). After being equilibrated for 1 h at 37°C, the urine samples were extracted as described under *Extraction and chiral derivatization for HPLC analysis*, and the extracts were analyzed by HPLC. For each concentration, triplicate samples were prepared and duplicated injections were made for each sample. The peak-area ratios of (−)- and (+)-moprolol to the (−)- and (+)-toliprolol (I.S.) were measured for each analysis. The data were fitted by linear regression equations $C_{(l)} = 3.8\ A_{(l)} - 0.14$ ($r^2 = 0.997$) and $C_{(d)} = 3.6\ A_{(d)} - 0.04$ ($r^2 = 0.999$), where $C_{(l)}$ and $C_{(d)}$ are the concentration of (−)- and (+)-moprolol in urine (μg/ml), respectively, $A_{(l)}$ and $A_{(d)}$ are the peak-area ratios of the (−)-MCF derivatives of (−)- and (+)-moprolol to that of (−)- and (+)-toliprolol (I.S.).

Recovery and precision

The extraction recovery of moprolol enantiomers from urine was assessed with spiked urine samples at five different concentrations, 0.3, 0.75, 1.5, 3.75, and 7.5 μg/ml for (−)- and (+)-moprolol. Sample preparation and HPLC analysis were performed as mentioned previously. The calculation of recovery was based on a comparison of the peak-area ratios of the (−)-MCF derivatives of the moprolol enantiomers to that of I.S. enantiomers from two different analysis. One set of data was obtained from the analysis of the spiked urine samples while the other set was from the analysis of standard solutions containing the same amount of moprolol and toliprolol enantiomers. The detection limit for each moprolol enantiomer was found to be 0.3 μg/ml (signal-to-noise ratio 3:1) when blank urine samples were spiked with known amounts of this drug.

Intra- and inter-assay variabilities were determined by replicate analysis of (−)- and (+)-moprolol enantiomers after spiking urine samples with (±)-moprolol (with the same concentration as in the recovery experiment) on the day of preparation and on different days, respectively (Table I).

Extraction and double derivatization procedure for GC and GC–MS analysis

The urine sample was extracted and derivatized with (−)-MCF as described above under *Extraction and chiral derivatization for HPLC analysis*. After derivatization, the solution was evaporated to dryness under a stream of nitrogen at 50°C. To the residue, 100 μl of MSTFA and 1 μl of TMSI were added. The solution was kept at 70°C for 30 min, and 1 μl was injected into the gas chromatograph for GC and GC–MS analysis.

Enzymatic hydrolysis of conjugates in urine and derivatization for GC analysis

An aliquot of 5.0 ml of urine was extracted with ethyl acetate to remove unconjugated compounds. Remaining traces of ethyl acetate were removed from the aqueous phase by heating the sample at 50°C and passing of a slow stream of nitrogen. The pH of the aqueous phase was adjusted to pH 5.0 with 0.1 *M* HCl. Subsequently the sample was incubated with β-glucuronidase (0.34 I.U.) at 37°C for 16 h. The hydrolysate was basified to pH 10–11 with potassium carbonate, and then 1.0 g of sodium chloride was added. The resulting sample was extracted with 6 ml of ethyl acetate. The organic layer was separated and dried with sodium sulfate. The organic solvent was evaporated under a stream

of nitrogen at 50°C. To the dried extract, 100 μl of a 0.4% triethylamine solution and 100 μl of 1% (−)-MCF were added. The solution was kept at room temperature for 1 h, then evaporated under a stream of nitrogen at 50°C. To the dried residue, 100 μl of MSTFA and 1 μl of TMSI were added. The resulting mixture was heated at 70°C for 30 min, and 1 μl was injected into the gas chromatograph for GC and GC–MS analysis.

Gas chromatography

Moprolol and its metabolites were detected by gas chromatography (GC) using an HP 5890 gas chromatograph equipped with FID (flame ionization detector) (Hewlett-Packard). The column was an HP-5 (crosslinked 5% phenyl methyl silicone) fused-silica capillary column (25 m × 0.2 mm I.D., 0.33 μm film thickness). The injections were made in the splitless mode (3 min delay with open splitter) with helium as carrier gas. The oven temperature was programmed from 120°C at 15°C/min to 250°C (1 min hold) and then at 1.5°C/min to 280°C (maintained for 10 min). The injector and the detector temperatures were 250°C and 300°C respectively.

Gas chromatography–mass spectrometry

Identification of moprolol and its enantiomeric metabolites was carried out using an HP 5970 mass-selective detector (Hewlett-Packard), equipped with an HP 5890 gas chromatograph fitted with an HP-5 fused-silica capillary column (25 m × 0.2 mm I.D., 0.33 μm film thickness). The injections were made in the splitless mode using helium as a carrier gas at a flow-rate of 0.8 ml/min. The oven temperature was programmed as mentioned previously under *Gas chromatography*. The mass spectrometer was operated in the full-scan mode for moprolol and its metabolites. Mass spectra of the (−)-MCF and TMS derivatives of the moprolol metabolites of interest were recorded at a scanning speed of 1 s per decade over the mass range 50–600 a.m.u.

RESULTS AND DISCUSSION

Chiral separation of moprolol and its enantiomeric metabolites

This study shows an efficient chiral separation

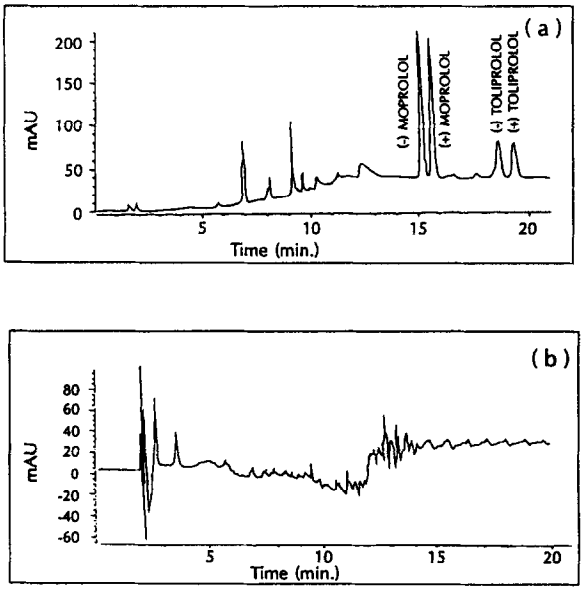

Fig. 1. (a) HPLC of standard (±)-moprolol enantiomers. (b) HPLC obtained from an extract of 2 ml of blank human urine sample.

of moprolol enantiomers and their enantiomeric metabolites in urine after oral administration of racemic parent drug to a human volunteer. With the chromatographic methods used in this investigation, the most successful separation of the enantiomeric compounds have been achieved after chemical derivatization with the chiral reagent [(−)MCF] to form diastereomer pairs. The resulting diastereomeric derivatives are no longer mirror images of each other, and have different physicochemical properties in solution, so that they could be separated on a non-chiral chromatographic system. In this experiment, after derivatization of the moprolol enantiomers with the chiral reagent (−)-MCF, racemic moprolol could be well separated on a non-chiral column with a mobile phase gradient consisting of 0.02 *M* phosphate buffer at pH 3.2 and acetonitrile. The standard racemic (±)-moprolol gave two peaks at retention times of 15.36 and 15.86 min (Fig. 1a). The peak of standard (−)-moprolol appeared at 15.36 min. The second peak at 15.86 min appeared to be (+)-moprolol, the mass spectrum of which was identical to its (−)-antipode. The retention times of (−)- and (+)-toliprolol were 18.89 and 19.60 min respec-

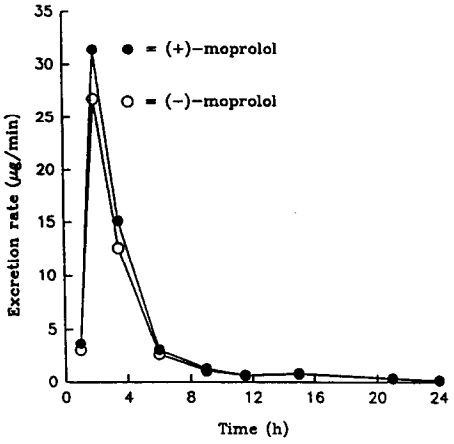

Fig. 2. Excretion rate profile of a subject after a 50-mg oral dose of (±)-moprolol.

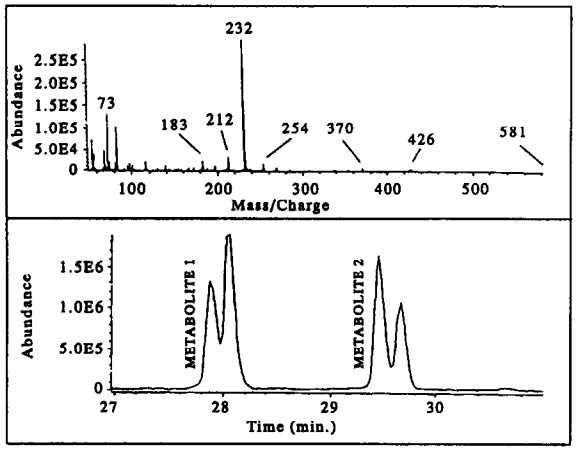

Fig. 4. GC–MS of (−)-MCF and MSTFA derivatives of moprolol metabolite 1 from urine sample.

tively. Fig. 1b shows an HPLC chromatogram of an extract from blank human urine. It shows the absence of interfering peaks at the retention times of moprolol and toliprolol (I.S.).

Recovery studies

The reproducibility of the results and the recovery of (+)- and (−)-moprolol are given in Table I. The overall coefficient of variation (C.V.) is below 10%.

Excretion rate

The urine excretion curves (Fig. 2) indicate that the compounds can be detected up to 24 h after moprolol administration. Excretion rates of moprolol in urine were maximum between 2 and 4 h after administration. Renal clearance of (−)- and (+)-moprolol is not significantly different, (+)-moprolol having a little higher excretion rate than (−)-moprolol.

Urinary excretion of unconjugated moprolol metabolites was first detected two hours after the administration of a 50-mg oral dose and remained detectable for a period of 9 h.

Identification of metabolites by GC–MS

The mass spectra of the (−)-MCF and TMS derivatives of (±)-moprolol and its metabolites obtained by GC–MS are shown in Figs. 3, 4 and 5. Interestingly, the corresponding mass spec-

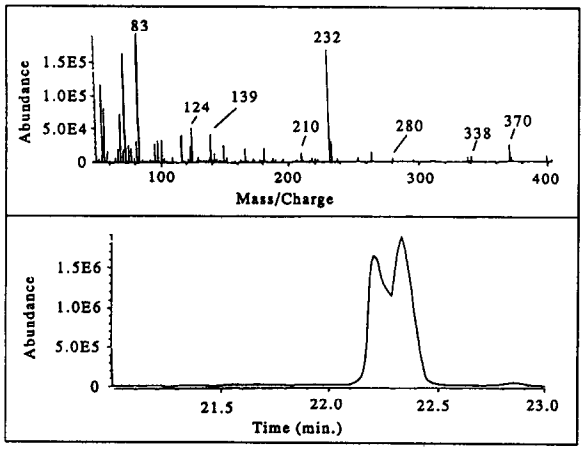

Fig. 3. GC–MS of standard (±)-moprolol derivatized with (−)-MCF and MSTFA.

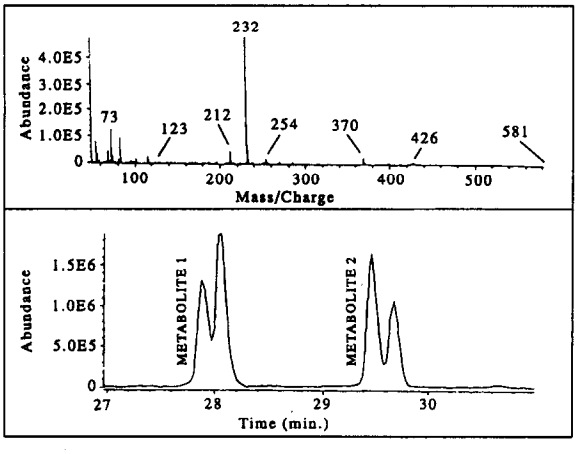

Fig. 5. GC–MS of (−)-MCF and MSTFA derivatives of moprolol metabolite 2 from urine sample.

TABLE I

RECOVERY OF (+)- AND (−)-MOPROLOL FROM HUMAN URINE SPIKED WITH (±)-MOPROLOL

Added (μg/ml)	Intra-assay (n = 3)		Inter-assay (n = 9)	
	Recovery (mean ± S.D.) (%)	C.V. (mean ± S.D.) (%)	Recovery (mean ± S.D.) (%)	C.V. (mean ± S.D.) (%)
1.5	(L) 78.2 ± 2.3	2.9	(L) 79.7 ± 5.1	6.5
	(D) 80.2 ± 0.3	0.4	(D) 82.6 ± 5.0	6.1
7.5	(L) 73.5 ± 0.8	1.2	(L) 70.8 ± 3.0	4.3
	(D) 75.7 ± 0.9	1.2	(D) 72.4 ± 1.1	1.5
15	(L) 76.4 ± 2.1	2.8	(L) 81.1 ± 1.9	2.3
	(D) 78.9 ± 2.5	3.2	(D) 80.7 ± 1.0	1.3

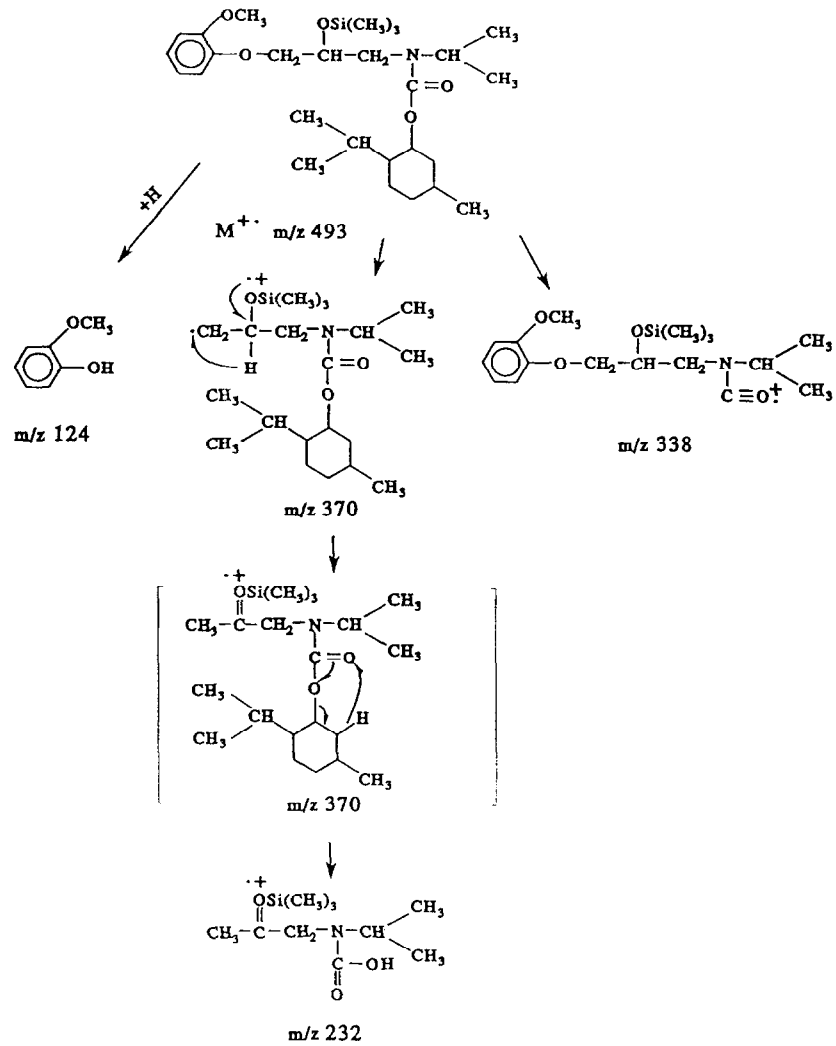

Fig. 6. Proposed fragmentation routes of standard moprolol.

trum of metabolite 1 is very similar to that of metabolite 2. Both metabolites exhibit identical fragmentation pathways and relative ion abundances, but they differ in their retention times (Figs. 4 and 5).

The molecular ion at m/z 581 indicated monohydroxylation of (±)-moprolol, whereas the diagnostic ions at m/z 426 and 212 (Figs. 6 and 7) indicated that the aromatic ring was the hydroxylation site of both metabolites. Indeed, the ions at m/z 426 and 212 are the OTMS analogs of the ions at m/z 338 and 124 in the mass spectrum of moprolol (Figs. 3, 4 and 5). By comparing the mass spectra of metabolites 1 and 2 with that of standard moprolol, it can be concluded that both metabolites are hydroxylated on the aromatic ring but at different positions (Fig. 8). Further confirmation will be done with the synthesized standard compounds by nuclear magnetic resonance and GC–MS analysis.

Profiles of the unconjugated and conjugated metabolites

The profiles of the urinary moprolol and its metabolites obtained by GC analysis of the

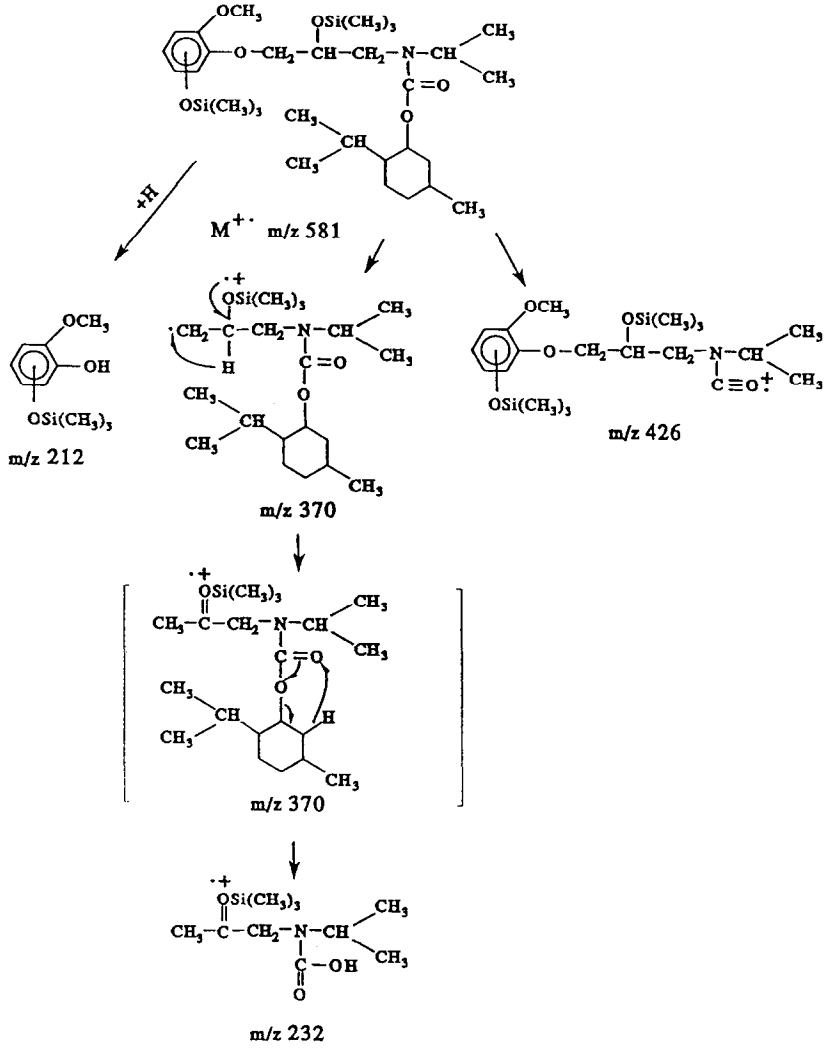

Fig. 7. Proposed fragmentation routes of metabolites 1 and 2.

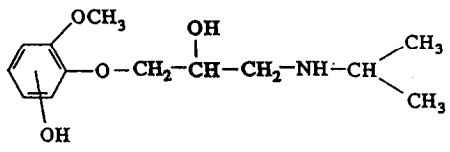

Fig. 8. Suggested chemical structure of metabolites 1 and 2.

unconjugated and the conjugated fractions are shown in Fig. 9a and b. They demonstrate clearly that moprolol is mainly excreted in urine in the unconjugated form. The data from the 6-h urine sample shows that the concentrations of the (−)- and (+)- forms of unconjugated moprolol are twofold higher those of conjugated moprolol; the concentrations of the (−)- and (+)- forms of the conjugated metabolite-1 are respectively 2.3 and 1.3 times higher than that of the unconjugated metabolite-1; the concentrations of the (−)- and (+)- forms of the conjugated metabolite-2 are respectively 2.5 and 5.3 times higher than that of the unconjugated metabolite-2.

Stereoselective metabolism

Since β-receptor binding for norepinephrine is highly stereoselective for the (−)-(*R*)-enantiomer, β-blockers also show analogous stereoselectivity. The stereoselectivity can vary widely with the L/D-enantiomer ratio for the binding affinity and the enantiomeric selectivity associated with beta receptor binding and the therapeutic actions of β-blockers. It is possible that stereoselective processes (absorption, dis-

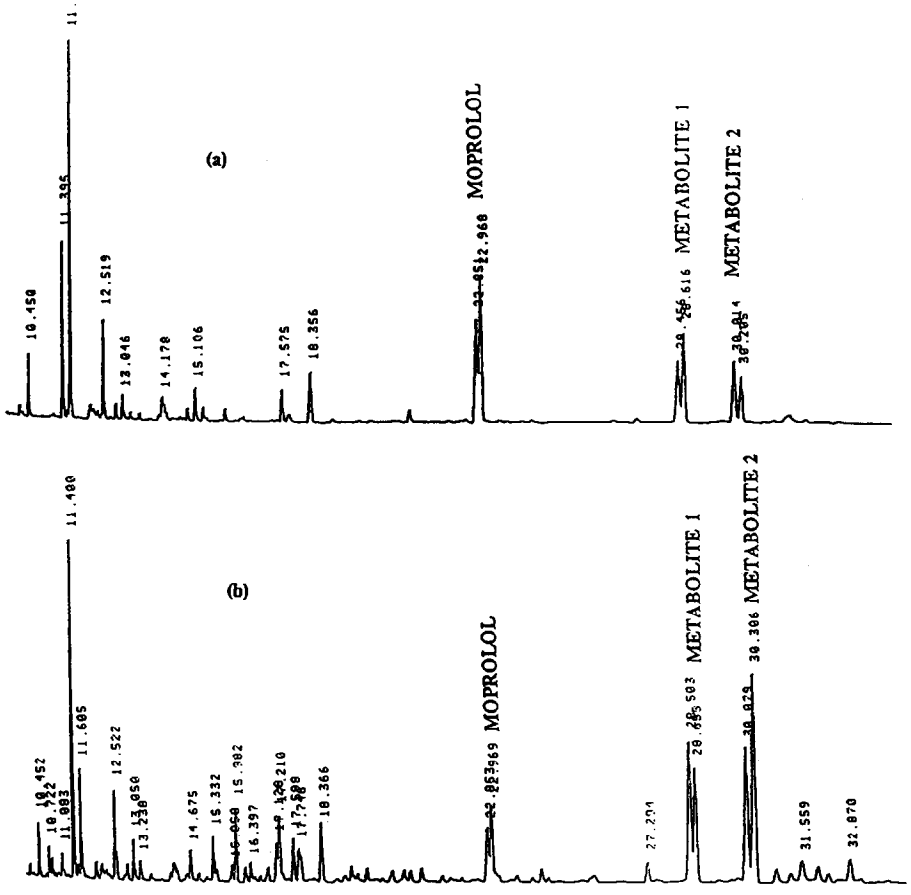

Fig. 9. GC of (−)-MCF and MSTFA derivatives of (a) unconjugated and (b) conjugated urinary moprolol and its metabolites.

tribution, metabolism and excretion) dramatically alter the stereochemical composition of β-blockers at their sites of action [6]. After oral administration of racemic moprolol to a human subject, the enantiomeric metabolites in urine were analyzed by GC and GC–MS. Moprolol was biotransformed to two major metabolites. In the unconjugated fraction, (+)-moprolol, (+)-metabolite-1 and (−)-metabolite-2 were mainly excreted in urine. In the conjugated fraction, (+)-moprolol, (−)-metabolite-1 and (+)-metabolite-2 were principal excretory components in urine. This phenomenon demonstrates that the mechanism of moprolol metabolism is stereoselective. It is yet unknown whether or not these metabolites contribute significantly to its β-adrenergic blocking activity and the mechanism of biotransformation.

CONCLUSIONS

We have presented a new assay for the determination of the enantiomers of moprolol and their metabolites in human urine using pre-column derivatization and reversed-phase HPLC. The assay method was evaluated using urine samples spiked with moprolol and the internal standard toliprolol. The assay was used to monitor excretion of (−)- and (+)-moprolol enantiomers in human urine following a 50-mg oral dose of racemic moprolol. The two metabolites of moprolol were identified by GC–MS. The method can be easily adapted for routine doping control and clinical laboratories. A comparison was made with the urinary profiles of metabolites obtained by GC.

ACKNOWLEDGEMENTS

This work was supported by grants from Canadian Centre for Drug-free Sport (CCDS). The authors thank Mrs. M. Peat, Miss D. Daoust and Miss Zhen Yang for technical assistance and Mrs. D. Lacoste for preparing diagrams.

REFERENCES

1 W.H. Frishman (Editor), *Clinical Pharmacology of The Beta-Adrenoceptor Blocking Drugs*, Prentice-Hall, New York, 1980.
2 G. Ferrari, R. Ferrini and C. Casagrande, *Boll. Chim. Farm.*, 107 (1968) 234–248.
3 V. Gianesello, E. Brenn, G. Figini and A. Gazzaniga, *J. Chromatogr.*, 473 (1989) 343–352.
4 C. Harvengt and J.P. Desager, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 20 (1982) 57–61.
5 J.P. Desager, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 129–132.
6 V. Marko (Editor), *Determination of Beta-Blockers in Biological Material*, Elsevier Science Publishers, Amsterdam, 1989.